#### 504956772 06/12/2018

#### PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1 Stylesheet Version v1.2 EPAS ID: PAT5003519

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

#### **CONVEYING PARTY DATA**

Name	Execution Date
SPOGEN BIOTECH INC.	09/14/2015

#### **RECEIVING PARTY DATA**

Name:	BAYER CROPSCIENCE LP
Street Address:	2 T.W. ALEXANDER DRIVE
City:	RESEARCH TRIANGLE PARK
State/Country:	NORTH CAROLINA
Postal Code:	27709

#### **PROPERTY NUMBERS Total: 1**

Property Type	Number
Application Number:	15511835

#### **CORRESPONDENCE DATA**

Fax Number: (916)661-3001

Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent

using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.

Phone: (916) 661-3454

michelle.samonek@bayer.com, ann.inglett@bayer.com, Email:

EMillard@senniger.com

**Correspondent Name:** MICHELLE L. SAMONEK Address Line 1: BAYER CROPSCIENCE LP Address Line 2: 890 EMBARCADERO DRIVE

Address Line 4: WEST SACRAMENTO, CALIFORNIA 95605

ATTORNEY DOCKET NUMBER:	149058 PCT-US/3010 US.N
NAME OF SUBMITTER:	ANN H. INGLETT
SIGNATURE:	/ahi/
DATE SIGNED:	06/12/2018

#### **Total Attachments: 167**

source=Assignment from Spogen to BCS LP for BCS149058 US#page1.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page2.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page3.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page4.tif

> **PATENT** REEL: 046350 FRAME: 0677

504956772

source=Assignment from Spogen to BCS LP for BCS149058 US#page5.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page6.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page7.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page8.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page9.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page10.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page11.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page12.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page13.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page14.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page15.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page16.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page17.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page18.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page19.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page20.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page21.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page22.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page23.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page24.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page25.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page26.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page27.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page28.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page29.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page30.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page31.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page32.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page33.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page34.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page35.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page36.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page37.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page38.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page39.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page40.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page41.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page42.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page43.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page44.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page45.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page46.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page47.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page48.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page49.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page50.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page51.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page52.tif

source=Assignment from Spogen to BCS LP for BCS149058 US#page53.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page54.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page55.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page56.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page57.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page58.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page59.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page60.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page61.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page62.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page63.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page64.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page65.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page66.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page67.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page68.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page69.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page70.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page71.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page72.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page73.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page74.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page75.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page76.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page77.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page78.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page79.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page80.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page81.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page82.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page83.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page84.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page85.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page86.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page87.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page88.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page89.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page90.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page91.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page92.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page93.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page94.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page95.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page96.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page97.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page98.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page99.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page100.tif

source=Assignment from Spogen to BCS LP for BCS149058 US#page101.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page102.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page103.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page104.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page105.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page106.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page107.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page108.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page109.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page110.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page111.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page112.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page113.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page114.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page115.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page116.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page117.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page118.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page119.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page120.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page121.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page122.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page123.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page124.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page125.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page126.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page127.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page128.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page129.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page130.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page131.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page132.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page133.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page134.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page135.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page136.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page137.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page138.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page139.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page140.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page141.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page142.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page143.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page144.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page145.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page146.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page147.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page148.tif

source=Assignment from Spogen to BCS LP for BCS149058 US#page149.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page150.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page151.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page152.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page153.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page154.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page155.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page156.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page157.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page158.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page159.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page160.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page161.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page162.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page163.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page164.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page165.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page166.tif source=Assignment from Spogen to BCS LP for BCS149058 US#page167.tif

#### ASSIGNMENT

Whereas, Spogen Biotech Inc., a Missouri corporation, having a place of business at 1685 Galt Industrial Boulevard, St. Louis, Missouri 63132, U.S.A., ("Assignor") owns certain rights, title and interest in and to the subject matter of:

# COMPOSITIONS COMPRISING RECOMBINANT BACILLUS CELLS AND A FUNGICIDE

described in an application for Letters Patent the specification of which:

was filed on: September 17, 2014
as United States Application No.: 62/051,915

Whereas, Bayer CropScience LP, a Delaware limited partnership, having a place of business at 2 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709, U.S.A., ("Assignee") desires to acquire Assignee's entire right, title, and interest in the Invention, as that term is defined below, and to any United States and foreign patents to be obtained therefor;

Now therefore, for valuable consideration, receipt whereof is hereby acknowledged,

The above-named Assignor, hereby sells, assigns and transfers to the above named Assignee, its successors, legal representatives, and assigns, its entire right, title and interest in the subject matter and the application for the United States of America only to the extent described in Attachment A hereto (the "Invention"), including all direct and indirect divisions, continuations, and continuations-in-part thereof, and all original, extended, reissued, reviewed, and reexamined Letters Patent of the United States, and all countries foreign thereto, that may be granted thereon, including rights of priority under the International Convention of Paris (1883) as amended, including the right to claim priority under 35 U.S.C. §119, and above-named Assignor requests the Director of the U.S. Patent and Trademark Office to issue any Letters Patent granted upon the Invention set forth in the application to the Assignee, its successors and assigns; and it hereby agrees that the Assignee may apply for foreign Letters Patent on the Invention and the above-named Assignor will without further consideration, communicate all facts known to it and execute without further consideration all papers deemed necessary by the Assignee in connection with the United States and foreign applications when called upon to do so by the Assignee, its successors, legal representatives, or assigns. The above-named Assignor further represents and warrants that it has the full right to convey the interest assigned by this assignment, and that it has not granted any rights inconsistent with the rights granted herein.

This Assignment may be executed in one or more counterparts, each of which shall be deemed an original and all of which may be taken together as one and the same Assignment.

This Assignment shall be binding upon the successors and assigns of Assignor and shall inure to the benefit of the successors and assigns of Assignee.

IN WITNESS WHEREOF, Assignor has caused this Patent Assignment to be executed as of the date set forth below.

Assignor SPOGEN BIOTECH INC. (a Missouri corporation)	Assignee BAYER CROPSCIENCE LP (a Delaware limited partnership)	
Name: Steve Trange	Name: Margaret Keating	
Title: Vice-Pres. dast	Title: Assistant Secretary	
Date: September 14, 2015	Date: September 14, 2015	

STATE OF MISSOURI COUNTY OF SAINT LOUIS

On this 14th day of September, 2015, before me, a Notary Public, personally appeared Steve Trampe to me known to be the person described in and who executed the foregoing assignment and acknowledged that he executed same as his free act and deed.

IN TESTIMONY WHEREOF, I have hereunto set my hand and seal and the date and year last above written.

My Commission Expires: No. 25, 2016

THOMAS E. PLACKE, JR.
Notary Public - Notary Seal
State of Missouri
Commissioned for St. Louis County
My Commission Expires: November 25, 2016
Commission Number: 12417541

This Assignment shall be binding upon the successors and assigns of Assignor and shall inure to the benefit of the successors and assigns of Assignee.

IN WITNESS WHEREOF, Assignor has caused this Patent Assignment to be executed as of the date set forth below.

Assignor	Assignee	
SPOGEN BIOTECH INC.	BAYER CROPSCIENCE LP	
(a Missouri corporation)	(a Delaware limited partnership)	
	Margaret Carling	
Name:	Name: Margaret Keating	
Title:	Title: Assistant Secretary	
Date: Sentember , 2015	Date: September 14, 2015	

#### INDIVIDUAL ACKNOWLEDGMENT

State/Commonwealth of Month Card	[]			
State/Commonwealth of Abrilia Cuso  County of Durham		SS.		
On this the 14 had day of 54	Month	,	, be	for <del>e</del>
me, <u>FYY: Same of Notary Publi</u>	106	, the unde	ersigned No	tary
Public, personally appeared	c <u>ing a ret</u> Name	Kecting (s) of Signer(s)	taranan dalah dari di panjangan padapapan	
		illy known to me –		
	☐ proved evidence	to me on the basi	s of satisfac	tory
THE COLLEGE OF THE PARTY OF THE	subscribed acknowled	person(s) whose to the within in ged to me tha ne same for the pu	nstrument, a t he/she/ti	and ney
	WITNESS	my hand and offici	al seal.	
EN COUNTY IN		Signature of Notary Po		
Place Notary Seal and/or Any Stamp Above	my our	emide and proper	1 June	13 2020.
	IONAL	***************************************	******************************	
Although the information in this section is not require persons relying on the document and could prevent from the form to another document.		and reattachment	Right Promise of Spiner Top of thumb h	iere
Description of Attached Document				
Title or Type of Document: Allarney Dock	let Nos. Bo	5149058		
Document Date: <u>9-/Y-20//</u> Number				
Signer(s) Other Than Named Above:	MA			

© 2002 National Notary Association • 9350 De Soto Ave., P.O. Box 2402 • Chatsworth, CA 91313-2402 • www.NationalNotary.org ttem No. 5936 Reorder: Call Toll-Free 1-800 US NOTARY (1-800-876-6827)

Attorney Docket Nos.: BCS149058 US; ELEN 3010.USP

### ATTACHMENT A

## COMPOSITIONS COMPRISING RECOMBINANT BACILLUS CELLS AND A FUNGICIDE

#### **CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/051,915, filed September 17, 2014, the content of which is incorporated herein by reference in its entirety.

#### REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII-formatted sequence listing with a file named "BCS149058WO\_ST25.txt" created on September 14, 2015, and having a size of 152 kilobytes, and is filed concurrently with the specification. The sequence listing contained in this ASCII-formatted document is part of the specification and is herein incorporated by reference in its entirety.

15

20

25

30

35

10

5

#### **BACKGROUND**

#### Field of the Invention

[0003] The present invention relates to a composition comprising (i) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (x) at least one plant growth stimulating protein or peptide; and (y) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and (ii) at least one fungicide selected from the particular fungicides disclosed herein that exhibits the ability to improve plant growth and/or health and/or activity against insects, mites, nematodes and/or phytopathogens in synergistically effective amounts. Furthermore, the present invention relates to the use of this composition as well as a method for enhancing plant growth, promoting plant health, and/or reducing overall damage of plants and plant parts.

#### **Background of the Invention**

[0004] In crop protection, there is a continuous need for applications that improve the health and/or the growth of plants. Healthier plants generally result in higher yields and/or better quality of a plant or its products.

[0005] In order to promote plant health, fertilizers are employed worldwide, based on both inorganic and organic substances. A fertilizer may be a single substance or a composition, and is used to provide nutrients to plants. A major breakthrough in the application of fertilizers was the development of nitrogen-based fertilizer by Justus von Liebig around 1840. Fertilizers, however, can lead to soil acidification and destabilization of nutrient balance in soil, including

depletion of minerals and enrichment of salt and heavy metals. In addition, excessive fertilizer use can lead to alteration of soil fauna as well as contaminate surface water and ground water. Further, unhealthful substances such as nitrate may become enriched in plants and fruits.

5

10

15

20

25

30

35

[0006] In addition, insecticides and fungicide are employed worldwide to control pests. Synthetic insecticides or fungicides often are non-specific and therefore can act on organisms other than the target organisms, including other naturally occurring beneficial organisms. Because of their chemical nature, they may also be toxic and non-biodegradable. Consumers worldwide are increasingly conscious of the potential environmental and health problems associated with the residuals of chemicals, particularly in food products. This has resulted in growing consumer pressure to reduce the use or at least the quantity of chemical (i.e., synthetic) pesticides. Thus, there is a need to manage food chain requirements while still allowing effective pest control.

[0007] A further problem arising with the use of synthetic insecticides or fungicides is that the repeated and exclusive application of an insecticide or fungicides often leads to selection of resistant animal pests or microorganisms. Normally, such strains are also cross-resistant against other active ingredients having the same mode of action. An effective control of the pathogens with said active compounds is then not possible any longer. However, active ingredients having new mechanisms of action are difficult and expensive to develop.

[0008] The use of biological control agents (BCAs), which act as plant health-enhancing and/or plant protection agents, is an alternative to fertilizers and synthetic pesticides. In some cases, the effectiveness of BCAs is not at the same level as for conventional insecticides and fungicides, especially in case of severe infection pressure. Consequently, in some circumstances, biological control agents, their mutants and metabolites produced by them are, in particular in low application rates, not entirely satisfactory. Thus, there is a constant need for developing new plant health-enhancing and/or plant protection compositions, including biological control agents used in conjunction with synthetic fungicides and insecticides, to strive to fulfill the above-mentioned requirements.

#### **SUMMARY**

[0009] In view of this, it was in particular an object of the present invention to provide compositions which have an enhanced ability to improve plant growth and/or to enhance plant health or which exhibit enhanced activity against insects, mites, nematodes and/or phytopathogens.

[00010] Accordingly, it was found that these objectives are achieved with the compositions according to the invention as defined in the following. By applying a) recombinant

exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound; an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source; and a protein or peptide that protects a plant from a pathogen or a pest; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one particular fungicide disclosed herein, one is able to enhance preferably in a superadditive manner (i) plant growth, plant yield and/or plant health and/or (ii) the activity against insects, mites, nematodes and/or phytopathogens.

5

10

15

20

25

30

35

[00011] References herein to targeting sequences, exosporium proteins, exosporium protein fragments, fusion proteins, and recombinant exosporium producing *Bacillus* cells that express such fusion proteins should not be considered to be stand-alone embodiments. Instead, throughout the present application, references to the targeting sequences, exosporium proteins, exosporium protein fragments, fusion proteins, and recombinant exosporium producing *Bacillus* cells that express such fusion proteins should be considered to be disclosed and claimed only in combination (and preferably in a synergistic combination) with one or more of the particular fungicides described herein. Furthermore, references to the "particular fungicide disclosed herein" are intended to encompass fungicides described below in paragraphs [000185]-[000187].

[00012] The present invention is directed to a composition comprising a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound; and an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source; or a protein or peptide that protects a plant from a pathogen; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one particular fungicide disclosed herein in a synergistically effective amount.

[00013] In some embodiments, the targeting sequence comprises: an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; a targeting sequence comprising amino acids 1–35 of SEQ ID NO: 1; a targeting sequence comprising amino acids 20–35 of SEQ ID NO: 1; a targeting sequence comprising amino acids 22–31 of SEQ ID NO: 1; a targeting sequence comprising amino acids 22–33 of SEQ ID NO: 1; a targeting sequence comprising amino acids 20–31 of SEQ ID NO: 1; a targeting sequence comprising SEQ ID NO: 1; or an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2.

[00014] In some embodiments, the exosporium-producing *Bacillus* cells are cells of a *Bacillus cereus* family member. The recombinant *Bacillus cereus* family member may be any one of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus samanii*, *Bacillus gaemokensis*, *Bacillus weihenstephensis*, *Bacillus toyoiensis* and combinations thereof. In a further embodiment, the recombinant *Bacillus* cells are cells of *Bacillus thuringiensis* BT013A.

5

10

15

20

25

30

In certain aspects, the fusion protein comprises an enzyme involved in the production or activation of a plant growth stimulating compound selected from the group consisting of an acetoin reductase, an indole-3-acetamide hydrolase, a tryptophan monooxygenase, an acetolactate synthetase, an α-acetolactate decarboxylase, a pyruvate decarboxylase, a diacetyl reductase, a butanediol dehydrogenase, an aminotransferase, a tryptophan decarboxylase, an amine oxidase, an indole-3-pyruvate decarboxylase, an indole-3acetaldehyde dehydrogenase, a tryptophan side chain oxidase, a nitrile hydrolase, a nitrilase, a peptidase, a protease, an adenosine phosphate isopentenyltransferase, a phosphatase, an adenosine kinase, an adenine phosphoribosyltransferase, CYP735A, a 5'ribonucleotide phosphohydrolase, an adenosine nucleosidase, a zeatin cis-trans isomerase, a zeatin Oglucosyltransferase, a β-glucosidase, a cis- hydroxylase, a CK cis-hydroxylase, a CK Nglucosyltransferase, a 2,5-ribonucleotide phosphohydrolase, an adenosine nucleosidase, a purine nucleoside phosphorylase, a zeatin reductase, a hydroxylamine reductase, a 2oxoglutarate dioxygenase, a gibberellic 2B/3B hydrolase, a gibberellin 3-oxidase, a gibberellin 20-oxidase, a chitosanase, a chitinase, a  $\beta$ -1,3- glucanase, a  $\beta$ -1,4-glucanase, a  $\beta$ -1,6-glucanase, an aminocyclopropane-1-carboxylic acid deaminase, and an enzyme involved in producing a nod factor.

**[00016]** In other aspects, the fusion protein comprises an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source selected from the group consisting of a cellulase, a lipase, a lignin oxidase, a protease, a glycoside hydrolase, a phosphatase, a nitrogenase, a nuclease, an amidase, a nitrate reductase, a nitrite reductase, an amylase, an ammonia oxidase, a ligninase, a glucosidase, a phospholipase, a phytase, a pectinase, a glucanase, a sulfatase, a urease, a xylanase, and a siderophore.

**[00017]** In still other aspects, the fusion protein comprises a protein or peptide that protects a plant from a pathogen and the protein or peptide has antibacterial activity, antifungal activity, or both antibacterial and antifungal activity. Such a protein may comprise a bacteriocin, a lysozyme, a lysozyme peptide, a siderophore, a non-ribosomal active peptide, a conalbumin, an albumin, a lactoferrin, a lactoferrin peptide, TasA, or streptavidin.

[00018] In some embodiments, the at least one fungicide is selected from the group consisting of bitertanol, bixafen, bromuconazole, carbendazim, carpropamid, dichlofluanid, fenamidone, fenhexamid, fentin acetate, fentin hydroxide, fluopicolide, fluopyram, fluoxastrobin, fluquinconazole, fosetyl, iprodione, iprovalicarb, isotianil, metominostrobin, ofurace, pencycuron, penflufen, prochloraz, propamocarb, propineb, prothioconazole, pyrimethanil, spiroxamine, tebuconazole, tolylfluanid, triadimefon, triadimenol, triazoxide, trifloxystrobin, N-[5-chloro-2-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, and 2,6-dimethyl-1H,5H-[1,4]dithiino[2,3-c:5,6-c']dipyrrole-1,3,5,7(2H,6H)-tetrone.

5

10

15

20

25

30

35

**[00019]** In other embodiments, the at least one fungicide is selected from the group consisting of carbendazim, fluquinconazole, isotianil, pencycuron, penflufen, prothioconazole, tebuconazole, and trifloxystrobin.

[00020] In some embodiments, the composition of the present invention comprises a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound and an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source or at least one protein or peptide that protects a plant from a pathogen; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one fungicide selected from the group consisting of carbendazim, fluquinconazole, isotianil, pencycuron, penflufen, prothioconazole, fluopyram, tebuconazole, and trifloxystrobin in a synergistically effective amount.

[00021] In a particular aspect of the above embodiments (i) the at least one fungicide is carbendazim; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringiensis* BT013A.

[00022] In a particular aspect of the above embodiments (i) the at least one fungicide is fluquinconazole; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises

5

endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

5

10

15

20

25

30

35

[00023] In a particular aspect of the above embodiments (i) the at least one fungicide is isotianil; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

[00024] In a particular aspect of the above embodiments (i) the at least one fungicide is pencycuron; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

[00025] In a particular aspect of the above embodiments (i) the at least one fungicide is penflufen; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

[00026] In a particular aspect of the above embodiments (i) the at least one fungicide is prothioconazole; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino

acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

5

10

15

20

25

30

[00027] In a particular aspect of the above embodiments (i) the at least one fungicide is fluopyram; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*.

[00028] In a particular aspect of the above embodiments (i) the at least one fungicide is trifloxystrobin; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

[00029] In a particular aspect of the above embodiments (i) the at least one fungicide is tebuconazole; (ii) the targeting sequence comprises an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%; (iii) the plant growth stimulating protein or peptide comprises endoglucanase, phospholipase or chitosinase, preferably with at least 95% sequence identity to SEQ ID NO: 107, 108 and 109, respectively; and (iv) the recombinant *Bacillus cereus* family member cells comprise cells of *Bacillus thuringiensis* or *Bacillus mycoides*. In yet another particular embodiment, the recombinant *Bacillus cereus* family member cells are cells of *Bacillus thuringeinsis* BT013A.

**[00030]** In some aspects, the composition further comprises at least one auxiliary selected from the group consisting of extenders, solvents, spontaneity promoters, carriers, emulsifiers, dispersants, frost protectants, thickeners and adjuvants.

[00031] In other aspects, the invention is directed to a seed treated with any of the compositions disclosed herein.

**[00032]** Furthermore, the present invention relates to use of the disclosed compositions as a fungicide and/or insecticide. In certain aspects, the disclosed compositions are used for reducing overall damage of plants and plant parts as well as losses in harvested fruits or vegetables caused by insects, mites, nematodes and/or phytopathogens. In other aspects, the disclosed compositions are used for enhancing plant growth and/or promoting plant health.

5

10

15

20

25

30

35

[00033] Additionally, the present invention is directed to a method of treating a plant, a plant part, such as a seed, root, rhizome, corm, bulb, or tuber, and/or a locus on which or near which the plant or the plant parts grow, such as soil, to enhance plant growth and/or promote plant health comprising the step of simultaneously or sequentially applying to a plant, a plant part and/or a plant loci: a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound; an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source; and a protein or peptide that protects a plant from a pathogen; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one fungicide selected from particular fungicides disclosed herein that exhibits activity against insects, mites, nematodes and/or phytopathogens in a synergistically effective amount.

[00034] In another embodiment, the present invention is a method for reducing overall damage of plants and plant parts as well as losses in harvested fruits or vegetables caused by insects, mites, nematodes and/or phytopathogens comprising the step of simultaneously or sequentially applying to a plant, a plant part, such as a seed, root, rhizome, corm, bulb, or tuber, and/or a locus on which or near which the plant or the plant parts grow, such as soil: a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound; an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source; and a protein or peptide that protects a plant from a pathogen; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one fungicide selected from the particular fungicides disclosed herein that exhibits activity against insects, mites, nematodes and/or phytopathogens in a synergistically effective amount.

[00035] In the above paragraphs, the term "comprise" or any derivative thereof (e.g., comprising, comprises) may be replaced with "consist of" or the applicable corresponding derivative thereof.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

[00036] FIG. 1 shows an alignment of the amino acid sequence of the amino-terminal portion of *Bacillus anthracis* Sterne strain BclA and with the corresponding region from various exosporium proteins from *Bacillus cereus* family members.

5

10

15

20

25

30

35

#### **DETAILED DESCRIPTION**

[00037] In general "pesticidal" means the ability of a substance to increase mortality or inhibit the growth rate of plant pests. The term is used herein, to describe the property of a substance to exhibit activity against insects, mites, nematodes and/or phytopathogens. In the sense of the present invention the term "pests" include insects, mites, nematodes and/or phytopathogens.

[00038] A variant of the indicated NRRL or ATCC Accession Number may also be defined as a strain having a genomic sequence that is greater than 85%, more preferably greater than 90% or more preferably greater than 95% sequence identity to the genome of the indicated NRRL or ATCC Accession Number. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example, those described in Current Protocols in Molecular Biology (F. M. Ausubel, et al., eds., 1987) Supplement 30, Section 7. 7. 18, Table 7. 7. 1.

[00039] NRRL is the abbreviation for the Agricultural Research Service Culture Collection, having the address National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, U.S.A.

[00040] ATCC is the abbreviation for the American Type Culture Collection, having the address ATCC Patent Depository, 10801 University Boulevard, Manassas, Virginia 10110, U.S.A.

[00041] All strains described herein and having an accession number in which the prefix is NRRL or ATCC have been deposited with the above-described respective depositary institution in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

[00042] An "enzyme involved in the production or activation of a plant growth stimulating compound" includes any enzyme that catalyzes any step in a biological synthesis pathway for a compound that stimulates plant growth or alters plant structure, or any enzyme that

9

catalyzes the conversion of an inactive or less active derivative of a compound that stimulates plant growth or alters plant structure to an active or more active form of the compound. Such compounds include, for example, but are not limited to, small molecule plant hormones such as auxins and cytokinins, bioactive peptides, and small plant growth stimulating molecules synthesized by bacteria or fungi in the rhizosphere (e.g., 2,3-butanediol).

[00043] A "plant immune system enhancer protein or peptide" as used herein includes any protein or peptide that has a beneficial effect on the immune system of a plant.

5

10

15

20

25

30

35

[00044] The term "plant growth stimulating protein or peptide" as used herein includes any protein or peptide that increases plant growth in a plant exposed to the protein or peptide.

[00045] The terms "promoting plant growth" and "stimulating plant growth" are used interchangeably herein, and refer to the ability to enhance or increase at least one of the plant's height, weight, leaf size, root size, or stem size, to increase protein yield from the plant or to increase grain yield of the plant.

[00046] A "protein or peptide that protects a plant from a pathogen" as used herein includes any protein or peptide that makes a plant exposed to the protein or peptide less susceptible to infection with a pathogen.

[00047] A "protein or peptide that enhances stress resistance in a plant" as used herein includes any protein or peptide that makes a plant exposed to the protein or peptide more resistant to stress.

[00048] The term "plant binding protein or peptide" refers to any peptide or protein capable of specifically or non-specifically binding to any part of a plant (e.g., roots or aerial portions of a plant such as leaves foliage, stems, flowers, or fruits) or to plant matter.

[00049] The term "targeting sequence" as used herein refers to a polypeptide sequence that results in the localization of a longer polypeptide or the protein to the exosporium of a *Bacillus cereus* family member.

#### Recombinant Exosporium-Producing Bacillus Cells Expressing Fusion Proteins

[00050] The fusion proteins contain a targeting sequence, an exosporium protein, or an exosporium protein fragment that targets the fusion protein to the exosporium of a *Bacillus cereus* family member and: (a) a plant growth stimulating protein or peptide; (b) a protein or peptide that protects a plant from a pathogen; (c) a protein or peptide that enhances stress resistance of a plant; (d) a plant binding protein or peptide; or (e) a plant immune system enhancer protein or peptide. When expressed in *Bacillus cereus* family member bacteria, these fusion proteins are targeted to the exosporium layer of the spore and are physically oriented such that the protein or peptide is displayed on the outside of the spore.

10

[00051] This *Bacillus* exosporium display (BEMD) system can be used to deliver peptides, enzymes, and other proteins to plants (e.g., to plant foliage, fruits, flowers, stems, or roots) or to a plant growth medium such as soil. Peptides, enzymes, and proteins delivered to the soil or another plant growth medium in this manner persist and exhibit activity in the soil for extended periods of time. Introduction of recombinant exosporium-producing *Bacillus* cells expressing the fusion proteins described herein into soil or the rhizosphere of a plant leads to a beneficial enhancement of plant growth in many different soil conditions. The use of the BEMD to create these enzymes allows them to continue to exert their beneficial results to the plant and the rhizosphere over the first months of a plants life.

10

15

5

#### Targeting Sequences, Exosporium Proteins, and Exosporium Protein Fragments

[00052] For ease of reference, the SEQ ID NOS. for the peptide and protein sequences referred to herein are listed in Table 1 below.

#### Table 1. Peptide and Protein Sequences

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
AA 1–41 of BcIA	SEQ ID NO: 1*
(B. anthracis Sterne)	
Full length BclA	SEQ ID NO: 2*
AA 1–33 of	SEQ ID NO: 3
BetA/BAS3290	
(B. anthracis Sterne)	
Full length BetA/BAS3290	SEQ ID NO: 4
Met + AA 2–43 of	SEQ ID NO: 5
BAS4623	
(B. anthracis Sterne)	
Full length BAS4623	SEQ ID NO: 6

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
AA 1–34 of BclB (B. anthracis Sterne)	SEQ ID NO: 7
Full length BclB	SEQ ID NO: 8
AA 1–30 of BAS1882 (B. anthracis Sterne)	SEQ ID NO: 9
Full length BAS1882	SEQ ID NO: 10
AA 1–39 of gene 2280 (B. weihenstephensis KBAB4)	SEQ ID NO: 11
Full length KBAB4 gene 2280	SEQ ID NO: 12
AA 1–39 of gene 3572 (B. weihenstephensis KBAB4)	SEQ ID NO: 13
Full Length KBAB4 gene 3572	SEQ ID NO: 14
AA 1–49 of Exosporium Leader Peptide (B. cereus VD200)	SEQ ID NO: 15
Full Length Exosporium Leader Peptide	SEQ ID NO: 16
AA 1–33 of Exosporium Leader Peptide (B. cereus VD166)	SEQ ID NO: 17
Full Length Exosporium Leader Peptide	SEQ ID NO: 18
AA 1–39 of hypothetical protein IKG_04663 (B. cereus VD200)	SEQ ID NO: 19
Full Length hypothetical protein IKG_04663, partial	SEQ ID NO: 20

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
AA 1–39 of YVTN β- propeller protein (B. weihenstephensis KBAB4)	SEQ ID NO: 21
Full length YVTN β- propeller protein KBAB4	SEQ ID NO: 22
AA 1–30 of hypothetical protein bcerkbab4_2363 (B. weihenstephensis KBAB4)	SEQ ID NO: 23
Full length hypothetical protein bcerkbab4_2363 KBAB4	SEQ ID NO: 24
AA 1–30 of hypothetical protein bcerkbab4_2131 (B. weihenstephensis KBAB4)	SEQ ID NO: 25
Full length hypothetical protein bcerkbab4_2131	SEQ ID NO: 26
AA 1–36 of triple helix repeat containing collagen (B. weihenstephensis KBAB4)	SEQ ID NO: 27
Full length triple helix repeat-containing collagen KBAB4	SEQ ID NO: 28
AA 1–39 of hypothetical protein bmyco0001_21660 (B. mycoides 2048)	SEQ ID NO: 29
Full length hypothetical protein bmyco0001_21660	SEQ ID NO: 30
AA 1–30 of hypothetical protein bmyc0001_22540 ( <i>B. mycoides</i> 2048)	SEQ ID NO: 31
Full length hypothetical protein bmyc0001_22540	SEQ ID NO: 32

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
AA 1–21 of hypothetical protein bmyc0001_21510 ( <i>B. mycoides</i> 2048)	SEQ ID NO: 33
Full length hypothetical protein bmyc0001_21510	SEQ ID NO: 34
AA 1–22 of collagen triple helix repeat protein (B. thuringiensis 35646)	SEQ ID NO: 35
Full length collagen triple helix repeat protein	SEQ ID NO: 36
AA 1–35 of hypothetical protein WP_69652 (B. cereus)	SEQ ID NO: 43
Full length hypothetical protein WP_69652	SEQ ID NO: 44
AA 1–41 of exosporium leader WP016117717 (B. cereus)	SEQ ID NO: 45
Full length exosporium leader WP016117717	SEQ ID NO: 46
AA 1–49 of exosporium peptide WP002105192 (B. cereus)	SEQ ID NO: 47
Full length exosporium peptide WP002105192	SEQ ID NO: 48
AA 1–38 of hypothetical protein WP87353 (B. cereus)	SEQ ID NO: 49
Full length hypothetical protein WP87353	SEQ ID NO: 50
AA 1–39 of exosporium peptide 02112369 (B. cereus)	SEQ ID NO: 51

14

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
Full length exosporium peptide 02112369	SEQ ID NO: 52
AA 1–39 of exosporium protein WP016099770 (B. cereus)	SEQ ID NO: 53
Full length exosporium protein WP016099770	SEQ ID NO: 54
AA 1–36 of hypothetical protein YP006612525 (B. thuringiensis)	SEQ ID NO: 55
Full length hypothetical protein YP006612525	SEQ ID NO: 56
AA 1–136 of hypothetical protein TIGR03720 (B. mycoides)	SEQ ID NO: 57**
Full length hypothetical protein TIGR03720	SEQ ID NO: 58**
AA 1–196 of BclA (B. anthracis Sterne)	SEQ ID NO: 59*
Met + AA 20–35 of BclA (B. anthracis Sterne)	SEQ ID NO: 60
Met + AA 12–27 of BetA/BAS3290 (B. anthracis Sterne)	SEQ ID NO: 61
Met + AA 18–33 of gene 2280 (B. weihenstephensis KBAB4)	SEQ ID NO: 62
Met + AA 18–33 of gene 3572 (B. weihenstephensis KBAB4)	SEQ ID NO: 63

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
Met + AA 12–27 of Exosporium Leader Peptide ( <i>B. cereus</i> VD166)	SEQ ID NO: 64
Met + AA 18–33 of YVTN β-propeller protein (B. weihenstephensis KBAB4)	SEQ ID NO: 65
Met + AA 9–24 of hypothetical protein bcerkbab4_2363 (B. weihenstephensis KBAB4)	SEQ ID NO: 66
Met + AA 9–24 of hypothetical protein bcerkbab4_2131 (B. weihenstephensis KBAB4)	SEQ ID NO: 67
Met + AA 9–24 of hypothetical protein bmyc0001_22540 ( <i>B. mycoides</i> 2048)	SEQ ID NO: 68
Met + AA 9–24 of BAS1882 ( <i>B. anthracis</i> Sterne)	SEQ ID NO: 69
Met + AA 20–35 of exosporium leader WP016117717 (B. cereus)	SEQ ID NO: 70
Full length InhA (B. mycoides)	SEQ ID NO: 71
Full length BAS1141 (ExsY) (B. anthracis Sterne)	SEQ ID NO: 72
Full length BAS1144 (BxpB/ExsFA) (B. anthracis Sterne)	SEQ ID NO: 73
Full length BAS1145 (CotY) (B. anthracis Sterne)	SEQ ID NO: 74

16

Protein, Protein Fragment, or Targeting Sequence	Sequence Identification Number
Full length BAS1140 (B. anthracis Sterne)	SEQ ID NO: 75
Full length ExsFB (B. anthracis H9401)	SEQ ID NO: 76
Full length InhA1 (B. thuringiensis HD74)	SEQ ID NO: 77
Full length ExsJ (B. cereus ATCC 10876)	SEQ ID NO: 78
Full length ExsH (B. cereus)	SEQ ID NO: 79
Full length YjcA (B. anthracis Ames)	SEQ ID NO: 80
Full length YjcB (B. anthracis)	SEQ ID NO: 81
Full length BclC (B. anthracis Sterne)	SEQ ID NO: 82
Full length acid phosphatase (Bacillus thuringiensis serovar konkukian str. 97-27)	SEQ ID NO: 83
Full length InhA2 (B. thuringiensis HD74)	SEQ ID NO: 84

AA = amino acids

\*B. anthracis Sterne strain BclA has 100% sequence identity with B. thuringiensis BclA. Thus, SEQ ID NOS: 1, 2, and 59 also represent amino acids 1–41 of B. thuringiensis BclA, full length B. thuringiensis BclA, and amino acids 1–196 of B. thuringiensis BclA, respectively.

17

Likewise, SEQ ID NO: 60 also represents a methionine residue plus amino acids 20–35 of *B. thuringiensis* BclA.

\*\* *B. mycoides* hypothetical protein TIGR03720 has 100% sequence identity with *B. mycoides* hypothetical protein WP003189234. Thus, SEQ ID NOS: 57 and 58 also represent amino acids 1–136 of *B. mycoides* hypothetical protein WP003189234 and full length *B. mycoides* hypothetical protein WP003189234, respectively.

5

10

15

20

25

30

35

[00053] Bacillus is a genus of rod-shaped bacteria. The Bacillus cereus family of bacteria includes the species Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus samanii, Bacillus gaemokensis, Bacillus toyoiensis, and Bacillus weihenstephensis. Under stressful environmental conditions, Bacillus cereus family bacteria undergo sporulation and form oval endospores that can stay dormant for extended periods of time. The outermost layer of the endospores is known as the exosporium and comprises a basal layer surrounded by an external nap of hair-like projections. Filaments on the hair-like nap are predominantly formed by the collagen-like glycoprotein BclA, while the basal layer is comprised of a number of different proteins. Another collagen-related protein, BclB, is also present in the exosporium and exposed on endospores of Bacillus cereus family members.

[00054] BclA, the major constituent of the surface nap, has been shown to be attached to the exosporium with its amino-terminus (N-terminus) positioned at the basal layer and its carboxy-terminus (C- terminus) extending outward from the spore.

[00055] It was previously discovered that certain sequences from the N-terminal regions of BclA and BclB could be used to target a peptide or protein to the exosporium of a *Bacillus cereus* endospore (*see* U.S. Patent Publication Nos. 2010/0233124 and 2011/0281316, and Thompson, et al., "Targeting of the BclA and BclB Proteins to the *Bacillus anthracis* Spore Surface," Molecular Microbiology, 70(2):421–34 (2008), the entirety of each of which is hereby incorporated by reference). It was also found that the BetA/BAS3290 protein of *Bacillus anthracis* localized to the exosporium.

[00056] In particular, amino acids 20–35 of BclA from *Bacillus anthracis* Sterne strain have been found to be sufficient for targeting to the exosporium. A sequence alignment of amino acids 1–41 of BclA (SEQ ID NO: 1) with the corresponding N-terminal regions of several other *Bacillus cereus* family exosporium proteins and *Bacillus cereus* family proteins having related sequences is shown in FIG. 1. As can be seen from FIG. 1, there is a region of high-homology among all of the proteins in the region corresponding to amino acids 20–41 of BclA. However, in these sequences, the amino acids corresponding to amino acids 36–41 of BclA contain secondary structure and are not necessary for fusion protein localization to the

exosporium. The conserved targeting sequence region of BclA (amino acids 20–35 of SEQ ID NO: 1) is shown in bold in FIG. 1 and corresponds to the minimal targeting sequence needed for localization to the exosporium. A more highly conserved region spanning amino acids 25–35 of BclA within the targeting sequence is underlined in the sequences in FIG. 1, and is the recognition sequence for ExsFA/BxpB/ExsFB and homologs, which direct and assemble the described proteins on the surface of the exosporium. The amino acid sequences of SEQ ID NOS: 3, 5, and 7 in FIG. 1 are amino acids 1–33 of *Bacillus anthracis*. Sterne strain BetA/BAS3290, a methionine followed by amino acids 2–43 of *Bacillus anthracis*. Sterne strain BAS4623, and amino acids 1–34 of *Bacillus anthracis*. Sterne strain BclB, respectively. (For BAS4623, it was found that replacing the valine present at position 1 in the native protein with a methionine resulted in better expression.) As can be seen from FIG. 1, each of these sequences contains a conserved region corresponding to amino acids 20–35 of BclA (SEQ ID NO: 1; shown in bold), and a more highly conserved region corresponding to amino acids 20–35 of BclA (underlined).

5

10

15

20

25

30

35

[00057] Additional proteins from Bacillus cereus family members also contain the conserved targeting region. In particular, in FIG. 1, SEQ ID NO: 9 is amino acids 1-30 of Bacillus anthracis Sterne strain BAS1882, SEQ ID NO: 11 is amino acids 1-39 of the Bacillus weihenstephensis KBAB4 2280 gene product, SEQ ID NO: 13 is amino acids 1-39 of the Bacillus weihenstephensis KBAB4 3572 gene product, SEQ ID NO: 15 is amino acids 1-49 of Bacillus cereus VD200 exosporium leader peptide, SEQ ID NO: 17 is amino acids 1-33 of Bacillus cereus VD166 exosporium leader peptide, SEQ ID NO: 19 is amino acids 1-39 of Bacillus cereus VD200 hypothetical protein IKG\_04663, SEQ ID NO: 21 is amino acids 1-39 of Bacillus weihenstephensis KBAB4 YVTN β-propeller protein, SEQ ID NO: 23 is amino acids 1-30 of Bacillus weihenstephensis KBAB4 hypothetical protein bcerkbab4\_2363, SEQ ID NO: 25 is amino acids 1-30 of Bacillus weihenstephensis KBAB4 hypothetical protein bcerkbab4\_2131, SEQ ID NO: 27 is amino acids 1-36 of Bacillus weihenstephensis KBAB4 triple helix repeat containing collagen, SEQ ID NO: 29 is amino acids 1-39 of Bacillus mycoides 2048 hypothetical protein bmyco0001\_21660, SEQ ID NO: 31 is amino acids 1-30 of Bacillus mycoides 2048 hypothetical protein bmyc0001\_22540, SEQ ID NO: 33 is amino acids 1-21 of Bacillus mycoides 2048 hypothetical protein bmyc0001\_21510, SEQ ID NO: 35 is amino acids 1-22 of Bacillus thuringiensis 35646 collagen triple helix repeat protein, SEQ ID NO: 43 is amino acids 1-35 of Bacillus cereus hypothetical protein WP\_69652, SEQ ID NO: 45 is amino acids 1–41 of *Bacillus cereus* exosporium leader WP016117717, SEQ ID NO: 47 is amino acids 1–49 of *Bacillus cereus* exosporium peptide WP002105192, SEO ID NO: 49 is amino acids 1-38 of Bacillus cereus hypothetical protein WP87353, SEQ ID NO: 51 is

amino acids 1–39 of *Bacillus cereus* exosporium peptide 02112369, SEQ ID NO: 53 is amino acids 1–39 of *Bacillus cereus* exosporium protein WP016099770, SEQ ID NO: 55 is amino acids 1–36 of *Bacillus thuringiensis* hypothetical protein YP006612525, and SEQ ID NO: 57 is amino acids 1–136 of *Bacillus mycoides* hypothetical protein TIGR03720. As shown in FIG. 1, each of the N-terminal regions of these proteins contains a region that is conserved with amino acids 20–35 of BclA (SEQ ID NO: 1), and a more highly conserved region corresponding to amino acids 25–35 of BclA.

5

10

15

20

25

30

35

[00058] Any portion of BclA which includes amino acids 20–35 can be used as the In addition, full-length exosporium proteins or exosporium protein fragments can be used for targeting the fusion proteins to the exosporium. Thus, full-length BclA or a fragment of BclA that includes amino acids 20-35 can be used for targeting to the exosporium. For example, full length BclA (SEQ ID NO: 2) or a midsized fragment of BclA that lacks the carboxy-terminus such as SEQ ID NO: 59 (amino acids 1-196 of BclA) can be used to target the fusion proteins to the exosporium. Midsized fragments such as the fragment of SEQ ID NO: 59 have less secondary structure than full length BclA and have been found to be suitable for use as a targeting sequence. The targeting sequence can also comprise much shorter portions of BclA which include amino acids 20-35, such as SEQ ID NO: 1 (amino acids 1-41 of BclA), amino acids 1-35 of SEQ ID NO: 1, amino acids 20-35 of SEQ ID NO: 1, or SEQ ID NO: 60 (a methionine residue linked to amino acids 20-35 of BclA). Even shorter fragments of BclA which include only some of amino acids 20–35 also exhibit the ability to target fusion proteins to the exosporium. For example, the targeting sequence can comprise amino acids 22-31 of SEQ ID NO: 1, amino acids 22-33 of SEQ ID NO: 1, or amino acids 20-31 of SEQ ID NO: 1.

[00059] Alternatively, any portion of BetA/BAS3290, BAS4623, BclB, BAS1882, the KBAB4 2280 gene product, the KBAB4 3572 gene product, *B. cereus* VD200 exosporium leader peptide, *B. cereus* VD200 hypothetical protein IKG\_04663, *B. weihenstephensis* KBAB4 YVTN β-propeller protein, *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2363, *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2131, *B. weihenstephensis* KBAB4 triple helix repeat containing collagen, *B. mycoides* 2048 hypothetical protein bmyco0001\_21660, *B. mycoides* 2048 hypothetical protein bmyco001\_22540, *B. mycoides* 2048 hypothetical protein bmyc0001\_21510, *B. thuringiensis* 35646 collagen triple helix repeat protein, *B. cereus* hypothetical protein WP\_69652, *B. cereus* exosporium leader WP016117717, *B. cereus* exosporium peptide WP002105192, *B. cereus* hypothetical protein WP87353, *B. cereus* exosporium peptide 02112369, *B. cereus* exosporium protein WP016099770, *B. thuringiensis* 

hypothetical protein YP006612525, or B. mycoides hypothetical protein TIGR03720 which includes the amino acids corresponding to amino acids 20-35 of BclA can serve as the targeting sequence. As can be seen from FIG. 1, amino acids 12–27 of BetA/BAS3290, amino acids 23-38 of BAS4623, amino acids 13-28 of BclB, amino acids 9-24 of BAS1882, amino acids 18-33 of KBAB4 2280 gene product, amino acids 18-33 of KBAB4 3572 gene product, amino acids 28-43 of B. cereus VD200 exosporium leader peptide, amino acids 12-27 of B. cereus VD166 exosporium leader peptide, amino acids 18–33 of B. cereus VD200 hypothetical protein IKG 04663, amino acids 18–33 B. weihenstephensis KBAB4 YVTN β-propeller amino acids 9–24 of *B. weihenstephensis* KBAB4 hypothetical bcerkbab4 2363, amino acids 9-24 of B. weihenstephensis KBAB4 hypothetical protein bcerkbab4\_2131, amino acids 15-30 of B. weihenstephensis KBAB4 triple helix repeat containing collagen, amino acids 18–33 of B. mycoides 2048 hypothetical protein acids 9–24 of B. mycoides protein bmyco0001\_21660, amino 2048 hypothetical bmyc0001\_22540, amino acids 1-15 of B. mycoides 2048 hypothetical protein bmyc0001\_21510, amino acids 1-16 of B. thuringiensis 35646 collagen triple helix repeat protein, amino acids 14–29 of B. cereus hypothetical protein WP\_69652, amino acids 20–35 of B. cereus exosporium leader WP016117717, amino acids 28-43 of B. cereus exosporium peptide WP002105192, amino acids 17–32 of B. cereus hypothetical protein WP87353, amino acids 18-33 of B. cereus exosporium peptide 02112369, amino acids 18-33 of B. cereus exosporium protein WP016099770, amino acids 15–30 of B. thuringiensis hypothetical protein YP006612525, and amino acids 115–130 of B. mycoides hypothetical protein TIGR03720 correspond to amino acids 20–35 of BclA. Thus, any portion of these proteins that includes the above-listed corresponding amino acids can serve as the targeting sequence.

5

10

15

20

25

30

[00060] Furthermore, any amino acid sequence comprising amino acids 20–35 of BclA, or any of the above-listed corresponding amino acids can serve as the targeting sequence.

[00061] Thus, the targeting sequence can comprise amino acids 1–35 of SEQ ID NO: 1, amino acids 20–35 of SEQ ID NO: 1, SEQ ID NO: 1, SEQ ID NO: 60, amino acids 22–31 of SEQ ID NO: 1, amino acids 22–33 of SEQ ID NO: 1, or amino acids 20–31 of SEQ ID NO: 1. Alternatively, the targeting sequence consists of amino acids 1–35 of SEQ ID NO: 1, amino acids 20–35 of SEQ ID NO: 1, SEQ ID NO: 1, or SEQ ID NO: 60. Alternatively, the targeting sequence can consist of amino acids 22–31 of SEQ ID NO: 1, amino acids 22–33 of SEQ ID NO: 1, or amino acids 20–31 of SEQ ID NO: 1. Alternatively, the exosporium protein can comprise full length BclA (SEQ ID NO: 2), or the exosporium protein fragment can comprise a midsized fragment of BclA that lacks the carboxy-terminus, such as SEQ ID NO: 59 (amino

acids 1–196 of BclA). Alternatively, the exosporium protein fragment can consist of SEQ ID NO: 59.

[00062] The targeting sequence can also comprise amino acids 1–27 of SEQ ID NO: 3, amino acids 12–27 of SEQ ID NO: 3, or SEQ ID NO: 3, or the exosporium protein can comprise full length BetA/BAS3290 (SEQ ID NO: 4). It has also been found that a methionine residue linked to amino acids 12–27 of BetA/BAS3290 can be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 61. The targeting sequence can also comprise amino acids 14–23 of SEQ ID NO: 3, amino acids 14–25 of SEQ ID NO: 3, or amino acids 12–23 of SEQ ID NO: 3.

5

10

15

20

25

30

[00063] The targeting sequence can also comprise amino acids 1–38 of SEQ ID NO: 5, amino acids 23–38 of SEQ ID NO: 5, or SEQ ID NO: 5, or the exosporium protein can comprise full length BAS4623 (SEQ ID NO: 6).

[00064] Alternatively, the targeting sequence can comprise amino acids 1–28 of SEQ ID NO: 7, amino acids 13–28 of SEQ ID NO: 7, or SEQ ID NO: 7, or the exosporium protein can comprise full length BclB (SEQ ID NO: 8).

[00065] The targeting sequence can also comprise amino acids 1–24 of SEQ ID NO: 9, amino acids 9–24 of SEQ ID NO: 9, or SEQ ID NO: 9, or the exosporium protein can comprise full length BAS1882 (SEQ ID NO: 10). A methionine residue linked to amino acids 9–24 of BAS1882 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 69.

[00066] The targeting sequence can also comprise amino acids 1–33 of SEQ ID NO: 11, amino acids 18–33 of SEQ ID NO: 11, or SEQ ID NO: 11, or the exosporium protein can comprise the full length *B. weihenstephensis* KBAB4 2280 gene product (SEQ ID NO: 12). A methionine residue linked to amino acids 18–33 of the *B. weihenstephensis* KBAB4 2280 gene product can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 62.

[00067] The targeting sequence can also comprise amino acids 1–33 of SEQ ID NO: 13, amino acids 18–33 of SEQ ID NO: 13, or SEQ ID NO: 13, or the exosporium protein can comprise the full length *B. weihenstephensis* KBAB4 3572 gene product (SEQ ID NO: 14). A methionine residue linked to amino acids 18–33 of the *B. weihenstephensis* KBAB4 3572 gene product can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 63.

[00068] Alternatively, the targeting sequence can comprise amino acids 1–43 of SEQ ID NO: 15, amino acids 28–43 of SEQ ID NO: 15, or SEQ ID NO: 15, or the exosporium

protein can comprise full length *B. cereus* VD200 exosporium leader peptide (SEQ ID NO: 16).

[00069] The targeting sequence can also comprise amino acids 1–27 of SEQ ID NO: 17, amino acids 12–27 of SEQ ID NO: 17, or SEQ ID NO: 17, or the exosporium protein can comprise full-length *B. cereus* VD166 exosporium leader peptide (SEQ ID NO: 18). A methionine residue linked to amino acids 12–27 of the *B. cereus* VD166 exosporium leader peptide can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 64.

5

10

15

20

25

30

35

[00070] The targeting sequence can also comprise amino acids 1–33 of SEQ ID NO: 19, amino acids 18–33 of SEQ ID NO: 19, or SEQ ID NO: 19, or the exosporium protein can comprise full length *B. cereus* VD200 hypothetical protein IKG\_04663 (SEQ ID NO: 20).

[00071] Alternatively, the targeting sequence comprises amino acids 1–33 of SEQ ID NO: 21, amino acids 18–33 of SEQ ID NO: 21, or SEQ ID NO: 21, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 YVTN β-propeller protein (SEQ ID NO: 22). A methionine residue linked to amino acids 18–33 of the *B. weihenstephensis* KBAB4 YVTN β-propeller protein can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 65.

[00072] The targeting sequence can also comprise amino acids 1–24 of SEQ ID NO: 23, amino acids 9–24 of SEQ ID NO: 23, or SEQ ID NO: 23, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2363 (SEQ ID NO: 24). A methionine residue linked to amino acids 9–24 of *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2363 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 66.

[00073] The targeting sequence comprise amino acids 1–24 of SEQ ID NO: 25, amino acids 9–24 of SEQ ID NO: 25, or SEQ ID NO: 25, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2131 (SEQ ID NO: 26). A methionine residue linked to amino acids 9–24 of *B. weihenstephensis* KBAB4 hypothetical protein bcerkbab4\_2131 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 67.

[00074] Alternatively, the targeting sequence comprises amino acids 1–30 of SEQ ID NO: 27, amino acids 15–30 of SEQ ID NO: 27, or SEQ ID NO: 27, or the exosporium protein can comprise full length *B. weihenstephensis* KBAB4 triple helix repeat containing collagen (SEQ ID NO: 28).

[00075] The targeting sequence can also comprise amino acids 1–33 of SEQ ID NO: 29, amino acids 18–33 of SEQ ID NO: 29, or SEQ ID NO: 29, or the exosporium protein can

23

comprise full length *B. mycoides* 2048 hypothetical protein bmyco0001\_21660 (SEQ ID NO: 30).

[00076] The targeting sequence can also comprise amino acids 1–24 of SEQ ID NO: 31, amino acids 9–24 of SEQ ID NO: 31, or SEQ ID NO: 31, or the exosporium protein can comprise full length *B. mycoides* 2048 hypothetical protein bmyc0001\_22540 (SEQ ID NO: 32). A methionine residue linked to amino acids 9–24 of *B. mycoides* 2048 hypothetical protein bmyc0001\_22540 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 68.

5

10

15

20

25

30

35

[00077] Alternatively, the targeting sequence comprises amino acids 1–15 of SEQ ID NO: 33, SEQ ID NO: 33, or the exosporium protein comprises full length *B. mycoides* 2048 hypothetical protein bmyc0001\_21510 (SEQ ID NO: 34).

[00078] The targeting sequence can also comprise amino acids 1–16 of SEQ ID NO: 35, SEQ ID NO: 35, or the exosporium protein can comprise full length *B. thuringiensis* 35646 collagen triple helix repeat protein (SEQ ID NO: 36).

[00079] The targeting sequence can comprise amino acids 1–29 of SEQ ID NO: 43, amino acids 14–29 of SEQ ID NO: 43, or SEQ ID NO: 43, or the exosporium protein can comprise full length *B. cereus* hypothetical protein WP\_69652 (SEQ ID NO: 44).

[00080] Alternatively, the targeting sequence can comprise amino acids 1–35 of SEQ ID NO: 45, amino acids 20–35 of SEQ ID NO: 45, or SEQ ID NO: 45, or the exosporium protein can comprise full length *B. cereus* exosporium leader WP016117717 (SEQ ID NO: 46). A methionine residue linked to amino acids 20–35 of *B. cereus* exosporium leader WP016117717 can also be used as a targeting sequence. Thus, the targeting sequence can comprise SEQ ID NO: 70.

[00081] The targeting sequence can comprise amino acids 1–43 of SEQ ID NO: 47, amino acids 28–43 of SEQ ID NO: 47, or SEQ ID NO: 47, or the exosporium protein can comprise full length *B. cereus* exosporium peptide WP002105192 (SEQ ID NO: 48).

[00082] The targeting sequence can comprise amino acids 1–32 of SEQ ID NO: 49, amino acids 17–32 of SEQ ID NO: 49, or SEQ ID NO: 49, or the exosporium protein can comprise full length *B. cereus* hypothetical protein WP87353 (SEQ ID NO: 50).

[00083] Alternatively, the targeting sequence can comprise amino acids 1–33 of SEQ ID NO: 51, amino acids 18–33 of SEQ ID NO: 51, or SEQ ID NO: 51, or the exosporium protein can comprise full length *B. cereus* exosporium peptide 02112369 (SEQ ID NO: 52).

[00084] The targeting sequence can comprise amino acids 1–33 of SEQ ID NO: 53, amino acids 18–33 of SEQ ID NO: 53, or SEQ ID NO: 53, or the exosporium protein can comprise full length *B. cereus* exosporium protein WP016099770 (SEQ ID NO: 54).

24

[00085] Alternatively, the targeting sequence can comprise acids 1–30 of SEQ ID NO: 55, amino acids 15–30 of SEQ ID NO: 55, or SEQ ID NO: 55, or the exosporium protein can comprise full length *B. thuringiensis* hypothetical protein YP006612525 (SEQ ID NO: 56).

[00086] The targeting sequence can also comprise amino acids 1–130 of SEQ ID NO: 57, amino acids 115–130 of SEQ ID NO: 57, or SEQ ID NO: 57, or the exosporium protein can comprise full length *B. mycoides* hypothetical protein TIGR03720 (SEQ ID NO: 58).

5

10

15

20

25

30

35

[00087] In addition, it can readily be seen from the sequence alignment in FIG. 1 that while amino acids 20–35 of BclA are conserved, and amino acids 25–35 are more conserved, some degree of variation can occur in this region without affecting the ability of the targeting sequence to target a protein to the exosporium. FIG. 1 lists the percent identity of each of corresponding amino acids of each sequence to amino acids 20–35 of BclA ("20–35% Identity") and to amino acids 25–35 of BclA ("25–35% Identity"). Thus, for example, as compared to amino acids 20–35 of BclA, the corresponding amino acids of BetA/BAS3290 are about 81.3% identical, the corresponding amino acids of BAS4623 are about 50.0% identical, the corresponding amino acids of BAS1882 are about 62.5% identical, the corresponding amino acids of the KBAB4 2280 gene product are about 81.3% identical, and the corresponding amino acids of the KBAB4 3572 gene product are about 81.3% identical. The sequence identities over this region for the remaining sequences are listed in FIG. 1.

[00088] With respect to amino acids 25–35 of BclA, the corresponding amino acids of BetA/BAS3290 are about 90.9% identical, the corresponding amino acids of BAS4623 are about 72.7% identical, the corresponding amino acids of BclB are about 54.5% identical, the corresponding amino acids of BAS1882 are about 72.7% identical, the corresponding amino acids of the KBAB4 2280 gene product are about 90.9% identical, and the corresponding amino acids of the KBAB4 3572 gene product are about 81.8% identical. The sequence identities over this region for the remaining sequences are listed in FIG. 1.

[00089] Thus, the targeting sequence can comprise an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%.

[00090] The targeting sequence can also comprise an amino acid sequence having at least about 50% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 63%. Alternatively the targeting sequence consists of an

amino acid sequence consisting of 16 amino acids and having at least about 50% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 63%.

[00091] The targeting sequence can also comprise an amino acid sequence having at least about 50% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 72%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 50% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 72%.

5

10

15

20

25

30

35

[00092] The targeting sequence can also comprise an amino acid sequence having at least about 56% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 63%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 56% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 63%.

[00093] Alternatively, the targeting sequence can comprise an amino sequence having at least about 62% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 72%. The targeting sequence can also consist of an amino acid sequence consisting of 16 amino acids and having at least about 62% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 of SEQ ID NO:1 is at least about 72%.

[00094] The targeting sequence can comprise an amino acid sequence having at least 68% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 81%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least 68% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 81%.

[00095] The targeting sequence can also comprises an amino sequence having at least about 75% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 72%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 75% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 of SEQ ID NO: 1 is at least about 72%.

[00096] The targeting sequence can also comprise an amino sequence having at least about 75% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 81%. Alternatively, the targeting sequence consists of an amino

acid sequence consisting of 16 amino acids and having at least about 75% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 of SEQ ID NO: 1 is at least about 81%.

[00097] The targeting sequence can also comprise an amino acid sequence having at least about 81% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 81%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 81% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 81%.

5

10

15

20

25

30

35

[00098] The targeting sequence can comprise an amino acid sequence having at least about 81% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 90%. Alternatively, the targeting sequence consists of an amino acid sequence consisting of 16 amino acids and having at least about 81% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 90%.

[00099] The skilled person will recognize that variants of the above sequences can also be used as targeting sequences, so long as the targeting sequence comprises amino acids 20–35 of BclA, the corresponding amino acids of BetA/BAS3290, BAS4263, BclB, BAS1882, the KBAB4 2280 gene product, or the KBAB 3572 gene product, or a sequence comprising any of the above noted sequence identities to amino acids 20–35 and 25–35 of BclA is present.

[000100] It has further been discovered that certain *Bacillus cereus* family exosporium proteins which lack regions having homology to amino acids 25–35 of BcIA can also be used to target a peptide or protein to the exosporium of a *Bacillus cereus* family member. In particular, the fusion proteins can comprise an exosporium protein comprising SEQ ID NO: 71 (*B. mycoides* InhA), an exosporium protein comprising SEQ ID NO: 72 (*B. anthracis* Sterne BAS1141 (ExsY)), an exosporium protein comprising SEQ ID NO: 73 (*B. anthracis* Sterne BAS1144 (BxpB/ExsFA)), an exosporium protein comprising SEQ ID NO: 74 (*B. anthracis* Sterne BAS1145 (CotY)), an exosporium protein comprising SEQ ID NO: 76 (*B. anthracis* H9401 ExsFB), an exosporium protein comprising SEQ ID NO: 77 (*B. thuringiensis* HD74 InhA1), an exosporium protein comprising SEQ ID NO: 79 (*B. cereus* ATCC 10876 ExsJ), an exosporium protein comprising SEQ ID NO: 80 (*B. anthracis* Ames YjcA), an exosporium protein comprising SEQ ID NO: 81 (*B. anthracis* YjcB), an exosporium protein comprising SEQ ID NO: 82 (*B. anthracis* Sterne BcIC), an exosporium protein comprising SEQ ID NO: 83 (*Bacillus thuringiensis* serovar

konkukian str. 97-27 acid phosphatase), or an exosporium protein comprising SEQ ID NO: 84 (*B. thuringiensis* HD74 InhA2). Inclusion of an exosporium protein comprising SEQ ID NO: 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84 in the fusion proteins described herein will result in targeting to the exosporium of a *B. cereus* family member.

[000101] Moreover, exosporium proteins having a high degree of sequence identity with any of the full-length exosporium proteins or the exosporium protein fragments described above can also be used to target a peptide or protein to the exosporium of a *Bacillus cereus* family member. Thus, the fusion protein can comprise an exosporium protein comprising an amino acid sequence having at least 85% identity with any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 59, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, and 84. Alternatively, the fusion protein can comprise an exosporium protein having at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 44, 46, 48, 50, 52, 54, 56, 58, 59, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, and 84.

[000102] Alternatively, the fusion protein can comprise an exosporium protein fragment consisting of an amino acid sequence having at least 85% identity with SEQ ID NO: 59. Alternatively, the fusion protein can comprise an exosporium protein fragment consisting of an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with SEQ ID NO: 59.

[000103] In any of the targeting sequences, exosporium proteins, or exosporium protein fragments described herein, the targeting sequence, exosporium protein, or exosporium protein fragment can comprise the amino acid sequence GXT at its carboxy terminus, wherein X is any amino acid.

**[000104]** In any of the targeting sequences, exosporium proteins, and exosporium protein fragments described herein, the targeting sequence, exosporium protein, or exosporium protein fragment, can comprise an alanine residue at the position of the targeting sequence that corresponds to amino acid 20 of SEQ ID NO: 1.

### 30 Fusion Proteins

5

10

15

20

25

35

[000105] The fusion proteins can comprise a targeting sequence, an exosporium protein, or an exosporium protein fragment, and at least one plant growth stimulating protein or peptide. The plant growth stimulating protein or peptide can comprise a peptide hormone, a non-hormone peptide, an enzyme involved in the production or activation of a plant growth stimulating compound or an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source.

The targeting sequence, exosporium protein, or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments described above.

[000106] The fusion proteins can comprise a targeting sequence, an exosporium protein, or an exosporium protein fragment, and at least one protein or peptide that protects a plant from a pathogen. The targeting sequence, exosporium protein, or exosporium protein fragment can be any of the targeting sequences, exosporium proteins, or exosporium protein fragments described above.

5

10

15

20

25

30

[000107] The fusion protein can be made using standard cloning and molecular biology methods known in the art. For example, a gene encoding a protein or peptide (e.g., a gene encoding a plant growth stimulating protein or peptide) can be amplified by polymerase chain reaction (PCR) and ligated to DNA coding for any of the above-described targeting sequences to form a DNA molecule that encodes the fusion protein. The DNA molecule encoding the fusion protein can be cloned into any suitable vector, for example a plasmid vector. The vector suitably comprises a multiple cloning site into which the DNA molecule encoding the fusion protein can be easily inserted. The vector also suitably contains a selectable marker, such as an antibiotic resistance gene, such that bacteria transformed, transfected, or mated with the vector can be readily identified and isolated. Where the vector is a plasmid, the plasmid suitably also comprises an origin of replication. The DNA encoding the fusion protein is suitably under the control of a sporulation promoter which will cause expression of the fusion protein on the exosporium of a *B. cereus* family member endospore (e.g., a native bclA promoter from a *B. cereus* family member). Alternatively, DNA coding for the fusion protein can be integrated into the chromosomal DNA of the *B. cereus* family member host.

[000108] The fusion protein can also comprise additional polypeptide sequences that are not part of the targeting sequence, exosporium protein, exosporium protein fragment, or the plant growth stimulating protein or peptide, the protein or peptide that protects a plant from a pathogen, the protein or peptide that enhances stress resistance in a plant, or the plant binding protein or peptide. For example, the fusion protein can include tags or markers to facilitate purification or visualization of the fusion protein (e.g., a polyhistidine tag or a fluorescent protein such as GFP or YFP) or visualization of recombinant exosporium-producing *Bacillus* cells spores expressing the fusion protein.

[000109] Expression of fusion proteins on the exosporium using the targeting sequences, exosporium proteins, and exosporium protein fragments described herein is enhanced due to a lack of secondary structure in the amino-termini of these sequences, which allows for native folding of the fused proteins and retention of activity. Proper folding can be further

enhanced by the inclusion of a short amino acid linker between the targeting sequence, exosporium protein, exosporium protein fragment, and the fusion partner protein.

[000110] Thus, any of the fusion proteins described herein can comprise an amino acid linker between the targeting sequence, the exosporium protein, or the exosporium protein fragment and the plant growth stimulating protein or peptide, the protein or peptide that protects a plant from a pathogen, the protein or peptide that enhances stress resistance in a plant, or the plant binding protein or peptide.

[000111] The linker can comprise a polyalanine linker or a polyglycine linker. A linker comprising a mixture of both alanine and glycine residues can also be used. For example, where the targeting sequence comprises SEQ ID NO: 1, a fusion protein can have one of the following structures:

No linker: SEQ ID NO: 1 – Fusion Partner Protein

5

10

15

20

25

Alanine Linker: SEQ ID NO: 1–An–Fusion Partner Protein Glycine Linker: SEQ ID NO: 1–Gn–Fusion Partner Protein

Mixed Alanine and Glycine Linker: SEQ ID NO: 1 – (A/G)n – Fusion Partner Protein

where An, Gn, and (A/G)n are any number of alanines, any number of glycines, or any number of a mixture of alanines and glycines, respectively. For example, n can be 1 to 25, and is preferably 6 to 10. Where the linker comprises a mixture of alanine and glycine residues, any combination of glycine and alanine residues can be used. In the above structures, "Fusion Partner Protein" represents the plant growth stimulating protein or peptide, the protein or peptide that protects a plant from a pathogen, the protein or peptide that enhances stress resistance in a plant, or the plant binding protein or peptide.

[000112] Alternatively or in addition, the linker can comprise a protease recognition site. Inclusion of a protease recognition site allows for targeted removal, upon exposure to a protease that recognizes the protease recognition site, of the plant growth stimulating protein or peptide, the protein or peptide that protects a plant from a pathogen, the protein or peptide that enhances stress resistance in a plant, or the plant binding protein or peptide.

### 30 Plant Growth Stimulating Proteins and Peptides

[000113] As noted above, the fusion proteins can comprise a targeting sequence, exosporium protein, or exosporium protein fragment and at least one plant growth stimulating protein or peptide. For example, the plant growth stimulating protein or peptide can comprise a peptide hormone, a non-hormone peptide, an enzyme involved in the production or activation of

a plant growth stimulating compound, or an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source.

[000114] For example, where the plant growth stimulating protein or peptide comprises a peptide hormone, the peptide hormone can comprise a phytosulfokine (e.g., phytosulfokine-α), clavata 3 (CLV3), systemin, ZmlGF, or a SCR/SP11.

5

10

15

20

25

30

[000115] Where the plant growth stimulating protein or peptide comprises a non-hormone peptide, the non-hormone peptide can comprise a RKN 16D10, Hg-Syv46, an eNOD40 peptide, melittin, mastoparan, Mas7, RHPP, POLARIS, or kunitz trypsin inhibitor (KTI).

[000116] The plant growth stimulating protein or peptide can comprise an enzyme involved in the production or activation of a plant growth stimulating compound. The enzyme involved in the production or activation of a plant growth stimulating compound can be any enzyme that catalyzes any step in a biological synthesis pathway for a compound that stimulates plant growth or alters plant structure, or any enzyme that catalyzes the conversion of an inactive or less active derivative of a compound that stimulates plant growth or alters plant structure into an active or more active form of the compound.

[000117] The plant growth stimulating compound can comprise a compound produced by bacteria or fungi in the rhizosphere, e.g., 2,3-butanediol.

[000118] Alternatively, the plant growth stimulating compound can comprise a plant growth hormone, e.g., a cytokinin or a cytokinin derivative, ethylene, an auxin or an auxin derivative, a gibberellic acid or a gibberellic acid derivative, abscisic acid or an abscisic acid derivative, or a jasmonic acid or a jasmonic acid derivative.

[000119] Where the plant growth stimulating compound comprises a cytokinin or a cytokinin derivative, the cytokinin or the cytokinin derivative can comprise kinetin, cis-zeatin, trans-zeatin, 6-benzylaminopurine, dihydroxyzeatin, N6-(D2-isopentenyl) adenine, ribosylzeatin, N6-(D2-isopentenyl) adenosine, 2-methylthio-cis-ribosylzeatin, cis-ribosylzeatin, trans-ribosylzeatin, 2-methylthio-trans-ribosylzeatin, ribosylzeatin-5-monosphosphate, N6-methylaminopurine, N6-dimethylaminopurine, 2'-deoxyzeatin riboside, 4-hydroxy-3-methyltrans-2-butenylaminopurine, ortho-topolin, meta-topolin, benzyladenine, ortho-methyltopolin, meta-methyltopolin, or a combination thereof.

**[000120]** Where the plant growth stimulating compound comprises an auxin or an auxin derivative, the auxin or the auxin derivative can comprise an active auxin, an inactive auxin, a conjugated auxin, a naturally occurring auxin, or a synthetic auxin, or a combination thereof. For example, the auxin or auxin derivative can comprise indole-3-acetic acid, indole-3- pyruvic acid, indole-3-acetaldoxime, indole-3-acetamide, indole-3-acetonitrile, indole-3-ethanol, indole-3-

pyruvate, indole-3-acetaldoxime, indole-3-butyric acid, a phenylacetic acid, 4- chloroindole-3-acetic acid, a glucose-conjugated auxin, or a combination thereof.

5

10

15

20

25

30

35

[000121] The enzyme involved in the production or activation of a plant growth stimulating compound can comprise an acetoin reductase, an indole-3-acetamide hydrolase, a tryptophan monooxygenase, an acetolactate synthetase, an α-acetolactate decarboxylase, a pyruvate decarboxylase, a diacetyl reductase, a butanediol dehydrogenase, an aminotransferase (e.g., tryptophan aminotransferase), a tryptophan decarboxylase, an amine oxidase, an indole-3pyruvate decarboxylase, an indole-3-acetaldehyde dehydrogenase, a tryptophan side chain oxidase, a nitrile hydrolase, a nitrilase, a peptidase, a protease, an adenosine phosphate isopentenyltransferase, a phosphatase, adenosine kinase. adenine an an phosphoribosyltransferase, CYP735A, a 5'ribonucleotide phosphohydrolase, an adenosine nucleosidase, a zeatin cis-trans isomerase, a zeatin O-glucosyltransferase, a β-glucosidase, a cishydroxylase, a CK cis-hydroxylase, a CK N-glucosyltransferase, a 2,5-ribonucleotide phosphohydrolase, an adenosine nucleosidase, a purine nucleoside phosphorylase, a zeatin reductase, a hydroxylamine reductase, a 2-oxoglutarate dioxygenase, a gibberellic 2B/3B hydrolase, a gibberellin 3-oxidase, a gibberellin 20-oxidase, a chitosinase, a chitinase, a β-1,3glucanase, a β-1,4-glucanase, a β-1,6-glucanase, an aminocyclopropane-1-carboxylic deaminase, or an enzyme involved in producing a nod factor (e.g., nodA, nodB, or nodI).

[000122] Where the enzyme comprises a protease or peptidase, the protease or peptidase can be a protease or peptidase that cleaves proteins, peptides, proproteins, or preproproteins to create a bioactive peptide. The bioactive peptide can be any peptide that exerts a biological activity.

[000123] Examples of bioactive peptides include RKN 16D10 and RHPP.

[000124] The protease or peptidase that cleaves proteins, peptides, proproteins, or preproproteins to create a bioactive peptide can comprise subtilisin, an acid protease, an alkaline protease, a proteinase, an endopeptidase, an exopeptidase, thermolysin, papain, pepsin, trypsin, pronase, a carboxylase, a serine protease, a glutamic protease, an aspartate protease, a cysteine protease, a threonine protease, or a metalloprotease.

[000125] The protease or peptidase can cleave proteins in a protein-rich meal (e.g., soybean meal or yeast extract).

[000126] The plant growth stimulating protein can also comprise an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source. Such enzymes include cellulases, lipases, lignin oxidases, proteases, glycoside hydrolases, phosphatases, nitrogenases, nucleases, amidases, nitrate reductases, nitrite reductases, amylases, ammonia oxidases, ligninases, glucosidases, phospholipases, phytases, pectinases, glucanases, sulfatases, ureases,

32

xylanases, and siderophores. When introduced into a plant growth medium or applied to a plant, seed, or an area surrounding a plant or a plant seed, fusion proteins comprising enzymes that degrade or modify a bacterial, fungal, or plant nutrient source can aid in the processing of nutrients in the vicinity of the plant and result in enhanced uptake of nutrients by the plant or by beneficial bacteria or fungi in the vicinity of the plant.

5

10

15

20

25

30

[000127] Suitable cellulases include endocellulases (e.g., an endogluconase such as a *Bacillus subtilis* endoglucanase, a *Bacillus thuringiensis* endoglucanase, a *Bacillus cereus* endoglucanase, or a *Bacillus clausii* endoglucanase), exocellulases (e.g., a *Trichoderma reesei* exocellulase), and  $\beta$ -glucosidases (e.g., a *Bacillus subtilis*  $\beta$ -glucosidase, a *Bacillus thuringiensis*  $\beta$ -glucosidase, a *Bacillus cereus*  $\beta$ -glucosidase, or a *Bacillus clausii*  $\beta$ -glucosidase).

[000128] The lipase can comprise a *Bacillus subtilis* lipase, a *Bacillus thuringiensis* lipase, a *Bacillus cereus* lipase, or a *Bacillus clausii* lipase.

[000129] In one embodiment, the lipase comprises a *Bacillus subtilis* lipase. The *Bacillus subtilis* lipase can be PCR amplified using the following primers: ggatccatggctgaacacaatcc (forward, SEQ ID NO: 37) and ggatccttaattcgtattctggcc (reverse, SEQ ID NO: 38).

[000130] In another embodiment, the cellulase is a *Bacillus subtilis* endoglucanase. The *Bacillus subtilis* endoglucanase can be PCR amplified using the following primers: ggatccatgaaacggtcaatc (forward, SEQ ID NO: 39) and ggatccttactaatttggttctgt (reverse, SEQ ID NO: 40).

[000131] In yet another embodiment, the fusion protein comprises an *E. coli* protease PtrB. The *E. coli* protease PtrB can be PCR amplified using the following primers: ggatccatgctaccaaaagcc (forward, SEQ ID NO: 41) and ggatccttagtccgcaggcgtagc (reverse, SEQ ID NO: 42).

[000132] In certain embodiments, the fusion protein contains an endoglucanase which derives from the nucleotide sequence in SEQ ID NO: 104.

[000133] The amino acid sequence for an exemplary endoglucanase that may be fused to the targeting sequence, an exosporium protein, or an exosporium protein fragment and, optionally, a linker sequence, such as a poly-A linker, is the fusion protein provided as SEQ ID NO: 107.

[000134] In other embodiments, the fusion protein contains a phospholipase that derives from the nucleotide sequence set forth in SEQ ID NO: 105.

[000135] The amino acid sequence for an exemplary phospholipase that may be fused to the targeting sequence, an exosporium protein, or an exosporium protein fragment and,

33

optionally, a linker sequence, such as a poly-A linker, is the fusion protein provided as SEQ ID NO: 108.

[000136] In still other embodiments, the fusion protein contains a chitosanase that derives from the nucleotide sequence set forth in SEQ ID NO: 106. The amino acid sequence for an exemplary chitosanase that may be fused to the targeting sequence, an exosporium protein, or an exosporium protein fragment and, optionally, a linker sequence, such as a poly-A linker, in the fusion protein is provided as SEQ ID NO: 109.

5

10

15

20

25

30

[000137] To create fusion constructs, genes may be fused to the native bclA promoter of *Bacillus thuringiensis* DNA encoding the first 35 amino acids of BclA (amino acids 1–35 of SEQ ID NO: 1) using the splicing by overlapping extension (SOE) technique. Correct amplicons are cloned into the *E. coli/Bacillus* shuttle vector pHP13, and correct clones screened by DNA sequencing. Correct clones are electroporated into *Bacillus thuringiensis* (Cry–, plasmid–) and screened for chloramphenicol resistance. Correct transformants are grown in brain heart infusion broth overnight at 30 °C, plated onto nutrient agar plates, and incubated at 30 °C for 3 days. Spores expressing the fusion construct (BEMD spores) may be collected off of the plates by washing in phosphate buffered saline (PBS) and purified by centrifugation and additional washes in PBS.

[000138] In such fusion proteins, the endoglucanase, phospholipase or chitosinase can comprise a nucleotide sequence encoding an amino acid sequence having at least 85% identity with SEQ ID NO: 107, 108 or 109, respectively.

[000139] In such fusion proteins, the endoglucanase, phospholipase or chitosinase can comprise an amino acid sequence having at least 90% identity with SEQ ID NO: 107, 108 or 109, respectively.

[000140] In such fusion proteins, the endoglucanase, phospholipase or chitosinase can comprise an amino acid sequence having at least 95% identity with SEQ ID NO: 107, 108 or 109, respectively.

[000141] In such fusion proteins, the endoglucanase, phospholipase or chitosinase can comprise an amino acid sequence having at least 98% identity with SEQ ID NO: 107, 108 or 109, respectively.

[000142] In such fusion proteins, the endoglucanase, phospholipase or chitosinase can compire an amino acid sequence having at least 99% identity with SEQ ID NO: 107, 108 or 109, respectively.

[000143] Suitable lignin oxidases comprise lignin peroxidases, laccases, glyoxal oxidases, ligninases, and manganese peroxidases.

[000144] The protease can comprise a subtilisin, an acid protease, an alkaline protease, a proteinase, a peptidase, an endopeptidase, an exopeptidase, a thermolysin, a papain, a pepsin, a trypsin, a pronase, a carboxylase, a serine protease, a glutamic protease, an aspartate protease, a cysteine protease, a threonine protease, or a metalloprotease.

[000145] The phosphatase can comprise a phosphoric monoester hydrolase, a phosphomonoesterase (e.g., PhoA4), a phosphoric diester hydrolase, a phosphodiesterase, a triphosphoric monoester hydrolase, a phosphoryl anhydride hydrolase, a pyrophosphatase, a phytase (e.g., *Bacillus subtilis* EE148 phytase or *Bacillus thuringiensis* BT013A phytase), a trimetaphosphatase, or a triphosphatase.

[000146] The nitrogenase can comprise a Nif family nitrogenase (e.g., *Paenibacillus massiliensis* NifBDEHKNXV).

# **Proteins and Peptides that Protects Plants from Pathogens**

5

10

15

20

25

30

35

[000147] The fusion proteins can comprise a targeting sequence, exosporium protein, or exosporium protein fragment, and at least one protein or peptide that protects a plant from a pathogen.

[000148] The protein or peptide can comprise a protein or peptide that stimulates a plant immune response. For example, the protein or peptide that stimulates a plant immune response can comprise a plant immune system enhancer protein or peptide. The plant immune system enhancer protein or peptide can be any protein or peptide that has a beneficial effect on the immune system of a plant. Suitable plant immune system enhancer proteins and peptides include harpins,  $\alpha$ -elastins,  $\beta$ -elastins, systemins, phenylalanine ammonia-lyase, elicitins, defensins, cryptogeins, flagellin proteins, and flagellin peptides (e.g., flg22).

[000149] Alternatively, the protein or peptide that protects a plant from a pathogen can be a protein or peptide that has antibacterial activity, antifungal activity, or both antibacterial and antifungal activity. Examples of such proteins and peptides include bacteriocins, lysozymes, lysozyme peptides (e.g., LysM), siderophores, non-ribosomal active peptides, conalbumins, albumins, lactoferrins, lactoferrin peptides (e.g., LfcinB), streptavidin and TasA.

[000150] The protein or peptide that protects a plant from a pathogen can also be a protein or peptide that has insecticidal activity, helminthicidal activity, suppresses insect or worm predation, or a combination thereof. For example, the protein or peptide that protects a plant from a pathogen can comprise an insecticidal bacterial toxin (e.g., a VIP insecticidal protein), an endotoxin, a Cry toxin (e.g., a Cry toxin from *Bacillus thuringiensis*), a protease inhibitor protein or peptide (e.g., a trypsin inhibitor or an arrowhead protease inhibitor), a cysteine protease, or a chitinase. Where the Cry toxin is a Cry toxin from *Bacillus thuringiensis*, the Cry toxin can be a

Cry5B protein or a Cry21A protein. Cry5B and Cry21A have both insecticidal and nematocidal activity.

[000151] The protein that protects a plant from a pathogen can comprise an enzyme. Suitable enzymes include proteases and lactonases. The proteases and lactonases can be specific for a bacterial signaling molecule (e.g., a bacterial lactone homoserine signaling molecule).

[000152] Where the enzyme is a lactonase, the lactonase can comprise 1,4-lactonase, 2-pyrone-4,6-dicarboxylate lactonase, 3-oxoadipate enol-lactonase, actinomycin lactonase, deoxylimonate A-ring-lactonase, gluconolactonase L-rhamnono-1,4-lactonase, limonin-D-ring-lactonase, steroid-lactonase, triacetate-lactonase, or xylono-1,4-lactonase.

[000153] The enzyme can also be an enzyme that is specific for a cellular component of a bacterium or fungus. For example, the enzyme can comprise a  $\beta$ -1,3-glucanase, a  $\beta$ -1,4-glucanase, a  $\beta$ -1,6-glucanase, a chitosinase, a chitosinase-like enzyme, a lyticase, a peptidase, a proteinase, a protease (e.g., an alkaline protease, an acid protease, or a neutral protease), a mutanolysin, a stapholysin, or a lysozyme

## Proteins and Peptides that Enhance Stress Resistance in Plants

[000154] The fusion proteins can comprise a targeting sequence, exosporium protein, or exosporium protein fragment and at least one protein or peptide that enhances stress resistance in a plant.

[000155] For example, the protein or peptide that enhances stress resistance in a plant comprises an enzyme that degrades a stress-related compound. Stress-related compounds include, but are not limited to, aminocyclopropane-1-carboxylic acid (ACC), reactive oxygen species, nitric oxide, oxylipins, and phenolics. Specific reactive oxygen species include hydroxyl, hydrogen peroxide, oxygen, and superoxide. The enzyme that degrades a stress-related compound can comprise a superoxide dismutase, an oxidase, a catalase, an aminocyclopropane-1-carboxylic acid deaminase, a peroxidase, an antioxidant enzyme, or an antioxidant peptide.

[000156] The protein or peptide that enhances stress resistance in a plant can also comprise a protein or peptide that protects a plant from an environmental stress. The environmental stress can comprise, for example, drought, flood, heat, freezing, salt, heavy metals, low pH, high pH, or a combination thereof. For instance, the protein or peptide that protects a plant from an environmental stress can comprises an ice nucleation protein, a prolinase, a phenylalanine ammonia lyase, an isochorismate synthase, an isochorismate pyruvate lyase, or a choline dehydrogenase.

36

35

5

10

15

20

25

30

## **Plant Binding Proteins and Peptides**

5

10

15

20

25

30

35

[000157] The fusion proteins can comprise a targeting sequence, exosporium protein, or exosporium protein fragment and at least plant binding protein or peptide. The plant binding protein or peptide can be any protein or peptide that is capable of specifically or non-specifically binding to any part of a plant (e.g., a plant root or an aerial portion of a plant such as a leaf, stem, flower, or fruit) or to plant matter. Thus, for example, the plant binding protein or peptide can be a root binding protein or peptide, or a leaf binding protein or peptide.

[000158] Suitable plant binding proteins and peptides include adhesins (e.g., rhicadhesin), flagellins, omptins, lectins, expansins, biofilm structural proteins (e.g., TasA or YuaB) pilus proteins, curlus proteins, intimins, invasins, agglutinins, and afimbrial proteins.

## Recombinant Bacillus that Express the Fusion Proteins

[000159] The fusion proteins described herein can be expressed by recombinant exosporium-producing *Bacillus* cells. The fusion protein can be any of the fusion proteins discussed above.

[000160] The recombinant exosporium-producing *Bacillus* cells can coexpress two or more of any of the fusion proteins discussed above. For example, the recombinant exosporium-producing *Bacillus* cells can coexpress at least one fusion protein that comprises a plant binding protein or peptide, together with at least one fusion protein comprising a plant growth stimulating protein or peptide, at least one fusion protein comprising a protein or peptide that protects a plant from a pathogen, or at least one protein or peptide that enhances stress resistance in a plant.

[000161] The recombinant exosporium-producing Bacillus cells can comprise Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, Bacillus samanii, Bacillus gaemokensis, Bacillus weihenstephensis, Bacillus toyoiensis or a combination thereof. For example, the recombinant exosporium-producing Bacillus cells can comprise Bacillus cereus, Bacillus thuringiensis, Bacillus pseudomycoides, or Bacillus mycoides. In particular, the recombinant exosporium-producing Bacillus cells can comprise Bacillus thuringiensis or Bacillus mycoides.

[000162] To generate a recombinant exosporium-producing *Bacillus* cells expressing a fusion protein, any *Bacillus cereus* family member can be conjugated, transduced, or transformed with a vector encoding the fusion protein using standard methods known in the art (e.g., by electroporation). The bacteria can then be screened to identify transformants by any method known in the art. For example, where the vector includes an antibiotic resistance gene, the bacteria can be screened for antibiotic resistance. Alternatively, DNA encoding the fusion protein can be integrated into the chromosomal DNA of a *B. cereus* family member host. The

37

recombinant exosporium-producing *Bacillus* cells can then exposed to conditions which will induce sporulation. Suitable conditions for inducing sporulation are known in the art. For example, the recombinant exosporium-producing *Bacillus* cells can be plated onto agar plates, and incubated at a temperature of about 30 °C for several days (e.g., 3 days).

[000163] Inactivated strains, non-toxic strains, or genetically manipulated strains of any of the above species can also suitably be used. For example, a *Bacillus thuringiensis* that lacks the Cry toxin can be used. Alternatively or in addition, once the recombinant *B. cereus* family spores expressing the fusion protein have been generated, they can be inactivated to prevent further germination once in use. Any method for inactivating bacterial spores that is known in the art can be used. Suitable methods include, without limitation, heat treatment, gamma irradiation, x-ray irradiation, UV-A irradiation, UV-B irradiation, chemical treatment (e.g., treatment with gluteraldehyde, formaldehyde, hydrogen peroxide, acetic acid, bleach, or any combination thereof), or a combination thereof. Alternatively, spores derived from nontoxigenic strains, or genetically or physically inactivated strains, can be used.

15

20

25

30

35

10

5

# Recombinant Exosporium-Producing *Bacillus* Cells Having Plant-Growth Promoting Effects and/or Other Beneficial Attributes

[000164] Many *Bacillus cereus* family member strains have inherent beneficial attributes. For example, some strains have plant-growth promoting effects. Any of the fusion proteins described herein can be expressed in such strains.

[000165] For example, the recombinant exosporium-producing *Bacillus* cells can comprise a plant-growth promoting strain of bacteria.

**[000166]** The plant-growth promoting strain of bacteria can comprise a strain of bacteria that produces an insecticidal toxin (e.g., a Cry toxin), produces a fungicidal compound (e.g., a  $\beta$ -1,3-glucanase, a chitosinase, a lyticase, or a combination thereof), produces a nematocidal compound (e.g., a Cry toxin), produces a bacteriocidal compound, is resistant to one or more antibiotics, comprises one or more freely replicating plasmids, binds to plant roots, colonizes plant roots, forms biofilms, solubilizes nutrients, secretes organic acids, or any combination thereof.

[000167] For example, where the recombinant exosporium-producing *Bacillus* cells comprises a plant-growth promoting strain of bacteria, the plant growth-promoting strain of bacteria can comprise *Bacillus mycoides* BT155 (NRRL No. B-50921), *Bacillus mycoides* EE118 (NRRL No. B-50918), *Bacillus mycoides* EE141 (NRRL No. B-50916), *Bacillus mycoides* BT46-3 (NRRL No. B-50922), *Bacillus cereus* family member EE128 (NRRL No. B-50917), *Bacillus thuringiensis* BT013A (NRRL No. B-50924), or *Bacillus cereus* family member EE349

(NRRL No. B-50928). *Bacillus thuringiensis* BT013A is also known as *Bacillus thuringiensis* 4Q7. Each of these strains was deposited with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), having the address 1815 North University Street, Peoria, Illinois 61604, U.S.A., on March 10, 2014, and is identified by the NRRL deposit number provided in parentheses.

[000168] These plant-growth promoting strains were isolated from the rhizospheres of various vigorous plants and were identified by their 16S rRNA sequences, and through biochemical assays. The strains were identified at least to their genus designation by means of conventional biochemistry and morphological indicators. Biochemical assays for confirmed Gram-positive strains such as *Bacillus* included growth on PEA medium and nutrient agar, microscopic examination, growth on 5% and 7.5% NaCl medium, growth at pH 5 and pH 9, growth at 42 °C and 50 °C, the ability to produce acid upon fermentation with cellobiose, lactose, glycerol, glucose, sucrose, d-mannitol, and starch; fluorescent pigment production; gelatin hydrolysis; nitrate reduction; catalase production, starch hydrolysis; oxidase reaction, urease production and motility.

[000169] For example, the recombinant exosporium-producing *Bacillus* cells comprising a plant-growth promoting strain of bacteria can comprise *Bacillus mycoides* BT155, *Bacillus mycoides* EE141, or *Bacillus thuringiensis* BT013A. The recombinant exosporium-producing *Bacillus* cells can express any of the fusion proteins described herein, e.g., a fusion protein comprising the targeting sequence of SEQ ID NO: 60 and a non-hormone peptide (e.g., kunitz trypsin inhibitor (KTI)), an enzyme involved in the production or activation of a plant growth stimulating compound (e.g., a chitosinase), a plant binding protein or peptide (e.g., TasA); a protein or peptide that protects a plant from a pathogen (e.g., TasA), or an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source (e.g., a phosphatase such as PhoA or phytase, or an endoglucanase).

### **Promoters**

5

10

15

20

25

30

[000170] In any of the recombinant *Bacillus cereus* family members described herein, the fusion protein can be expressed under the control of a promoter that is native to the targeting sequence, the exosporium protein, or the exosporium protein fragment of the fusion protein. For example, where the fusion protein comprises a targeting sequence derived from *B. anthracis* Sterne BclA (e.g., amino acids 20–35 of SEQ ID NO: 1, amino acids 1–35 of SEQ ID NO: 1, SEQ ID NO: 1, or SEQ ID NO: 60) or where the fusion protein comprises full length BclA (SEQ ID NO: 2) or a fragment of full length BclA (e.g., SEQ ID NO: 59), the fusion

protein can be expressed under the control of a promoter that is normally associated with the *BclA* gene in the genome of *B. anthracis* Sterne (e.g., the promoter of SEQ ID NO: 85).

[000171] Alternatively, the fusion protein can be expressed under the control of a high-expression sporulation promoter. In some cases, the promoter that is native to the targeting sequence, exosporium protein, or exosporium protein fragment will be a high-expression sporulation promoter. In other cases, the promoter that is native to the targeting sequence, exosporium protein, or exosporium protein fragment will not be a high-expression sporulation promoter. In the latter cases, it may be advantageous to replace the native promoter with a high-expression sporulation promoter. Expression of the fusion protein under the control of a high-expression sporulation promoter provides for increased expression of the fusion protein on the exosporium of the *Bacillus cereus* family member.

[000172] The high-expression sporulation promoter can comprise one or more sigma-K sporulation-specific polymerase promoter sequences.

[000173] Suitable high-expression sporulation promoters for use in expressing the fusion proteins in a *Bacillus cereus* family member include those listed in Table 2 below:

**Table 2. Promoter Sequences** 

5

10

15

Promoter (SEQ ID NO.)	Sequence
BcIA promoter (B. anthracis Sterne) (SEQ ID NO: 85)	TAATCACCCTCTTCCAAATCAAT <u>CATATGTTA</u> TA <u>CATATACTA</u> AACT TTCCATTTTTTAAATTGTTCAAGTAGTTTAAGATTTCTTTTCAATAAT TCAAATGTCCGTGTCATTTTCTTTCGGTTTTG <u>CATCTACTA</u> TATAATG AACGCTTTATGGAGGTGAATTTATG
BetA promoter (B. anthracis Sterne) (SEQ ID NO: 86)	ATTTATTCATTCAATTTTCCTATTTAGTACCTACCGCACTCACAAAA AGCACCTCTCATTAATTTATATATTATAGTCATTGAAATCTAATTTAATGA AATCAT <u>CATACTATA</u> TGTTTTATAAGAAGTAAAGGTAC <u>CATACTTAA</u> TTAATACATATCTATACACTTCAATATCACAGCATGCAGTTGAATTAT ATCCAACTTTCATTTCA
BAS1882 promoter (B. anthracis Sterne) (SEQ ID NO: 87)	AATTACATAACAAGAACTACATTAGGGAGCAAGCAGTCTAGCGAAAG CTAACTGCTTTTTTATTAAATAACTATTTTATTAAATTTCATATATACA ATCGCTTGTCCATTTCATTT

	T
Promoter (SEQ ID NO.)	Sequence
Gene 3572 promoter (B. weihenstephensis KBAB 4) (SEQ ID NO: 88)	CTATGATTTAAGATACACAATAGCAAAAGAGAAAACAAAC
YVTN β-propeller protein promoter (B. weihenstephensis KBAB 4) (SEQ ID NO: 89)	GGTAGGTAGATTTGAAATATGATGAAGAAAAGGAATAACTAAAAGGA GTCGATATCCGACTCCTTTTAGTTATAAATAATGTGGAATTAGAGTAT AATTTTATATAGGTATATTGTATTAGATGAACGCTTTATCCTTTAATTG TGATTAATGATGGATTGTAAGAGAAGGGGCTTACAGTCCTTTTTTAT GGTGTTCTATAAGCCTTTTTAAAAGGGGTACCACCCCACACCCAAAAA CAGGGGGGGTTATAACTACATATTGGATGTTTTGTAACGTACAAGAAT CGGTATTAATTACCCTGTAAATAAGTTATGTGTATATAAAGGTAACTTT ATATATTCTCCTACAATAAAATAA
Cry1A promoter (B. thuringiensis HD-73) (SEQ ID NO: 90)	AACCCTTAATGCATTGGTTAAACATTGTAAAGTCTAAAGCATGGATAA TGGGCGAGAAGTAAGTAGATTGTTAACACCCCTGGGTCAAAAATTGAT ATTTAGTAAAATTAGTTGCACTTTGTGCATTTTTTCATAAGATGAGTC ATATGTTTTAAAATTGTAGTAATGAAAAACAGTATTATATCATAATGA ATTGGTATCTTAATAAAAAGAGATGGAGGTAACTTA
ExsY promoter (B. thuringiensis serovar konkukian str. 97-27) (SEQ ID NO: 91)	TAATTCCACCTTCCCTTATCCTCTTTCGCCTATTTAAAAAAAA
CotY promoter (B. thuringiensis Al Hakam) (SEQ ID NO: 92)	AGGATGTCTTTTTTATATTGTATTATGTACATCCCTACTATATAAATT CCCTGCTTTTATCGTAAGAATTAACGTAATATCAACCATATCCCGTT <u>C</u> ATATTGTAGTAGTGTATGTCAGAACTCACGAGAAGGAGTGAACATAA
YjcA promoter (B. thuringiensis serovar kurstaki str. HD73) (SEQ ID NO: 93)	TTAATGTCACTCCTTATCTTCTTGTTTGTATTTACATT <u>AATAAGATA</u> TT GGAGTTGAGGAGATTTGGTCACAATCTCAAGACCTTTTTTTT
YjcB promoter (B. thuringiensis serovar kurstaki str. HD73) (SEQ ID NO: 94)	ATATATTTCATAATACGAGAAAAAGCGGAGTTTAAAAGAATGAGGG AACGGAAATAAAGAGTTGTT <u>CATATAGTA</u> AATAGACAGAATTGACAG TAGAGGAGA

Promoter	
(SEQ ID NO.)	Sequence
BxpB promoter (B. thuringiensis Al Hakam) (SEQ ID NO: 95)	AAACTAAATAATGAGCTAAGCATGGATTGGGTGGCAGAATTATCTGC CACCCAATC <u>CATGCTTAA</u> CGAGTATTATTATGTAAATTTCTTAAAATT GGGAACTTGTCTAGAACATAGAACCTGTCCTTTT <u>CATTAACTG</u> AAAG TAGAAACAGATAAAGGAGTGAAAAACA
Rhamnose promoter (B. thuringiensis Al Hakam) (SEQ ID NO: 96)	ATTCACTACAACGGGGATGAGTTTGATGCGGATA <u>CATATGAGA</u> AGTA CCGGAAAGTGTTTGTAGAA <u>CATTACAA</u> AGATATATTATCTCCATCATA AAGGAGAGATGCAAAG
CotY/CotZ promoter (B. anthracis Sterne) (SEQ ID NO: 97)	CGCGCACCACTTCGTCGTACAACAACGCAAGAAGAAGTTGGGGATAC AGCAGTATTCTTATTCAGTGATTTAGCACGCGGCGTAACAGGAGAAA ACATTCACGTTGATTCAGGGTAT <u>CATATCTTA</u> GGATAAATATATT AATTTTAAAGGACAATCTCTACATGTTGAGATTGTCCTTTTTATTTGTT CTTAGAAAGAACGATTTTTAACGAAAGTTCTTACCACGTTATGAATAT AAGTATAATAGTACACGATTTATTCAGCTACGTA
BclC promoter (B. anthracis Sterne) (SEQ ID NO: 98)	TGAAGTATCTAGAGCTAATTTACGCAAAGGAATCTCAGGACAACACT TTCGCAACACCTATATTTTAAATTTAATAAAAAAAGAGACTCCGGAGT CAGAAATTATAAAGCTAGCTGGGTTCAAATCAAA
Sigma K promoter (B. anthracis Sterne) (SEQ ID NO: 99)	TATATCATATGTAAAATTAGTTCTTATTCCCA <u>CATATCATA</u> TAGAATC GC <u>CATATTATA</u> CATGCAGAAAACTAAGTATGGTATTATTCTTAAATTG TTTAGCACCTTCTAATATTACAGATAGAATCCGTCATTTTCAACAGTG AACATGGATTTCTTCTGAACACACAACTCTTTTTCTTTCCTTATTTCCAAA AAGAAAAGCAGCCCATTTTAAAAATACGGCTGCTTGTAATGTACATTA
InhA promoter (B. thuringiensis Al Hakam) (SEQ ID NO: 100)	TATCACATAACTCTTTATTTTTAATATTTCGACATAAAGTGAAACTTT AATCAGTGGGGGCTTTGTTCATCCCCCCACTGATTATTAATTGAACCA AGGGATAAAAAGATAGAGGGTCTGACCAGAAAACTGGAGGGCATGA TTCTATAACAAAAAGCTTAATGTTTATAGAATTATGTCTTTTTATATAG GGAGGGTAGTAAACAGAGATTTGGACAAAAATGCACCGATTTATCTG AATTTTAAGTTTTATAAAGGGGAGAAAATG
BclA cluster glycosyl transferase operon 1 ( <i>B. thuringiensis</i> serovar konkukian str. 97-27)	ATTTTTACTTAGCAGTAAAACTGATATCAGTTTTACTGCTTTTTCATT TTTAAATTCAATCATTAAATCTTCCTTTTCTACATAGT <u>CATAATGTT</u> GT ATGACATTCCGTAGGAGGCACTTATA
(SEQ ID NO: 101)	

Promoter (SEQ ID NO.)	Sequence
BclA cluster glycosyl transferase operon 2 ( <i>B. thuringiensis</i> serovar kurstaki str. HD73) (SEQ ID NO: 102)	ACATAAATTCACCTCCATAAAGCGTTCATTATATAGTAGATGCAAAAC CGAAAGAAAATGACACGGACATTTGAATTATTGAAAAGAAATCTTAA ACTACTTGAACAATTTAAAAAAATGGAAAGTTTAGTATATGTATAA $\underline{\mathbf{C}}$ $\underline{\mathbf{ATATGATT}}$ GATTTGGAAGAGGGTGATTA
Glycosyl transferase promoter (B. thuringiensis Al Hakam) (SEQ ID NO: 103)	TTCTATTTTCCAA <u>CATAACATG</u> CTACGATTAAATGGTTTTTTGCAAAT GCCTTCTTGGGAAGAAGGATTAGAGCGTTTTTTTATAGAAACCAAAAG TCATTAACAATTTTAAGTTAATGACTTTTTTGTTTGCCTTTAAGAGGTT TTATGTTACTATAATTATAGTATCAGGTACTAATAACAAGTATAAGTA TTTCTGGGAGGATATATCA

[000174] In the promoter sequences listed in Table 2 above, the locations of the sigma-K sporulation-specific polymerase promoter sequences are indicated by bold and underlined text. The Cry1A promoter (*B. thuringiensis* HD-73; SEQ ID NO: 90) has a total of four sigma-K sequences, two of which overlap with one another, as indicated by the double underlining in Table 2.

5

10

15

20

[000175] Preferred high-expression sporulation promoters for use in expressing the fusion proteins in a *Bacillus cereus* family member include the BetA promoter (*B. anthracis* Sterne; SEQ ID NO: 86), the BclA promoter (*B. anthracis* Sterne; SEQ ID NO: 85), the BclA cluster glycosyl transferase operons 1 and 2 promoters (*B. anthracis* Sterne; SEQ ID NOs: 101 and 102), and the YVTN β-propeller protein promoter (*B. weihenstephensis* KBAB 4; SEQ ID NO: 89).

[000176] In any of the recombinant *Bacillus cereus* family members described herein, the fusion protein can be expressed under the control of a sporulation promoter comprising a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity with a nucleic acid sequence of any one of SEQ ID NOS: 85–103.

[000177] When the sporulation promoter comprising a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity with a nucleic acid sequence of any one of SEQ ID NOS: 85–103, the sigma-K sporulation-specific polymerase promoter sequence or sequences preferably have 100% identity with the corresponding nucleotides of SEQ ID NO: 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, or 103. For example, as illustrated in Table 2 above, the BclA promoter of *B. anthracis* Sterne (SEQ ID NO: 85) has sigma-K sporulation-specific polymerase promoter sequences at

nucleotides 24–32, 35–43, and 129–137. Thus, if the sporulation promoter comprises a sequence having at least 90% identity with the nucleic acid sequence of SEQ ID NO: 85, it is preferred that the nucleotides of the sporulation promoter corresponding to nucleotides 24–32, 35–43, and 129–137 of SEQ ID NO: 85 have 100% identity with nucleotides 24–32, 35–43, and 129–137 of SEQ ID NO: 85.

[000178] In any of the methods described herein for stimulating plant growth, plants grown in the plant growth medium comprising the recombinant exosporium-producing *Bacillus* cells and at least one fungicide selected from the particular fungicides disclosed herein exhibit increased growth as compared to the growth of plants in the identical plant growth medium that does not contain the recombinant exosporium-producing *Bacillus* cells.

[000179] In any of the compositions and methods described herein for stimulating plant growth, the recombinant exosporium-producing *Bacillus* cells can comprise any of the recombinant plant-growth promoting strains of bacteria described above.

[000180] In any of the compositions or methods for stimulating plant growth disclosed herein, the fusion protein can be expressed under the control of any of the promoters described above.

# **Fungicides**

5

10

15

20

25

30

35

[000181] In general, "fungicidal" means the ability of a substance to increase mortality or inhibit the growth rate of fungi.

[000182] The term "fungus" or "fungi" includes a wide variety of nucleated sporebearing organisms that are devoid of chlorophyll. Examples of fungi include yeasts, molds, mildews, rusts, and mushrooms.

[000183] The composition according to the present invention comprises at least one particular fungicide disclosed herein.

[000184] The active compounds specified herein by their common name are known and described, for example, in "Pesticide Manual" or on the Internet (for example: http://www.alanwood.net/pesticides).

[000185] In some embodiments, fungicides are selected from the group consisting of bitertanol, bixafen, bromuconazole, carbendazim, carpropamid, dichlofluanid, fenamidone, fenhexamid, fentin acetate, fentin hydroxide, fluopicolide, fluopyram, fluoxastrobin, fluquinconazole, fosetyl, iprodione, iprovalicarb, isotianil, metominostrobin, ofurace, pencycuron, penflufen, prochloraz, propamocarb, propineb, prothioconazole, pyrimethanil, spiroxamine, tebuconazole, tolylfluanid, triadimefon, triadimenol, triazoxide, trifloxystrobin, N-[5-chloro-2-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-

44

pyrazole-4-carboxamide, and 2,6-dimethyl-1H,5H-[1,4]dithiino[2,3-c:5,6-c']dipyrrole-1,3,5,7(2H,6H)-tetrone.

[000186] In another embodiment of the present invention the fungicide is selected from the group consisting carbendazim, fluquinconazole, isotianil, pencycuron, penflufen, prothioconazole, tebuconazole, and trifloxystrobin.

[000187] In yet another embodiment, the fungicide is N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide.

## **Compositions According to the Present Invention**

[000188] According to the present invention the composition comprises a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound; an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source; and a protein or peptide that protects a plant from a pathogen; and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one particular fungicide disclosed herein in a synergistically effective amount.

[000189] A "synergistically effective amount" according to the present invention represents a quantity of a combination of the recombinant exosporium-producing Bacillus cells that express a fusion protein and at least one particular fungicide as described herein that is more effective against insects, mites, nematodes and/or phytopathogens than the recombinant exosporium-producing Bacillus cells that expresses a fusion protein or such fungicide alone. A "synergistically effective amount" according to the present invention also represents a quantity of a combination of the recombinant exosporium-producing Bacillus cells that express a fusion protein and at least one particular fungicide as disclosed herein that is more effective at enhancing plant growth and/or promoting plant health than the recombinant exosporium-producing Bacillus cells that expresses a fusion protein or the fungicide alone.

[000190] The present invention comprises each and every combination of each of the fungicides mentioned herein with the recombinant exosporium-producing *Bacillus* cells.

### **Further Additives**

[000191] One aspect of the present invention is to provide a composition as described above additionally comprising at least one auxiliary selected from the group consisting of extenders, solvents, spontaneity promoters, carriers, emulsifiers, dispersants, frost protectants, thickeners and adjuvants. Those compositions are referred to as formulations.

45

PATENT REEL: 046350 FRAME: 0732

5

10

15

20

25

30

[000192] Accordingly, in one aspect of the present invention such formulations, and application forms prepared from them, are provided as crop protection agents and/or pesticidal agents, such as drench, drip and spray liquors, comprising the composition of the invention. The application forms may comprise further crop protection agents and/or pesticidal agents, and/or activity-enhancing adjuvants such as penetrants, examples being vegetable oils such as, for example, rapeseed oil, sunflower oil, mineral oils such as, for example, liquid paraffins, alkyl esters of vegetable fatty acids, such as rapeseed oil or soybean oil methyl esters, or alkanol alkoxylates, and/or spreaders such as, for example, alkylsiloxanes and/or salts, examples being organic or inorganic ammonium or phosphonium salts, examples being ammonium sulphate or diammonium hydrogen phosphate, and/or retention promoters such as dioctyl sulphosuccinate or hydroxypropylguar polymers and/or humectants such as glycerol and/or fertilizers such as ammonium, potassium or phosphorous fertilizers, for example.

5

10

15

20

25

30

[000193] Examples of typical formulations include water-soluble liquids (SL), emulsifiable concentrates (EC), emulsions in water (EW), suspension concentrates (SC, SE, FS, OD), water-dispersible granules (WG), granules (GR) and capsule concentrates (CS); these and other possible types of formulation are described, for example, by Crop Life International and in Pesticide Specifications, Manual on Development and Use of FAO and WHO Specifications for Pesticides, FAO Plant Production and Protection Papers – 173, prepared by the FAO/WHO Joint Meeting on Pesticide Specifications, 2004, ISBN: 9251048576. The formulations may comprise active agrochemical compounds other than one or more active compounds of the invention.

[000194] The formulations or application forms in question preferably comprise auxiliaries, such as extenders, solvents, spontaneity promoters, carriers, emulsifiers, dispersants, frost protectants, biocides, thickeners and/or other auxiliaries, such as adjuvants, for example. An adjuvant in this context is a component which enhances the biological effect of the formulation, without the component itself having a biological effect. Examples of adjuvants are agents which promote the retention, spreading, attachment to the leaf surface, or penetration.

[000195] These formulations are produced in a known manner, for example by mixing the active compounds with auxiliaries such as, for example, extenders, solvents and/or solid carriers and/or further auxiliaries, such as, for example, surfactants. The formulations are prepared either in suitable plants or else before or during the application.

[000196] Suitable for use as auxiliaries are substances which are suitable for imparting to the formulation of the active compound or the application forms prepared from these formulations (such as, e.g., usable crop protection agents, such as spray liquors or seed dressings) particular properties such as certain physical, technical and/or biological properties.

[000197] Suitable extenders are, for example, water, polar and nonpolar organic chemical liquids, for example from the classes of the aromatic and non-aromatic hydrocarbons (such as paraffins, alkylbenzenes, alkylnaphthalenes, chlorobenzenes), the alcohols and polyols (which, if appropriate, may also be substituted, etherified and/or esterified), the ketones (such as acetone, cyclohexanone), esters (including fats and oils) and (poly)ethers, the unsubstituted and substituted amines, amides, lactams (such as N-alkylpyrrolidones) and lactones, the sulphones and sulphoxides (such as dimethyl sulphoxide).

5

10

15

20

25

30

[000198] If the extender used is water, it is also possible to employ, for example, organic solvents as auxiliary solvents. Essentially, suitable liquid solvents are: aromatics such as xylene, toluene or alkylnaphthalenes, chlorinated aromatics and chlorinated aliphatic hydrocarbons such as chlorobenzenes, chloroethylenes or methylene chloride, aliphatic hydrocarbons such as cyclohexane or paraffins, for example petroleum fractions, mineral and vegetable oils, alcohols such as butanol or glycol and also their ethers and esters, ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, strongly polar solvents such as dimethylformamide and dimethyl sulphoxide, and also water.

[000199] In principle it is possible to use all suitable solvents. Suitable solvents are, for example, aromatic hydrocarbons, such as xylene, toluene or alkylnaphthalenes, for example, chlorinated aromatic or aliphatic hydrocarbons, such as chlorobenzene, chloroethylene or methylene chloride, for example, aliphatic hydrocarbons, such as cyclohexane, for example, paraffins, petroleum fractions, mineral and vegetable oils, alcohols, such as methanol, ethanol, isopropanol, butanol or glycol, for example, and also their ethers and esters, ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, for example, strongly polar solvents, such as dimethyl sulphoxide, and water.

[000200] All suitable carriers may in principle be used. Suitable carriers are in particular: for example, ammonium salts and ground natural minerals such as kaolins, clays, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, and ground synthetic minerals, such as finely divided silica, alumina and natural or synthetic silicates, resins, waxes and/or solid fertilizers. Mixtures of such carriers may likewise be used. Carriers suitable for granules include the following: for example, crushed and fractionated natural minerals such as calcite, marble, pumice, sepiolite, dolomite, and also synthetic granules of inorganic and organic meals, and also granules of organic material such as sawdust, paper, coconut shells, maize cobs and tobacco stalks.

[000201] Liquefied gaseous extenders or solvents may also be used. Particularly suitable are those extenders or carriers which at standard temperature and under standard

pressure are gaseous, examples being aerosol propellants, such as halogenated hydrocarbons, and also butane, propane, nitrogen and carbon dioxide.

5

10

15

20

25

30

[000202] Examples of emulsifiers and/or foam-formers, dispersants or wetting agents having ionic or nonionic properties, or mixtures of these surface-active substances, are salts of polyacrylic acid, salts of lignosulphonic acid, salts of phenolsulphonic acid or naphthalenesulphonic acid, polycondensates of ethylene oxide with fatty alcohols or with fatty acids or with fatty amines, with substituted phenols (preferably alkylphenols or arylphenols), salts of sulphosuccinic esters, taurine derivatives (preferably alkyltaurates), phosphoric esters of polyethoxylated alcohols or phenols, fatty acid esters of polyols, and derivatives of the compounds containing sulphates, sulphonates and phosphates, examples being alkylaryl polyglycol ethers, alkylsulphonates, alkyl sulphates, arylsulphonates, protein hydrolysates, lignin-sulphite waste liquors and methylcellulose. The presence of a surface-active substance is advantageous if one of the active compounds and/or one of the inert carriers is not soluble in water and if application takes place in water.

[000203] Further auxiliaries that may be present in the formulations and in the application forms derived from them include colorants such as inorganic pigments, examples being iron oxide, titanium oxide, Prussian Blue, and organic dyes, such as alizarin dyes, azo dyes and metal phthalocyanine dyes, and nutrients and trace nutrients, such as salts of iron, manganese, boron, copper, cobalt, molybdenum and zinc.

[000204] Stabilizers, such as low-temperature stabilizers, preservatives, antioxidants, light stabilizers or other agents which improve chemical and/or physical stability may also be present. Additionally present may be foam-formers or defoamers.

[000205] Furthermore, the formulations and application forms derived from them may also comprise, as additional auxiliaries, stickers such as carboxymethylcellulose, natural and synthetic polymers in powder, granule or latex form, such as gum arabic, polyvinyl alcohol, polyvinyl acetate, and also natural phospholipids, such as cephalins and lecithins, and synthetic phospholipids. Further possible auxiliaries include mineral and vegetable oils.

[000206] There may possibly be further auxiliaries present in the formulations and the application forms derived from them. Examples of such additives include fragrances, protective colloids, binders, adhesives, thickeners, thixotropic substances, penetrants, retention promoters, stabilizers, sequestrants, complexing agents, humectants and spreaders. Generally speaking, the active compounds may be combined with any solid or liquid additive commonly used for formulation purposes.

[000207] Suitable retention promoters include all those substances which reduce the dynamic surface tension, such as dioctyl sulphosuccinate, or increase the viscoelasticity, such as hydroxypropylguar polymers, for example.

5

10

15

20

25

30

[000208] Suitable penetrants in the present context include all those substances which are typically used in order to enhance the penetration of active agrochemical compounds into plants. Penetrants in this context are defined in that, from the (generally aqueous) application liquor and/or from the spray coating, they are able to penetrate the cuticle of the plant and thereby increase the mobility of the active compounds in the cuticle. This property can be determined using the method described in the literature (Baur, et al., 1997, Pesticide Science, 51, 131-152). Examples include alcohol alkoxylates such as coconut fatty ethoxylate (10) or isotridecyl ethoxylate (12), fatty acid esters such as rapeseed or soybean oil methyl esters, fatty amine alkoxylates such as tallowamine ethoxylate (15), or ammonium and/or phosphonium salts such as ammonium sulphate or diammonium hydrogen phosphate, for example.

[000209] The formulations preferably comprise between 0.0001% and 98% by weight of active compound or, with particular preference, between 0.01% and 95% by weight of active compound, more preferably between 0.5% and 90% by weight of active compound, based on the weight of the formulation. The content of the active compound is defined as the sum of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein.

[000210] The active compound content of the application forms (crop protection products) prepared from the formulations may vary within wide ranges. The active compound concentration of the application forms may be situated typically between 0.0001% and 95% by weight of active compound, preferably between 0.0001% and 1% by weight, based on the weight of the application form. Application takes place in a customary manner adapted to the application forms.

[000211] Furthermore, in one aspect of the present invention a kit of parts is provided comprising recombinant exosporium-producing *Bacillus* cells and at least one particular fungicide disclosed herein in a synergistically effective amount in a spatially separated arrangement.

[000212] In a futher embodiment of the present invention the above-mentioned kit of parts further comprises at least one additional fungicide and/or at least one insecticide. The fungicide and/or the insecticide can be present either in the recombinant exosporium-producing *Bacillus* cells component of the kit of parts or in the fungicide component of the kit of parts being spatially separated or in both of these components. Preferably, the fungicide and the insecticide

are present in the recombinant *Bacillus cereus* family member-based biological control agent component.

[000213] Moreover, the kit of parts according to the present invention can additionally comprise at least one auxiliary selected from the group consisting of extenders, solvents, spontaneity promoters, carriers, emulsifiers, dispersants, frost protectants, thickeners and adjuvants as mentioned below. This at least one auxiliary can be present either in the recombinant exosporium-producing *Bacillus* cells component of the kit of parts or in the fungicide component of the kit of parts being spatially separated or in both of these components.

5

10

15

20

25

30

[000214] In another aspect of the present invention the composition as described above is used for reducing overall damage of plants and plant parts as well as losses in harvested fruits or vegetables caused by insects, mites, nematodes and/or phytopathogens.

[000215] Furthermore, in another aspect of the present invention the composition as described above increases the overall plant health.

[000216] The term "plant health" generally comprises various sorts of improvements of plants that are not connected to the control of pests. For example, advantageous properties that may be mentioned are improved crop characteristics including: emergence, crop yields, protein content, oil content, starch content, more developed root system, improved root growth, improved root size maintenance, improved root effectiveness, improved stress tolerance (e.g., against drought, heat, salt, UV, water, cold), reduced ethylene (reduced production and/or inhibition of reception), tillering increase, increase in plant height, bigger leaf blade, less dead basal leaves, stronger tillers, greener leaf color, pigment content, photosynthetic activity, less input needed (such as fertilizers or water), less seeds needed, more productive tillers, earlier flowering, early grain maturity, less plant verse (lodging), increased shoot growth, enhanced plant vigor, increased plant stand and early and better germination.

[000217] With regard to the use according to the present invention, improved plant health preferably refers to improved plant characteristics including: crop yield, more developed root system (improved root growth), improved root size maintenance, improved root effectiveness, tillering increase, increase in plant height, bigger leaf blade, less dead basal leaves, stronger tillers, greener leaf color, photosynthetic activity, more productive tillers, enhanced plant vigor, and increased plant stand.

**[000218]** With regard to the present invention, improved plant health preferably especially refers to improved plant properties selected from crop yield, more developed root system, improved root growth, improved root size maintenance, improved root effectiveness, tillering increase, and increase in plant height.

[000219] The effect of a composition according to the present invention on plant health as defined herein can be determined by comparing plants which are grown under the same environmental conditions, whereby a part of said plants is treated with a composition according to the present invention and another part of said plants is not treated with a composition according to the present invention. Instead, said other part is not treated at all or treated with a placebo (i.e., an application without a composition according to the invention such as an application without all active ingredients (i.e., without a the recombinant *Bacillus cereus* family member-based biological control agent as described herein and without a fungicide as described herein), or an application without the recombinant *Bacillus cereus* family member-based biological control agent as described herein, or an application without a fungicide as described herein.

5

10

15

20

25

30

35

[000220] The composition according to the present invention may be applied in any desired manner, such as in the form of a seed coating, soil drench, and/or directly in-furrow and/or as a foliar spray and applied either pre-emergence, post-emergence or both. In other words, the composition can be applied to the seed, the plant or to harvested fruits and vegetables or to the soil wherein the plant is growing or wherein it is desired to grow (plant's locus of growth).

[000221] Reducing the overall damage of plants and plant parts often results in healthier plants and/or in an increase in plant vigor and yield.

[000222] Preferably, the composition according to the present invention is used for treating conventional or transgenic plants or seed thereof.

[000223] The present invention also relates to methods for stimulating plant growth using any of the compositions described above comprising recombinant exosporium-producing *Bacillus* cells that express a fusion protein and at least one particular fungicide disclosed herein. The method for stimulating plant growth comprises applying to a plant, a plant part, to the locus surrounding the plant or in which the plant will be planted (e.g., soil or other growth medium) a composition comprising recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide; and (ii) a targeting sequence, exosporium protein, or exosporium protein fragment, and at least one further particular fungicide disclosed herein in a synergistically effective amount.

[000224] In another aspect of the present invention a method for reducing overall damage of plants and plant parts as well as losses in harvested fruits or vegetables caused by insects, mites, nematodes and/or phytopathogens is provided comprising the step of simultaneously or sequentially applying the recombinant exosporium-producing *Bacillus* cells and at least one particular fungicide disclosed herein in a synergistically effective amount.

[000225] In another embodiment of the present invention, the composition comprises at least one insecticide and/or at least one fungicide in addition to the recombinant exosporium-producing *Bacillus* cells and the particular fungicide disclosed herein. In one embodiment, the at least one insecticide is a synthetic insecticide.

[000226] The method of the present invention includes the following application methods, namely both of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein mentioned before may be formulated into a single, stable composition with an agriculturally acceptable shelf life (so called "solo-formulation"), or being combined before or at the time of use (so called "combined-formulations").

5

10

15

20

25

30

35

[000227] If not mentioned otherwise, the expression "combination" stands for the various combinations of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide, in a solo-formulation, in a single "ready-mix" form, in a combined spray mixture composed from solo-formulations, such as a "tank-mix", and especially in a combined use of the single active ingredients when applied in a sequential manner, i.e., one after the other within a reasonably short period, such as a few hours or days, e.g., 2 hours to 7 days. The order of applying the composition according to the present invention is not essential for working the present invention. Accordingly, the term "combination" also encompasses the presence of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or insecticide on or in a plant to be treated or its surrounding, habitat or storage space, e.g., after simultaneously or consecutively applying the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide to a plant its surrounding, habitat or storage space.

[000228] If the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide are employed or used in a sequential manner, it is preferred to treat the plants or plant parts (which includes seeds and plants emerging from the seed), harvested fruits and vegetables according to the following method: Firstly applying the at least one particular fungicide disclosed herein and optionally the at least one additional fungicide and/or the at least one insecticide on the plant or plant parts, and secondly applying the recombinant exosporium-producing *Bacillus* cells to the same plant or plant parts. By this application manner the amount of residues of insecticides/fungicides on the plant upon harvesting is as low as possible. The time periods between the first and the second application within a (crop) growing cycle may vary and depend on the effect to be achieved. For example, the first application is done to prevent an

infestation of the plant or plant parts with insects, mites, nematodes and/or phytopathogens (this is particularly the case when treating seeds) or to combat the infestation with insects, mites, nematodes and/or phytopathogens (this is particularly the case when treating plants and plant parts) and the second application is done to prevent or control the infestation with insects, mites, nematodes and/or phytopathogens and/or to promote plant growth. Control in this context means that the recombinant exosporium-producing *Bacillus* cells are not able to fully exterminate the pests or phytopathogenic fungi but are able to keep the infestation on an acceptable level.

5

10

15

20

25

30

[000229] The present invention also provides methods of enhancing the killing, inhibiting, preventative and/or repelling activity of the compositions of the present invention by multiple applications. In some other embodiments, the compositions of the present invention are applied to a plant and/or plant part for two times, during any desired development stages or under any predetermined pest pressure, at an interval of about 1 hour, about 5 hours, about 10 hours, about 24 hours, about two days, about 3 days, about 4 days, about 5 days, about 1 week, about 10 days, about two weeks, about three weeks, about 1 month or more. Still in some embodiments, the compositions of the present invention are applied to a plant and/or plant part for more than two times, for example, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, or more, during any desired development stages or under any predetermined pest pressure, at an interval of about 1 hour, about 5 hours, about 10 hours, about 24 hours, about two days, about 3 days, about 4 days, about 5 days, about 1 week, about 10 days, about two weeks, about three weeks, about 1 month or more. The intervals between each application can vary if it is desired. One skilled in the art will be able to determine the application times and length of interval depending on plant species, plant pest species, and other factors.

[000230] By following the before mentioned steps, a very low level of residues of the at least one fungicide and/or at least one insecticide on the treated plant, plant parts, and the harvested fruits and vegetables can be achieved.

[000231] If not mentioned otherwise the treatment of plants or plant parts (which includes seeds and plants emerging from the seed), harvested fruits and vegetables with the composition according to the invention is carried out directly or by action on their surroundings, habitat or storage space using customary treatment methods, for example dipping, spraying, atomizing, irrigating, evaporating, dusting, fogging, broadcasting, foaming, painting, spreading-on, watering (drenching), drip irrigating. It is furthermore possible to apply the recombinant exosporium-producing *Bacillus* cells, the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide as solo-formulation or combined-formulations by the ultra-low volume method, or to inject the

composition according to the present invention as a composition or as sole-formulations into the soil (in-furrow).

[000232] The term "plant to be treated" encompasses every part of a plant including its root system and the material - e.g., soil or nutrition medium - which is in a radius of at least 10 cm, 20 cm, 30 cm around the caulis or bole of a plant to be treated or which is at least 10 cm, 20 cm, 30 cm around the root system of said plant to be treated, respectively.

5

10

15

20

25

30

35

[000233] The amount of the recombinant exosporium-producing *Bacillus* cells which are used or employed in combination with at least one particular fungicide disclosed herein, optionally in the presence of at least one additional fungicide and/or the at least one insecticide, depends on the final formulation as well as size or type of the plant, plant parts, seeds, harvested fruits and vegetables to be treated. Usually, the recombinant exosporium-producing *Bacillus* cells to be employed or used according to the invention is present in about 1% to about 80% (w/w), preferably in about 1% to about 60% (w/w), more preferably about 10% to about 50% (w/w) of its solo-formulation or combined-formulation with the at least one particular fungicide disclosed herein, and optionally the additional fungicide and/or the at least one insecticide.

[000234] Also the amount of the at least one particular fungicide disclosed herein which is used or employed in combination with the recombinant exosporium-producing *Bacillus* cells, optionally in the presence of at least one additional fungicide and/or the at least one insecticide, depends on the final formulation as well as size or type of the plant, plant parts, seeds, harvested fruit or vegetable to be treated. Usually, the particular fungicide to be employed or used according to the invention is present in about 0.1% to about 80% (w/w), preferably 1% to about 60% (w/w), more preferably about 10% to about 50% (w/w) of its solo-formulation or combined-formulation with the recombinant exosporium-producing *Bacillus* cells, and optionally the at least one additional fungicide and/or the at least one insecticide.

[000235] Application of the recombinant exosporium-producing *Bacillus* cells may be effected as a foliar spray, as a soil treatment, and/or as a seed treatment/dressing. When used as a foliar treatment, in one embodiment, about 1/16 to about 5 gallons of whole broth are applied per acre. When used as a soil treatment, in one embodiment, about 1 to about 5 gallons of whole broth are applied per acre. When used for seed treatment about 1/32 to about 1/4 gallons of whole broth are applied per acre. For seed treatment, the end-use formulation contains at least at least  $1 \times 10^4$ , at least  $1 \times 10^5$ , at least  $1 \times 10^6$ ,  $1 \times 10^7$ , at least  $1 \times 10^8$ , at least  $1 \times 10^9$ , at least  $1 \times 10^{10}$  colony forming units per gram.

[000236] The recombinant exosporium-producing *Bacillus* cells and at least one particular fungicide disclosed herein, and if present preferably also the additional fungicide and/or the insecticide are used or employed in a synergistic weight ratio. The skilled person is

able to find out the synergistic weight ratios for the present invention by routine methods. The skilled person understands that these ratios refer to the ratio within a combined-formulation as well as to the calculative ratio of the recombinant exosporium-producing *Bacillus* cells described herein and the at least one particular fungicide disclosed herein when both components are applied as mono-formulations to a plant to be treated. The skilled person can calculate this ratio by simple mathematics since the volume and the amount of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide, respectively, in a mono-formulation is known to the skilled person.

5

10

15

20

25

30

35

[000237] The ratio can be calculated based on the amount of the at least one particular fungicide disclosed herein, at the time point of applying said component of a combination according to the invention to a plant or plant part and the amount of recombinant exosporium-producing *Bacillus* cells shortly prior (e.g., 48 h, 24 h, 12 h, 6 h, 2 h, 1 h) or at the time point of applying said component of a combination according to the invention to a plant or plant part.

[000238] The application of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein to a plant or a plant part can take place simultaneously or at different times as long as both components are present on or in the plant after the application(s). In cases where the recombinant exosporium-producing *Bacillus* cells and the particular fungicide disclosed herein are applied at different times and the particular fungicide disclosed herein is applied prior to the recombinant exosporium-producing *Bacillus* cells, the skilled person can determine the concentration of fungicide on/in a plant by chemical analysis known in the art, at the time point or shortly before the time point of applying the recombinant exosporium-producing *Bacillus* cells. Vice versa, when the recombinant exosporium-producing *Bacillus* cells can be determined using tests which are also known in the art, at the time point or shortly before the time point of applying the fungicide.

[000239] In particular, in one embodiment the synergistic weight ratio of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein lies in the range of 1:1000 to 1000:1, preferably in the range of 1:500 to 500:1, more preferably in the range of 1:300 to 500:1. Especially preferred ratios are between 20:1 and 1:20, such as 10:1, 5:1 or 2:1. It has to be noted that these ratio ranges refer to the recombinant *Bacillus cereus* family member-based biological control agent (to be combined with at least one particular fungicide disclosed herein or a preparation of at least one particular fungicide disclosed herein). For example, a ratio of 100:1 means 100 weight parts of a spore preparation of the recombinant exosporium-producing *Bacillus*-based biological control agent and 1 weight part of the particular fungicide disclosed herein are combined (either as a solo formulation, a combined

formulation or by separate applications to plants so that the combination is formed on the plant). In one aspect of this embodiment, the spore preparation of the recombinant exosporium-producing *Bacillus* cells is a dried spore preparation containing at least about 1 x  $10^4$  cfu/g, at least about 1 x  $10^5$  cfu/g, at least about 1 x  $10^6$  cfu/g at least about 1 x  $10^7$  cfu/g, at least about 1 x  $10^8$  cfu/g, at least about 1 x  $10^9$  cfu/g, at least about 1 x  $10^{10}$  cfu/g, or at least about 1 x  $10^{11}$  cfu/g.

5

10

15

20

25

30

35

[000240] In another embodiment, the synergistic weight ratio of the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein is in the range of 1:100 to 20,000:1, preferably in the range of 1:50 to 10,000:1 or even in the range of 1:50 to 1000:1.

[000241] In one embodiment of the present invention, the concentration of the recombinant exosporium-producing *Bacillus* cells after dispersal is at least 50 g/ha, such as 50 - 7500 g/ha, 50 - 2500 g/ha, 50 - 1500 g/ha; at least 250 g/ha (hectare), at least 500 g/ha or at least 800 g/ha.

[000242] The application rate of composition to be employed or used according to the present invention may vary. The skilled person is able to find the appropriate application rate by way of routine experiments.

[000243] In another aspect of the present invention a seed treated with the composition as described above is provided.

[000244] The control of insects, mites, nematodes and/or phytopathogens by treating the seed of plants has been known for a long time and is a subject of continual improvements. Nevertheless, the treatment of seed entails a series of problems which cannot always be solved in a satisfactory manner. Thus, it is desirable to develop methods for protecting the seed and the germinating plant that remove the need for, or at least significantly reduce, the additional delivery of crop protection compositions in the course of storage, after sowing or after the emergence of the plants. It is desirable, furthermore, to optimize the amount of active ingredient employed in such a way as to provide the best-possible protection to the seed and the germinating plant from attack by insects, mites, nematodes and/or phytopathogens, but without causing damage to the plant itself by the active ingredient employed. In particular, methods for treating seed ought also to take into consideration the intrinsic insecticidal and/or nematicidal properties of pest-resistant or pest-tolerant transgenic plants, in order to achieve optimum protection of the seed and of the germinating plant with a minimal use of crop protection compositions.

[000245] The present invention therefore also relates in particular to a method for protecting seed and germinating plants from attack by pests, by treating the seed with the recombinant exosporium-producing *Bacillus* cells as defined above and at least one particular

fungicide disclosed herein in a synergistically effective amount. The method of the invention for protecting seed and germinating plants from attack by pests encompasses a method in which the seed is treated simultaneously in one operation with the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide. It also encompasses a method in which the seed is treated at different times with the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide.

5

10

15

20

25

30

35

[000246] The invention likewise relates to the use of the composition of the invention for treating seed for the purpose of protecting the seed and the resultant plant against insects, mites, nematodes and/or phytopathogens.

[000247] The invention also relates to seed which at the same time has been treated with the recombinant exosporium-producing *Bacillus* cells and at least one particular fungicide disclosed herein, and optionally at least one additional fungicide and/or the at least one insecticide. The invention further relates to seed which has been treated at different times with the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein and optionally the at least one additional fungicide and/or the at least one insecticide. In the case of seed which has been treated at different times with the recombinant exosporium-producing *Bacillus* cells and the at least one particular fungicide disclosed herein, and optionally the at least one additional fungicide and/or the at least one insecticide, the individual active ingredients in the composition of the invention may be present in different layers on the seed.

[000248] Furthermore, the invention relates to seed which, following treatment with the composition of the invention, is subjected to a film-coating process in order to prevent dust abrasion of the seed.

[000249] One of the advantages of the present invention is that, owing to the particular systemic properties of the compositions of the invention, the treatment of the seed with these compositions provides protection from insects, mites, nematodes and/or phytopathogens not only to the seed itself but also to the plants originating from the seed, after they have emerged. In this way, it may not be necessary to treat the crop directly at the time of sowing or shortly thereafter.

[000250] A further advantage is to be seen in the fact that, through the treatment of the seed with composition of the invention, germination and emergence of the treated seed may be promoted.

[000251] It is likewise considered to be advantageous composition of the invention may also be used, in particular, on transgenic seed.

[000252] It is also stated that the composition of the invention may be used in combination with agents of the signalling technology, as a result of which, for example, colonization with symbionts is improved, such as rhizobia, mycorrhiza and/or endophytic bacteria, for example, is enhanced, and/or nitrogen fixation is optimized.

5

10

15

20

25

30

[000253] The compositions of the invention are suitable for protecting seed of any variety of plant which is used in agriculture, in greenhouses, in forestry or in horticulture. More particularly, the seed in question is that of cereals (e.g., wheat, barley, rye, oats and millet), maize, cotton, soybeans, rice, potatoes, sunflower, coffee, tobacco, canola, oilseed rape, beets (e.g., sugar beet and fodder beet), peanuts, vegetables (e.g., tomato, cucumber, bean, brassicas, onions and lettuce), fruit plants, lawns and ornamentals. Particularly important is the treatment of the seed of cereals (such as wheat, barley, rye and oats) maize, soybeans, cotton, canola, oilseed rape and rice.

[000254] As already mentioned above, the treatment of transgenic seed with the composition of the invention is particularly important. The seed in question here is that of plants which generally contain at least one heterologous gene that controls the expression of a polypeptide having, in particular, insecticidal and/or nematicidal properties. These heterologous genes in transgenic seed may come from microorganisms such as *Bacillus*, *Rhizobium*, *Pseudomonas*, *Serratia*, *Trichoderma*, *Clavibacter*, *Glomus* or *Gliocladium*. The present invention is particularly suitable for the treatment of transgenic seed which contains at least one heterologous gene from *Bacillus sp*. With particular preference, the heterologous gene in question comes from *Bacillus thuringiensis*.

[000255] For the purposes of the present invention, the composition of the invention is applied alone or in a suitable formulation to the seed. The seed is preferably treated in a condition in which its stability is such that no damage occurs in the course of the treatment. Generally speaking, the seed may be treated at any point in time between harvesting and sowing. Typically, seed is used which has been separated from the plant and has had cobs, hulls, stems, husks, hair or pulp removed. Thus, for example, seed may be used that has been harvested, cleaned and dried to a moisture content of less than 15% by weight. Alternatively, seed can also be used that after drying has been treated with water, for example, and then dried again.

[000256] When treating seed it is necessary, generally speaking, to ensure that the amount of the composition of the invention, and/or of other additives, that is applied to the seed is selected such that the germination of the seed is not adversely affected, and/or that the plant which emerges from the seed is not damaged. This is the case in particular with active ingredients which may exhibit phytotoxic effects at certain application rates.

[000257] The compositions of the invention can be applied directly, in other words without comprising further components and without having been diluted. As a general rule, it is preferable to apply the compositions in the form of a suitable formulation to the seed. Suitable formulations and methods for seed treatment are known to the skilled person and are described in, for example, the following documents: U.S. Patent Nos. 4,272,417 A; 4,245,432 A; 4,808,430 A; 5,876,739 A; U.S. Patent Publication No. 2003/0176428 A1; WO 2002/080675 A1; WO 2002/028186 A2.

5

10

15

20

25

30

[000258] The combinations which can be used in accordance with the invention may be converted into the customary seed-dressing formulations, such as solutions, emulsions, suspensions, powders, foams, slurries or other coating compositions for seed, and also ULV formulations.

[000259] These formulations are prepared in a known manner, by mixing composition with customary adjuvants, such as, for example, customary extenders and also solvents or diluents, colorants, wetters, dispersants, emulsifiers, antifoams, preservatives, secondary thickeners, stickers, gibberellins, and also water.

[000260] Colorants which may be present in the seed-dressing formulations which can be used in accordance with the invention include all colorants which are customary for such purposes. In this context it is possible to use not only pigments, which are of low solubility in water, but also water-soluble dyes. Examples include the colorants known under the designations Rhodamin B, C.I. Pigment Red 112 and C.I. Solvent Red 1.

[000261] Wetters which may be present in the seed-dressing formulations which can be used in accordance with the invention include all of the substances which promote wetting and which are customary in the formulation of active agrochemical ingredients. Use may be made preferably of alkylnaphthalenesulphonates, such as diisopropyl- or diisobutylnaphthalenesulphonates.

[000262] Dispersants and/or emulsifiers which may be present in the seed-dressing formulations which can be used in accordance with the invention include all of the nonionic, anionic and cationic dispersants that are customary in the formulation of active agrochemical ingredients. Use may be made preferably of nonionic or anionic dispersants or of mixtures of nonionic or anionic dispersants. Suitable nonionic dispersants are, in particular, ethylene oxide-propylene oxide block polymers, alkylphenol polyglycol ethers and also tristryrylphenol polyglycol ethers, and the phosphated or sulphated derivatives of these. Suitable anionic dispersants are, in particular, lignosulphonates, salts of polyacrylic acid, and arylsulphonate-formaldehyde condensates.

[000263] Antifoams which may be present in the seed-dressing formulations which can be used in accordance with the invention include all of the foam inhibitors that are customary in the formulation of active agrochemical ingredients. Use may be made preferably of silicone antifoams and magnesium stearate.

[000264] Preservatives which may be present in the seed-dressing formulations which can be used in accordance with the invention include all of the substances which can be employed for such purposes in agrochemical compositions. Examples include dichlorophen and benzyl alcohol hemiformal.

5

10

15

20

25

30

35

[000265] Secondary thickeners which may be present in the seed-dressing formulations which can be used in accordance with the invention include all substances which can be used for such purposes in agrochemical compositions. Those contemplated with preference include cellulose derivatives, acrylic acid derivatives, xanthan, modified clays and highly disperse silica.

[000266] Stickers which may be present in the seed-dressing formulations which can be used in accordance with the invention include all customary binders which can be used in seed-dressing products. Preferred mention may be made of polyvinylpyrrolidone, polyvinyl acetate, polyvinyl alcohol and tylose.

[000267] Gibberellins which may be present in the seed-dressing formulations which can be used in accordance with the invention include preferably the gibberellins A1, A3 (= gibberellic acid), A4 and A7, with gibberellic acid being used with particular preference. The gibberellins are known (cf. R. Wegler, "Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel", Volume 2, Springer Verlag, 1970, pp. 401-412).

[000268] The seed-dressing formulations which can be used in accordance with the invention may be used, either directly or after prior dilution with water, to treat seed of any of a wide variety of types. Accordingly, the concentrates or the preparations obtainable from them by dilution with water may be employed to dress the seed of cereals, such as wheat, barley, rye, oats and triticale, and also the seed of maize, rice, oilseed rape, peas, beans, cotton, sunflowers and beets, or else the seed of any of a very wide variety of vegetables. The seed-dressing formulations which can be used in accordance with the invention, or their diluted preparations, may also be used to dress seed of transgenic plants. In that case, additional synergistic effects may occur in interaction with the substances formed through expression.

[000269] For the treatment of seed with the seed-dressing formulations which can be used in accordance with the invention, or with the preparations produced from them by addition of water, suitable mixing equipment includes all such equipment which can typically be employed for seed dressing. More particularly, the procedure when carrying out seed dressing is to place the seed in a mixer, to add the particular desired amount of seed-dressing formulations,

60

either as such or following dilution with water beforehand, and to carry out mixing until the distribution of the formulation on the seed is uniform. This may be followed by a drying operation.

[000270] The application rate of the seed-dressing formulations which can be used in accordance with the invention may be varied within a relatively wide range. It is guided by the particular amount of the recombinant *Bacillus cereus* family member-based biological control agent and the at least one particular fungicide disclosed herein in the formulations, and by the seed. The application rates in the case of the composition are situated generally at between 0.001 and 50 g per kilogram of seed, preferably between 0.01 and 15 g per kilogram of seed.

5

10

15

20

25

30

[000271] Furthermore, the composition according to the present invention preferably has potent microbicidal activity and can be used for control of unwanted microorganisms, such as fungi and bacteria, in crop protection and in the protection of materials.

[000272] The invention also relates to a method for controlling unwanted microorganisms, characterized in that the inventive composition is applied to the phytopathogenic fungi, phytopathogenic bacteria and/or their habitat.

[000273] Fungicides can be used in crop protection for control of phytopathogenic fungi. They are characterized by an outstanding efficacy against a broad spectrum of phytopathogenic fungi, including soilborne pathogens, which are in particular members of the classes *Plasmodiophoromycetes*, *Peronosporomycetes* (Syn. *Oomycetes*), *Chytridiomycetes*, *Zygomycetes*, *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* (Syn. *Fungi imperfecti*). Some fungicides are systemically active and can be used in plant protection as foliar, seed dressing or soil fungicide. Furthermore, they are suitable for combating fungi, which inter alia infest wood or roots of plant.

[000274] Bactericides can be used in crop protection for control of *Pseudomonadaceae*, *Rhizobiaceae*, *Enterobacteriaceae*, *Corynebacteriaceae* and *Streptomycetaceae*.

[000275] Non-limiting examples of pathogens of fungal diseases which can be treated in accordance with the invention include:

[000276] diseases caused by powdery mildew pathogens, for example *Blumeria* species, for example *Blumeria graminis*; *Podosphaera* species, for example *Podosphaera leucotricha*; *Sphaerotheca* species, for example *Sphaerotheca fuliginea*; *Uncinula* species, for example *Uncinula necator*;

[000277] diseases caused by rust disease pathogens, for example *Gymnosporangium* species, for example *Gymnosporangium sabinae*; *Hemileia* species, for example *Hemileia* vastatrix; *Phakopsora* species, for example *Phakopsora pachyrhizi* and *Phakopsora meibomiae*;

Puccinia species, for example Puccinia recondite, P. triticina, P. graminis or P. striiformis or P. hordei; Uromyces species, for example Uromyces appendiculatus;

[000278] diseases caused by pathogens from the group of the *Oomycetes*, for example *Albugo* species, for example *Algubo candida*; *Bremia* species, for example *Bremia lactucae*; *Peronospora* species, for example *Peronospora pisi*, *P. parasitica* or *P. brassicae*; Phytophthora species, for example *Phytophthora infestans*; *Plasmopara* species, for example *Plasmopara viticola*; *Pseudoperonospora* species, for example *Pseudoperonospora humuli* or *Pseudoperonospora cubensis*; *Pythium* species, for example *Pythium ultimum*;

5

10

15

20

25

30

35

[000279] leaf blotch diseases and leaf wilt diseases caused, for example, by Alternaria species, for example Alternaria solani; Cercospora species, for example Cercospora beticola; Cladiosporium species, for example Cladiosporium cucumerinum; Cochliobolus species, for example Cochliobolus sativus (conidia form: Drechslera, Syn: Helminthosporium), Cochliobolus miyabeanus; Colletotrichum species, for example Colletotrichum lindemuthanium; Cycloconium species, for example Cycloconium oleaginum; Diaporthe species, for example Diaporthe citri; Elsinoe species, for example Elsinoe fawcettii; Gloeosporium species, for example Gloeosporium laeticolor; Glomerella species, for example Glomerella cingulata; Guignardia species, for example Guignardia bidwelli; Leptosphaeria species, for example Leptosphaeria maculans, Leptosphaeria nodorum; Magnaporthe species, for example Magnaporthe grisea; Microdochium species, for example Microdochium nivale; Mycosphaerella species, for example Mycosphaerella graminicola, M. arachidicola and M. fijiensis; Phaeosphaeria species, for example Phaeosphaeria nodorum; Pyrenophora species, for example Pyrenophora teres, Pyrenophora tritici repentis; Ramularia species, for example Ramularia collo-cygni, Ramularia areola; Rhynchosporium species, for example Rhynchosporium secalis; Septoria species, for example Septoria apii, Septoria lycopersii; Typhula species, for example Typhula incarnata; Venturia species, for example Venturia inaequalis;

[000280] root and stem diseases caused, for example, by *Corticium* species, for example *Corticium* graminearum; Fusarium species, for example Fusarium oxysporum; Gaeumannomyces species, for example Gaeumannomyces graminis; Rhizoctonia species, such as, for example Rhizoctonia solani; Sarocladium diseases caused for example by Sarocladium oryzae; Sclerotium diseases caused for example by Sclerotium oryzae; Tapesia species, for example Tapesia acuformis; Thielaviopsis species, for example Thielaviopsis basicola;

[000281] ear and panicle diseases (including corn cobs) caused, for example, by Alternaria species, for example Alternaria spp.; Aspergillus species, for example Aspergillus flavus; Cladosporium species, for example Cladosporium cladosporioides; Claviceps species, for example Claviceps purpurea; Fusarium species, for example Fusarium culmorum; Gibberella

62

species, for example *Gibberella zeae*; *Monographella* species, for example *Monographella nivalis*; *Septoria* species, for example *Septoria nodorum*;

[000282] diseases caused by smut fungi, for example *Sphacelotheca* species, for example *Sphacelotheca reiliana*; *Tilletia* species, for example *Tilletia caries*, *T. controversa*; *Urocystis* species, for example *Urocystis occulta*; *Ustilago* species, for example *Ustilago nuda*, *U. nuda tritici*;

5

10

15

20

25

30

35

[000283] fruit rot caused, for example, by Aspergillus species, for example Aspergillus flavus; Botrytis species, for example Botrytis cinerea; Penicillium species, for example Penicillium expansum and P. purpurogenum; Sclerotinia species, for example Sclerotinia sclerotiorum; Verticilium species, for example Verticilium alboatrum;

[000284] seed and soilborne decay, mould, wilt, rot and damping-off diseases caused, for example, by Alternaria species, caused for example by Alternaria brassicicola; Aphanomyces species, caused for example by Aphanomyces euteiches; Ascochyta species, caused for example by Ascochyta lentis; Aspergillus species, caused for example by Aspergillus flavus; Cladosporium species, caused for example by Cladosporium herbarum; Cochliobolus species, caused for example by Cochliobolus sativus; (Conidiaform: Drechslera, Bipolaris Syn: Helminthosporium); Colletotrichum species, caused for example by Colletotrichum coccodes; Fusarium species, caused for example by Fusarium culmorum; Gibberella species, caused for example by Gibberella zeae; Macrophomina species, caused for example by Macrophomina phaseolina; Monographella species, caused for example by Monographella nivalis; Penicillium species, caused for example by *Penicillium expansum*; *Phoma* species, caused for example by Phoma lingam; Phomopsis species, caused for example by Phomopsis sojae; Phytophthora species, caused for example by Phytophthora cactorum; Pyrenophora species, caused for example by Pyrenophora graminea; Pyricularia species, caused for example by Pyricularia oryzae; Pythium species, caused for example by Pythium ultimum; Rhizoctonia species, caused for example by Rhizoctonia solani; Rhizopus species, caused for example by Rhizopus oryzae; Sclerotium species, caused for example by Sclerotium rolfsii; Septoria species, caused for example by Septoria nodorum; Typhula species, caused for example by Typhula incarnata; Verticillium species, caused for example by Verticillium dahliae;

[000285] cancers, galls and witches' broom caused, for example, by *Nectria* species, for example *Nectria galligena*;

[000286] wilt diseases caused, for example, by *Monilinia* species, for example *Monilinia laxa*;

[000287] leaf blister or leaf curl diseases caused, for example, by *Exobasidium* species, for example *Exobasidium vexans*;

63

[000288] Taphrina species, for example Taphrina deformans

5

10

15

20

25

30

35

[000289] decline diseases of wooden plants caused, for example, by Esca disease, caused for example by *Phaemoniella clamydospora*, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea*; Eutypa dyeback, caused for example by *Eutypa lata*; Ganoderma diseases caused for example by *Ganoderma boninense*; Rigidoporus diseases caused for example by *Rigidoporus lignosus*;

[000290] diseases of flowers and seeds caused, for example, by *Botrytis* species, for example *Botrytis cinerea*;

[000291] diseases of plant tubers caused, for example, by *Rhizoctonia* species, for example *Rhizoctonia solani*; *Helminthosporium* species, for example *Helminthosporium solani*;

[000292] Club root caused, for example, by *Plasmodiophora* species, for example *Plamodiophora brassicae*;

[000293] diseases caused by bacterial pathogens, for example *Xanthomonas* species, for example *Xanthomonas* campestris pv. oryzae; Pseudomonas species, for example Pseudomonas syringae pv. lachrymans; Erwinia species, for example Erwinia amylovora.

[000294] The following diseases of soya beans can be controlled with preference:

[000295] Fungal diseases on leaves, stems, pods and seeds caused, for example, by Alternaria leaf spot (Alternaria spec. atrans tenuissima), Anthracnose (Colletotrichum gloeosporoides dematium var. truncatum), brown spot (Septoria glycines), cercospora leaf spot and blight (Cercospora kikuchii), choanephora leaf blight (Choanephora infundibulifera trispora (Syn.)), dactuliophora leaf spot (Dactuliophora glycines), downy mildew (Peronospora manshurica), drechslera blight (Drechslera glycini), frogeye leaf spot (Cercospora sojina), leptosphaerulina leaf spot (Leptosphaerulina trifolii), phyllostica leaf spot (Phyllosticta sojaecola), pod and stem blight (Phomopsis sojae), powdery mildew (Microsphaera diffusa), pyrenochaeta leaf spot (Pyrenochaeta glycines), rhizoctonia aerial, foliage, and web blight (Rhizoctonia solani), rust (Phakopsora pachyrhizi, Phakopsora meibomiae), scab (Sphaceloma glycines), stemphylium leaf blight (Stemphylium botryosum), target spot (Corynespora cassiicola).

[000296] Fungal diseases on roots and the stem base caused, for example, by black root rot (Calonectria crotalariae), charcoal rot (Macrophomina phaseolina), fusarium blight or wilt, root rot, and pod and collar rot (Fusarium oxysporum, Fusarium orthoceras, Fusarium semitectum, Fusarium equiseti), mycoleptodiscus root rot (Mycoleptodiscus terrestris), neocosmospora (Neocosmospora vasinfecta), pod and stem blight (Diaporthe phaseolorum), stem canker (Diaporthe phaseolorum var. caulivora), phytophthora rot (Phytophthora megasperma), brown stem rot (Phialophora gregata), pythium rot (Pythium aphanidermatum,

Pythium irregulare, Pythium debaryanum, Pythium myriotylum, Pythium ultimum), rhizoctonia root rot, stem decay, and damping-off (*Rhizoctonia solani*), sclerotinia stem decay (*Sclerotinia sclerotiorum*), sclerotinia southern blight (*Sclerotinia rolfsii*), thielaviopsis root rot (*Thielaviopsis basicola*).

[000297] The inventive compositions can be used for curative or protective/preventive control of phytopathogenic fungi. The invention therefore also relates to curative and protective methods for controlling phytopathogenic fungi by the use of the inventive composition, which is applied to the seed, the plant or plant parts, the fruit or the soil in which the plants grow.

5

10

15

20

25

30

35

[000298] The fact that the composition is well tolerated by plants at the concentrations required for controlling plant diseases allows the treatment of above-ground parts of plants, of propagation stock and seeds, and of the soil.

[000299] According to the invention all plants and plant parts can be treated. By plants is meant all plants and plant populations such as desirable and undesirable wild plants, cultivars and plant varieties (whether or not protectable by plant variety or plant breeder's rights). Cultivars and plant varieties can be plants obtained by conventional propagation and breeding methods which can be assisted or supplemented by one or more biotechnological methods such as by use of double haploids, protoplast fusion, random and directed mutagenesis, molecular or genetic markers or by bioengineering and genetic engineering methods. By plant parts is meant all above ground and below ground parts and organs of plants such as shoot, leaf, blossom and root, whereby for example leaves, needles, stems, branches, blossoms, fruiting bodies, fruits and seed as well as roots, corms and rhizomes are listed. Crops and vegetative and generative propagating material, for example cuttings, corms, rhizomes, runners and seeds also belong to plant parts.

[000300] The inventive composition, when it is well tolerated by plants, has favourable homeotherm toxicity and is well tolerated by the environment, is suitable for protecting plants and plant organs, for enhancing harvest yields, for improving the quality of the harvested material. It can preferably be used as crop protection composition. It is active against normally sensitive and resistant species and against all or some stages of development.

[000301] Plants which can be treated in accordance with the invention include the following main crop plants: maize, soya bean, alfalfa, cotton, sunflower, *Brassica* oil seeds such as *Brassica napus* (e.g., canola, rapeseed), *Brassica rapa*, *B. juncea* (e.g., (field) mustard) and *Brassica carinata*, *Arecaceae* sp. (e.g., oilpalm, coconut), rice, wheat, sugar beet, sugar cane, oats, rye, barley, millet and sorghum, triticale, flax, nuts, grapes and vine and various fruit and vegetables from various botanic taxa, e.g., *Rosaceae* sp. (e.g., pome fruits such as apples and pears, but also stone fruits such as apricots, cherries, almonds, plums and peaches, and berry

fruits such as strawberries, raspberries, red and black currant and gooseberry), Ribesioidae sp., Juglandaceae sp., Betulaceae sp., Anacardiaceae sp., Fagaceae sp., Moraceae sp., Oleaceae sp. (e.g., olive tree), Actinidaceae sp., Lauraceae sp. (e.g., avocado, cinnamon, camphor), Musaceae sp. (e.g., banana trees and plantations), Rubiaceae sp. (e.g., coffee), Theaceae sp. (e.g., tea), Sterculiceae sp., Rutaceae sp. (e.g., lemons, oranges, mandarins and grapefruit); Solanaceae sp. (e.g., tomatoes, potatoes, peppers, capsicum, aubergines, tobacco), Liliaceae sp., Compositae sp. (e.g., lettuce, artichokes and chicory – including root chicory, endive or common chicory), Umbelliferae sp. (e.g., carrots, parsley, celery and celeriac), Cucurbitaceae sp. (e.g., cucumbers – including gherkins, pumpkins, watermelons, calabashes and melons), Alliaceae sp. (e.g., leeks and onions), Cruciferae sp. (e.g., white cabbage, red cabbage, broccoli, cauliflower, Brussels sprouts, pak choi, kohlrabi, radishes, horseradish, cress and chinese cabbage), Leguminosae sp. (e.g., peanuts, peas, lentils and beans – e.g., common beans and broad beans), Chenopodiaceae sp. (e.g., Swiss chard, fodder beet, spinach, beetroot), Linaceae sp. (e.g., hemp), Cannabeacea sp. (e.g., cannabis), Malvaceae sp. (e.g., okra, cocoa), Papaveraceae (e.g., poppy), Asparagaceae (e.g., asparagus); useful plants and ornamental plants in the garden and woods including turf, lawn, grass and *Stevia rebaudiana*; and in each case genetically modified types of these plants.

5

10

15

20

25

30

35

[000302] Depending on the plant species or plant cultivars, their location and growth conditions (soils, climate, vegetation period, diet), using or employing the composition according to the present invention the treatment according to the invention may also result in super-additive ("synergistic") effects. Thus, for example, by using or employing inventive composition in the treatment according to the invention, reduced application rates and/or a widening of the activity spectrum and/or an increase in the activity better plant growth, increased tolerance to high or low temperatures, increased tolerance to drought or to water or soil salt content, increased flowering performance, easier harvesting, accelerated maturation, higher harvest yields, bigger fruits, larger plant height, greener leaf color, earlier flowering, higher quality and/or a higher nutritional value of the harvested products, higher sugar concentration within the fruits, better storage stability and/or processability of the harvested products are possible, which exceed the effects which were actually to be expected.

[000303] At certain application rates of the inventive composition in the treatment according to the invention may also have a strengthening effect in plants. The defense system of the plant against attack by unwanted phytopathogenic fungi and/or microorganisms and/or viruses is mobilized. Plant-strengthening (resistance-inducing) substances are to be understood as meaning, in the present context, those substances or combinations of substances which are capable of stimulating the defense system of plants in such a way that, when subsequently inoculated with unwanted phytopathogenic fungi and/or microorganisms and/or viruses, the

treated plants display a substantial degree of resistance to these phytopathogenic fungi and/or microorganisms and/or viruses, Thus, by using or employing composition according to the present invention in the treatment according to the invention, plants can be protected against attack by the abovementioned pathogens within a certain period of time after the treatment. The period of time within which protection is effected generally extends from 1 to 10 days, preferably 1 to 7 days, after the treatment of the plants with the active compounds.

5

10

15

20

25

30

[000304] Plants and plant cultivars which are also preferably to be treated according to the invention are resistant against one or more biotic stresses, i.e., said plants show a better defense against animal and microbial pests, such as against nematodes, insects, mites, phytopathogenic fungi, bacteria, viruses and/or viroids.

[000305] Plants and plant cultivars which may also be treated according to the invention are those plants which are resistant to one or more abiotic stresses, i.e., that already exhibit an increased plant health with respect to stress tolerance. Abiotic stress conditions may include, for example, drought, cold temperature exposure, heat exposure, osmotic stress, flooding, increased soil salinity, increased mineral exposure, ozone exposure, high light exposure, limited availability of nitrogen nutrients, limited availability of phosphorus nutrients, shade avoidance. Preferably, the treatment of these plants and cultivars with the composition of the present invention additionally increases the overall plant health (cf. above).

[000306] Plants and plant cultivars which may also be treated according to the invention, are those plants characterized by enhanced yield characteristics, i.e., that already exhibit an increased plant health with respect to this feature. Increased yield in said plants can be the result of, for example, improved plant physiology, growth and development, such as water use efficiency, water retention efficiency, improved nitrogen use, enhanced carbon assimilation, improved photosynthesis, increased germination efficiency and accelerated maturation.

[000307] Yield can furthermore be affected by improved plant architecture (under stress and non-stress conditions), including but not limited to, early flowering, flowering control for hybrid seed production, seedling vigor, plant size, internode number and distance, root growth, seed size, fruit size, pod size, pod or ear number, seed number per pod or ear, seed mass, enhanced seed filling, reduced seed dispersal, reduced pod dehiscence and lodging resistance. Further yield traits include seed composition, such as carbohydrate content, protein content, oil content and composition, nutritional value, reduction in anti-nutritional compounds, improved processability and better storage stability. Preferably, the treatment of these plants and cultivars with the composition of the present invention additionally increases the overall plant health (cf. above).

[000308] Plants that may be treated according to the invention are hybrid plants that already express the characteristic of heterosis or hybrid vigor which results in generally higher yield, vigor, health and resistance towards biotic and abiotic stress factors. Such plants are typically made by crossing an inbred male-sterile parent line (the female parent) with another inbred male-fertile parent line (the male parent). Hybrid seed is typically harvested from the male sterile plants and sold to growers. Male sterile plants can sometimes (e.g., in corn) be produced by detasseling, i.e., the mechanical removal of the male reproductive organs (or males flowers) but, more typically, male sterility is the result of genetic determinants in the plant genome. In that case, and especially when seed is the desired product to be harvested from the hybrid plants it is typically useful to ensure that male fertility in the hybrid plants is fully restored. This can be accomplished by ensuring that the male parents have appropriate fertility restorer genes which are capable of restoring the male fertility in hybrid plants that contain the genetic determinants responsible for male-sterility. Genetic determinants for male sterility may be located in the cytoplasm. Examples of cytoplasmic male sterility (CMS) were for instance described in Brassica species. However, genetic determinants for male sterility can also be located in the nuclear genome. Male sterile plants can also be obtained by plant biotechnology methods such as genetic engineering. A particularly useful means of obtaining male-sterile plants is described in WO 89/10396 in which, for example, a ribonuclease such as barnase is selectively expressed in the tapetum cells in the stamens. Fertility can then be restored by expression in the tapetum cells of a ribonuclease inhibitor such as barstar.

[000309] Plants or plant cultivars (obtained by plant biotechnology methods such as genetic engineering) which may be treated according to the invention are herbicide-tolerant plants, i.e., plants made tolerant to one or more given herbicides. Such plants can be obtained either by genetic transformation, or by selection of plants containing a mutation imparting such herbicide tolerance.

# **EXAMPLES**

# **Example 1: Formula for the Efficacy of the Combination of Multiple Active Ingredients**

**[000310]** A synergistic effect of active ingredients is present when the activity of the active ingredient combinations exceeds the total of the activities of the active ingredients when applied individually. The expected activity for a given combination of two active ingredients can be calculated as follows (cf. Colby, S.R., "Calculating Synergistic and Antagonistic Responses of Herbicide Combinations", *Weeds* **1967**, *15*, 20-22):

35

5

10

15

20

25

30

# [000311] If

X is the efficacy when active ingredient A is applied at an application rate of m ppm (or g/ha),

Y is the efficacy when active ingredient B is applied at an application rate of n ppm (or g/ha),

E is the efficacy when the active ingredients A and B are applied at application rates of m and n ppm (or g/ha), respectively, and

then

5

10

15

20

25

$$E = X + Y - \frac{X \cdot Y}{100}$$

[000312] If the actual activity exceeds the calculated value, then the activity of the combination is superadditive, i.e., a synergistic effect exists. In this case, the efficacy which was actually observed must be greater than the value for the expected efficacy (E) calculated from the above-mentioned formula.

[000313] For instance, the formula and analysis can be applied to an evaluation of plant growth promotion. Such an assay is evaluated several days after the applications to plants. 100% means plant weight which corresponds to that of the untreated control plant. Efficacy means in this case the additional % of plant weight in comparison to that of the untreated control. For example, a treatment that resulted in plant weights that were 120% compared to the untreated control plant would have an efficacy of 20%. If the plant growth promotion effect for the combination (i.e., the observed efficacy for % plant weights of plants treated with the combination) exceeds the calculated value, then the activity of the combination is superadditive, i.e., a synergistic effect exists.

[000314] The formula and analysis can also be used to evaluate synergy in disease control assays. The degree of efficacy expressed in % is denoted. 0% means an efficacy which corresponds to that of the control while an efficacy of 100% means that no disease is observed.

[000315] If the actual insecticidal or fungicidal activity exceeds the calculated value, then the activity of the combination is superadditive, i.e., a synergistic effect exists. In this case, the efficacy which is actually observed must be greater than the value for the expected efficacy (E) calculated from the above-mentioned formula.

[000316] A further way of demonstrating a synergistic effect is the method of Tammes (cf. "Isoboles, a graphic representation of synergism in pesticides" in *Neth. J. Plant Path.*, 1964, 70, 73-80).

# Example 2: Plant Growth Promotion with Trifloxystrobin and Recombinant *Bacillus* thuringiensis Cells

[000317] Experiments were conducted to analyze efficacy of a combination of trifloxystrobin and a fermentation product of recombinant *Bacillus thuringiensis* cells expressing endoglucanase ("BEE"). Maize seeds were grown in sterile mixture of synthetic media and sand in small three-inch square pots on light racks in a plant growth room at 25-28 °C and 50% humidity for about 14 days. Two seeds were planted in each pot. At planting, the growing media in each pot was drenched with the treatments described below. After 14 days, plants were measured for whole plant biomass. In the table below, UTC refers to untreated control. "Calculated" refers to the expected effect calculated using the above-described Colby equation and "Efficacy" refers to the actual effect observed.

5

10

15

20

25

30

35

[000318] The TRILEX® product, which contains trifloxystrobin as its active ingredient (22% trifloxystrobin), was diluted in 50 mL water and the diluted solution was used to drench the growing media. The application rate shown below refers to the amount of active ingredient (i.e., trifloxystrobin) applied to the growing media.

[000319] A recombinant *Bacillus cereus* family member (*Bacillus thuringiensis* BT013A) expressing endoglucanase on its exosporium (BEE) was generated as follows. To generate plasmids for expression of fusion proteins in *Bacillus cereus* family members, PCR fragments were generated that encoded the BclA promoter (SEQ ID NO: 85), a methionine start codon, and amino acids 20–35 of BclA (SEQ ID NO:1) followed by a six alanine linker sequence fused in frame to *Bacillus thuringiensis* BT013A endoglucanase (SEQ ID NO: 107). These PCR fragments were digested with XhoI and ligated into the SalI site of the pSUPER plasmid to generate the plasmids pSUPER-BclA 20–35-Endoglucanase. The pSUPER plasmid was generated through fusion of the pUC57 plasmid (containing an ampicillin resistance cassette) with the pBC16-1 plasmid from *Bacillus* (containing a tetracycline resistance). This 5.5 kbp plasmid can replicate in both *E. coli* and *Bacillus spp*. The pSUPER-BclA 20–35-Phospholipase plasmids were transformed into and propagated in dam methylase negative *E. coli* strains and finally were transformed into *Bacillus thuringiensis* BT013A.

[000320] To obtain whole broth cultures of BEE, 15 mL conicals containing brain heart infusion media (BHI) were inoculated with BEE and grown for 7-8 hours at around 30 °C at a shaker setting of 300 rpm. The next day, 250 µl aliquots from each flask were inoculated into 250 mL flasks containing 50 mL of a yeast extract-based media and grown at about 30 °C. After approximately 2 days of incubation, when sporulation was at least 95% completed, the culture broth was harvested and colony forming units calculated. The fermentation broth was diluted to 5% in 50 mL water and the following colony forming units applied to each pot.

[000321] Results of the experiment are shown in Table 3, below.

Table 3

5

10

15

Treatment	Application Rate	Whole Plant Biomass (g)	Found %	Efficacy %	Calculated %	
UTC		2.67	100			
	0.024					
Trifloxystrobin	mg/pot	2.69	101	1		
	$7.85 \times 10^8$					
BEE 5%	CFU/pot	2.89	108	8		
	0.024					
	mg/pot +					
Trifloxystrobin	$7.85 \times 10^{8}$					
+ BEE 5%	CFU/pot	3.06	115	15	8.92	

[000321] Results indicate a superadditive plant yield effect when combining trifloxystrobin and BEE.

# Example 3: Plant Growth Promotion with Isotianil and Recombinant *Bacillus thuringiensis* Cells

[000322] Maize seeds will be grown in loamy sand in the greenhouse at 20 °C and 70% humidity for about 11 days. After about 11 days from the time of treatment the seedlings will be cut off above the soil and the fresh weight will be determined.

[000322] Recombinant *Bacillus thuringiensis* cells expressing an endoglucanase encoded by SEQ ID NO: 107 or a phospholipase C encoded by SEQ ID NO: 108 and prepared as described above will be applied at about  $50 \mu g/kernel$ . Isotianil will also be applied at about  $250 \mu g/kernel$ .

[000323] It is expected that the maize plants treated with the recombinant *Bacillus* thuringiensis in combination with the isotianil will have % shoot weights that exceed the calculated value based on the % shoot weights from the maize plants treated with the two active ingredients alone, i.e., a synergistic effect will be observed.

# **CLAIMS**

What is claimed is:

5

10

15

20

25

- 1. A composition comprising:
  - a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising:
  - (i) at least one protein or peptide selected from the group consisting of a plant growth stimulating protein or peptide and a protein or peptide that protects a plant from a pathogen; and
    - (ii) a targeting sequence, exosporium protein, or exosporium protein fragment; and
    - b) at least one fungicide selected from the group consisting bitertanol, bixafen, bromuconazole, carbendazim, carpropamid, dichlofluanid, fenamidone, fenhexamid, fentin acetate, fentin hydroxide, fluopicolide, fluoxastrobin, fluquinconazole, fosetyl, iprodione, iprovalicarb, isotianil, metominostrobin, ofurace. pencycuron, penflufen, prochloraz, propamocarb, propineb, prothioconazole, pyrimethanil, spiroxamine, tolylfluanid, triadimefon, triadimenol, triazoxide, trifloxystrobin, N-[5-chloro-2-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-

carboxamide, 2,6-dimethyl-1H,5H-[1,4]dithiino[2,3-c:5,6-c']dipyrrole-1,3,5,7(2H,6H)-tetrone, and N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide; in a synergistically effective amount.

- 2. The composition of Claim 1, wherein the at least one protein or peptide is a plant growth stimulating protein or peptide selected from the group consisting of an enzyme involved in the production or activation of a plant growth stimulating compound and an enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source.
  - 3. The composition of Claim 1, wherein the exosporium-producing *Bacillus* cells are cells of a *Bacillus cereus* family member.
- 4. The composition according of Claim 3, wherein the *Bacillus cereus* family member is selected from the group consisting of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus samanii*, *Bacillus gaemokensis*, *Bacillus weihenstephensis*, *Bacillus toyoiensis* and combinations thereof.
- 5. The composition according to any one of Claims 1 to 4, wherein the targeting sequence or exosporium protein comprises:

72

an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%;

a targeting sequence comprising amino acids 1–35 of SEQ ID NO: 1; a targeting sequence comprising amino acids 20–35 of SEQ ID NO: 1; a targeting sequence comprising amino acids 22–31 of SEQ ID NO: 1; a targeting sequence comprising amino acids 22–33 of SEQ ID NO: 1; a targeting sequence comprising amino acids 20–31 of SEQ ID NO: 1; a targeting sequence comprising SEQ ID NO: 1; or

5

10

15

20

25

35

an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2.

- 6. The composition according to any one of Claims 2 to 5, wherein the enzyme involved in the production or activation of a plant growth stimulating compound is selected from the group consisting of an acetoin reductase, an indole-3-acetamide hydrolase, a tryptophan monooxygenase, an acetolactate synthetase, an α-acetolactate decarboxylase, a pyruvate decarboxylase, a diacetyl reductase, a butanediol dehydrogenase, aminotransferase, a tryptophan decarboxylase, an amine oxidase, an indole-3-pyruvate decarboxylase, an indole-3-acetaldehyde dehydrogenase, a tryptophan side chain oxidase, a nitrile hydrolase, a nitrilase, a peptidase, a protease, an adenosine phosphate isopentenyltransferase, phosphatase, adenosine kinase, adenine a an an phosphoribosyltransferase, CYP735A, a 5'ribonucleotide phosphohydrolase, an adenosine nucleosidase, a zeatin cis-trans isomerase, a zeatin O-glucosyltransferase, a β-glucosidase, a cis- hydroxylase, a CK cis-hydroxylase, a CK N-glucosyltransferase, a 2,5-ribonucleotide phosphohydrolase, an adenosine nucleosidase, a purine nucleoside phosphorylase, a zeatin reductase, a hydroxylamine reductase, a 2-oxoglutarate dioxygenase, a gibberellic 2B/3B hydrolase, a gibberellin 3-oxidase, a gibberellin 20-oxidase, a chitosanase, a chitinase, a β-1,3- glucanase, a β-1,4-glucanase, a β-1,6-glucanase, an aminocyclopropane-1-carboxylic acid deaminase, and an enzyme involved in producing a nod factor.
- 7. The composition of Claim 6, wherein the enzyme involved in the production or activation of a plant growth stimulating compound is a chitosanase.
- 30 8. The composition of Claim 7, wherein the fusion protein comprises SEQ ID NO: 109.
  - 9. The composition according to any one of Claims 2 to 5, wherein the enzyme that degrades or modifies a bacterial, fungal, or plant nutrient source is selected from the group consisting of a cellulase, a lipase, a lignin oxidase, a protease, a glycoside hydrolase, a phosphatase, a nitrogenase, a nuclease, an amidase, a nitrate reductase, a nitrite reductase, an

amylase, an ammonia oxidase, a ligninase, a glucosidase, a phospholipase, a phytase, a pectinase, a glucanase, a sulfatase, a urease, a xylanase, and a siderophore.

- 10. The composition of Claim 9, wherein the enzyme is a cellulase selected from the group consisting of an endocellulase, an exocellulase, and a  $\beta$ -glucosidase.
- 5 11. The composition of Claim 10, wherein the fusion protein comprises a *Bacillus* subtilis endoglucanase.
  - 12. The composition of Claim 11, wherein the fusion protein comprises SEQ ID NO: 107.
- 13. The composition of Claim 12, wherein the recombinant *Bacillus* cells are derived from *Bacillus thuringiensis* BT013A.
  - 14. The composition of Claim 9, wherein the enzyme is a phospholipase.
  - 15. The composition of Claim 14, wherein the fusion protein comprises SEQ ID NO: 108.
  - 16. The composition according to any one of Claims 1 to 15, wherein the fusion protein is expressed under the control of a sporulation promoter native to the targeting sequence, exosporium protein, or exosporium protein fragment of the fusion protein.

15

20

25

30

- 17. The composition according to any one of Claims 1 to 16, wherein the fusion protein is expressed under the control of a high-expression sporulation promoter.
- 18. The composition of Claim 17, wherein the high-expression sporulation promoter comprises a sigma-K sporulation-specific polymerase promoter sequence.
  - 19. The composition according to any one of Claims 16 to 18, wherein the sporulation promoter comprises a nucleic acid sequence having at least 80% identity with a nucleic acid sequence of any one of SEQ ID NOS: 85–103.
- 20. The composition according to any one of Claims 1 to 19, wherein the at least one fungicide is selected from the group consisting of carbendazim, fluquinconazole, isotianil, pencycuron, penflufen, prothioconazole, and trifloxystrobin.
  - 21. The composition according to Claim 20, wherein the fungicide is trifloxystrobin.
  - 22. The composition according to any one of Claims 1 to 19, wherein the at least one fungicide is isotianil.
    - 23. A seed treated with the composition according to any one of Claims 1 to 22.
  - 24. A use of the composition according to any one of Claims 1 to 23 for enhancing plant growth and/or promoting plant health.
  - 25. The use according to Claim 24 for treating conventional or transgenic plants or seed thereof.

- 26. A method of treating a plant, a plant part, or the locus surrounding the plant to enhance plant growth and/or promote plant health comprising the step of simultaneously or sequentially applying:
  - a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising:
    - (i) at least one plant growth stimulating protein or peptide; and

5

10

15

25

- (ii) a targeting sequence, exosporium protein, or exosporium protein fragment; and
- b) at least one fungicide selected from the group consisting bitertanol, bixafen, bromuconazole, carbendazim, carpropamid, dichlofluanid, fenamidone, fenhexamid, fentin acetate, fentin hydroxide, fluopicolide, fluoxastrobin, fluquinconazole, fosetyl, iprodione, iprovalicarb, isotianil, metominostrobin, ofurace, pencycuron, penflufen, prochloraz, propamocarb, propineb, prothioconazole, pyrimethanil, spiroxamine, tolylfluanid, triadimefon, triadimenol, triazoxide, trifloxystrobin, N-[5-chloro-2-(trifluoromethyl)benzyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide, 2,6-dimethyl-1H,5H-[1,4]dithiino[2,3-c:5,6-c']dipyrrole-1,3,5,7(2H,6H)-tetrone, and N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide.
- 27. The method according to Claim 26, wherein the targeting sequence or exosporium protein comprises:
  - an amino acid sequence having at least about 43% identity with amino acids 20–35 of SEQ ID NO: 1, wherein the identity with amino acids 25–35 is at least about 54%;
    - a targeting sequence comprising amino acids 1–35 of SEQ ID NO: 1;
    - a targeting sequence comprising amino acids 20-35 of SEQ ID NO: 1;
    - a targeting sequence comprising amino acids 22–31 of SEQ ID NO: 1;
    - a targeting sequence comprising amino acids 22-33 of SEQ ID NO: 1;
    - a targeting sequence comprising amino acids 20–31 of SEQ ID NO: 1;
    - a targeting sequence comprising SEQ ID NO: 1; or
- an exosporium protein comprising an amino acid sequence having at least 85% identity with SEQ ID NO: 2.

# ATTACHMENT A Attorney Docket No. BCS149058 WO

# **ABSTRACT**

[000324] The present invention relates to a composition comprising a) recombinant exosporium-producing *Bacillus* cells that express a fusion protein comprising: (i) at least one plant growth stimulating protein or peptide and (ii) a targeting sequence that localizes the fusion protein to the exosporium of the *Bacillus* cells; and b) at least one particular fungicide disclosed herein in a synergistically effective amount. Furthermore, the present invention relates to the use of this composition as well as a method for enhancing plant growth, promoting plant health, and/or reducing overall damage of plants and plant parts.

5

PATENT REEL: 046350 FRAME: 0763

# Attorney Docket No. BCS149058 WO

**FIG. 1** 

	SEQ	20-35	25-35
		%Identity	%Identity
	NO.		
MSNNNYSNGLNPDESLSASAFDPN <u>LVGPTLPPIPP</u> FTLPTG	1	100%	100%
MSEKYIILHGTALEPN <u>LIGPTLPPIPP</u> FTFPNG	3	81.3%	%6.06
MVKVVEGNGGKSKIKSPLNSNFKILSDLVGPTFPPVPTGMTGIT	5	50.0%	72.7%
MKQNDKLWLDKGIIGPENIGPTFPVLPPIHIPTG	7	43.8%	54.5%
MDEFLSSAALNPGSVGPTLPPMQPFQFRTG	6	62.5%	72.7%
MFDKNEIQKINGILQANALNPNLIGPTLPPIPPFTLPTG	11	81.3%	%6.06
MFDKNEMKKTNEVLQANALDPNIIGPTLPPIPPFTLPTG	13	81.3%	81.8%
MSRKDKFNRSRMSRKDRFNSPKIKSEISISPDLVGPTFPPIPSFTLPTG	15	62.5%	81.8%
MNEEYSILHGPALEPNLIGPTLPSIPPFFTFPTG	17	75.0%	81.8%
MKNRDNNRKQNSLSSNF <b>RIPPE<u>LIGPTFPPVPT</u></b> GFTGIG	19	50.0%	63.6%
MSDKHQMKKISEVLQAHALDPNLIGPPLPPITPFTG	21	75.0%	72.7%
MDEFLSFAALNPGSIGPTLPPVPPFQFPTG	23	62.5%	72.7%
MDEFLSSTALNPC <u>SIGPTLPPMQP</u> FQFPTG	25	56.2%	63.6%
MKERDRQNSLNSNF <b>RISPN<u>LIGPTFPPVPT</u></b> GFTGIG	27	56.2%	63.6%
VFDKNEIQKINGILQAN <b>ALNPN<u>LIGPTLPPIPP</u></b> FTLPTG	29	81.3%	%6.06
MDEFLYFA <b>ALNPG<u>SIGPTLPPVQP</u>FQFPT</b> G	31	56.2%	63.6%
MDSK <u>NIGPTFPPLPS</u> INFPTG	33	43.8%	54.5%
MIGPE <u>NIGPTFPILPP</u> IYIPTG	35	43.8%	54.5%
MSNNNIPSPFFFNNFNPE <u>LIGPTFPPIPP</u> LTLPTG	43	68.8%	81.8%
MFSEKKRKDLIPDNFLSAP <b>ALDPN<u>LIGPTFPPIPS</u>FTLPT</b> G	45	75.0%	72.7%
MTRKDKFNRSRISRRDRFNSPKIKSEIL <b>ISPD<u>LVGPTFPPIPS</u>FTLPT</b> G	47	62.5%	81.8%
MSRKDRFNSPKIKSEISISPD <u>LVGPTFPPIPS</u> FTLPTG	49	62.5%	81.8%
MKERDNKGKQHSLNSNF <b>RIPPE<u>LIGPTFPPVPT</u></b> GFTGIG	51	50.0%	63.6%
MRERDNKRQQHSLNPNF <b>RISPE<u>LIGPTFPPVPT</u></b> GFTGIG	53	50.0%	63.6%
MKNRDNKGKQQSNF <b>RIPPE<u>LIGPTFPPVPT</u></b> GFTGIG	55	50.0%	63.6%
MKFSKKSTVDSSIVGKRVVSKVNILRFYDARSCQDKDVDGFVDVGELFTIFRKLNMEGSVQFKAHNSI GRTVVTTINEVVVEVTVI I OVSTI IGGSVVEDKNEIOKINGII OANAL NPNI IGPTI PPIPPETI PTG	57	81.3%	%6.06
OINT THINE THE TAKE THE COST TENDER CONTRACTOR OF THE TENDER OF THE TEND			

# BCS149058WO\_ST25.txt SEQUENCE LISTING

<110> Bayer CropScience LP <120> COMPOSITIONS COMPRISING RECOMBINANT BACILLUS CELLS AND A **FUNGICIDE** <130> BCS149058 WO <150> US 62/051,915 2014-09-17 <151> <160> 109 <170> PatentIn version 3.5 <210> <211> 41 <212> PRT <213> Bacillus anthracis <400> 1 Met Ser Asn Asn Asn Tyr Ser Asn Gly Leu Asn Pro Asp Glu Ser Leu Ser Ala Ser Ala Phe Asp Pro Asn Leu Val Gly Pro Thr Leu Pro Pro 20 25 30 Ile Pro Pro Phe Thr Leu Pro Thr Gly <210> 332 <211> <212> PRT <213> Bacillus anthracis <400> 2 Met Ser Asn Asn Asn Tyr Ser Asn Gly Leu Asn Pro Asp Glu Ser Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Ser Ala Ser Ala Phe Asp Pro Asn Leu Val Gly Pro Thr Leu Pro Pro Ile Pro Pro Phe Thr Leu Pro Thr Gly Pro Thr Gly Pro Phe Thr Thr 35 Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly 50 55 60 Pro Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Thr Thr Gly Pro 65 70 75 80

Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr

Page 1

BCS149058WO\_ST25.txt 85 90 95

Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro
100 105 110 Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Thr Thr Gly Pro Thr 115 120 125 Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly 130 135 140 Thr Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro 145  $\,$  150  $\,$  155  $\,$  160 Thr Gly Pro Thr Gly Pro Thr Phe Thr Gly Pro Thr Gly 165 170 175 Pro Thr Gly Ala Thr Gly Leu Thr Gly Pro Thr Gly Pro 180 185 190 Ser Gly Leu Gly Leu Pro Ala Gly Leu Tyr Ala Phe Asn Ser Gly Gly 195 200 205 Ile Ser Leu Asp Leu Gly Ile Asn Asp Pro Val Pro Phe Asn Thr Val 210 220 Gly Ser Gln Phe Phe Thr Gly Thr Ala Ile Ser Gln Leu Asp Ala Asp 225 230 235 240 Thr Phe Val Ile Ser Glu Thr Gly Phe Tyr Lys Ile Thr Val Ile Ala 245 250 255 Asn Thr Ala Thr Ala Ser Val Leu Gly Gly Leu Thr Ile Gln Val Asn 260 265 270 Gly Val Pro Val Pro Gly Thr Gly Ser Ser Leu Ile Ser Leu Gly Ala 275 280 285 Pro Phe Thr Ile Val Ile Gln Ala Ile Thr Gln Ile Thr Thr Pro Ser Leu Val Glu Val Ile Val Thr Gly Leu Gly Leu Ser Leu Ala Leu 305 310 315 320 Gly Thr Ser Ala Ser Ile Ile Ile Glu Lys Val Ala 325 330

Page 2

BCS149058WO\_ST25.txt

<210> 3

<211> 33 <212> PRT

<213> Bacillus anthracis

<400> 3

Met Ser Glu Lys Tyr Ile Ile Leu His Gly Thr Ala Leu Glu Pro Asn  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro Pro Phe Thr Phe Pro Asn 20 25 30

Gly

<210> 4

<211> 209

<212> PRT

<213> Bacillus anthracis

<400> 4

Met Ser Glu Lys Tyr Ile Ile Leu His Gly Thr Ala Leu Glu Pro Asn  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro Pro Phe Thr Phe Pro Asn 20 25 30

Gly Pro Thr Gly Ile Thr Gly Pro Thr Gly Ala Thr Gly Phe Thr Gly

Ile Gly Ile Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Ile Gly 50 60

Ile Thr Gly Pro Thr Gly Ala Thr Gly Leu Gly Ile Leu Pro Val Phe 65 70 75 80

Gly Thr Ile Thr Thr Asp Val Gly Ile Gly Phe Ser Val Ile Val Asn 85 90 95

Thr Asn Ile Asn Phe Thr Leu Pro Gly Pro Val Ser Gly Thr Thr Leu 100 105 110

Asn Pro Val Asp Asn Ser Ile Ile Ile Asn Thr Thr Gly Val Tyr Ser 115 120 125

Val Ser Phe Ser Ile Val Phe Val Ile Gln Ala Ile Ser Ser Ile 130 135 140

Leu Asn Leu Thr Ile Asn Asp Ser Ile Gln Phe Ala Ile Glu Ser Arg Page 3

BCS149058WO\_ST25.txt 145 150 160 Ile Gly Gly Pro Gly Val Arg Ala Thr Ser Ala Arg Thr Asp Leu 165 170 175 Leu Ser Leu Asn Gln Gly Asp Val Leu Arg Val Arg Ile Arg Glu Ala 180 185 190 Thr Gly Asp Ile Ile Tyr Ser Asn Ala Ser Leu Val Val Ser Lys Val 195 200 205 Asp <210> <211> 44 <212> PRT Bacillus anthracis <213> 5 <400> Met Val Lys Val Val Glu Gly Asn Gly Gly Lys Ser Lys Ile Lys Ser 1 5 10 15 Pro Leu Asn Ser Asn Phe Lys Ile Leu Ser Asp Leu Val Gly Pro Thr Phe Pro Pro Val Pro Thr Gly Met Thr Gly Ile Thr <210> 6 647 <211> <212> PRT <213> Bacillus anthracis <400> Val Val Lys Val Val Glu Gly Asn Gly Gly Lys Ser Lys Ile Lys Ser 1 10 15 Pro Leu Asn Ser Asn Phe Lys Ile Leu Ser Asp Leu Val Gly Pro Thr 20 25 30 Phe Pro Pro Val Pro Thr Gly Met Thr Gly Ile Thr Gly Ser Thr Gly 35 40 45 Ala Thr Gly Asn Thr Gly Pro Thr Gly Glu Thr Gly Ala Thr Gly Ser 50 60 Ala Gly Ile Thr Gly Ser Thr Gly Pro Thr Gly Asn Thr Gly Gly Thr 65 70 75 80 Page 4

# BCS149058WO\_ST25.txt

Gly Ser Thr Gly Pro Thr Gly Asn Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Val Thr Gly Ser 100 105 110 Thr Gly Val Thr Gly Ser Thr Gly Pro Thr Gly Glu Thr Gly Gly Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Ala Thr Gly Ser Thr Gly 130 140 Val Thr Gly Asn Thr Gly Pro Thr Gly Ser Thr Gly Ala Thr Gly Asn 150 Thr Gly Ser Ile Gly Glu Thr Gly Gly Thr Gly Ser Met Gly Pro Thr 165 170 175Gly Glu Thr Gly Val Thr Gly Ser Thr Gly Gly Thr Gly Ser Thr Gly Val Thr Gly Asn Thr Gly Pro Thr Gly Ser Thr Gly Val Thr Gly Ser 195 200 205 Thr Gly Val Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Val Thr Gly Asn Met Gly Pro Thr Gly Ser Thr Gly Val Thr Gly Asn 245 250 255 Thr Gly Ser Thr Gly Thr Thr Gly Ala Thr Gly Glu Thr Gly Pro Met Gly Ser Thr Gly Ala Thr Gly Thr Thr Gly Pro Thr Gly Glu Thr Gly 275 280 285 Glu Thr Gly Glu Thr Gly Gly Thr Gly Ser Thr Gly Pro Thr Gly Asn 290 295 300 290 Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Val Thr 310 305 320 Gly Ser Thr Gly Val Thr Gly Glu Thr Gly Pro Thr Gly Ser Thr Gly Page 5

BCS149058WO\_ST25.txt 325 330 335

Ala Thr Gly Asn Thr Gly Pro Thr Gly Glu Thr Gly Gly Thr Gly Ser 340 350 Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Asn Thr Gly Pro Thr 355 360 365 Gly Ser Thr Gly Val Thr Gly Asn Thr Gly Ala Thr Gly Glu Thr Gly 370 375 380Pro Thr Gly Asn Thr Gly Ala Thr Gly Asn Thr Gly Pro Thr Gly Glu 385 390 395 400 Thr Gly Val Thr Gly Ser Thr Gly Pro Thr Gly Glu Thr Gly Val Thr 405 410 415 Gly Ser Thr Gly Pro Thr Gly Asn Thr Gly Ala Thr Gly Glu Thr Gly 420 425 430 Ala Thr Gly Ser Thr Gly Val Thr Gly Asn Thr Gly Ser Thr Gly Glu 435 440 445 Thr Gly Pro Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly Ala Thr 450 455 460 Gly Val Thr Gly Asn Thr Gly Pro Thr Gly Ser Thr Gly Ala Thr Gly 465 470 475 480 Ala Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly Thr Thr Gly Asn 485 490 495 Thr Gly Val Thr Gly Asp Thr Gly Pro Thr Gly Ala Thr Gly Val Ser 500 510 Thr Thr Ala Thr Tyr Ala Phe Ala Asn Asn Thr Ser Gly Ser Val Ile 515 520 525 Ser Val Leu Leu Gly Gly Thr Asn Ile Pro Leu Pro Asn Asn Gln Asn 530 540 Ile Gly Pro Gly Ile Thr Val Ser Gly Gly Asn Thr Val Phe Thr Val Ala Asn Ala Gly Asn Tyr Tyr Ile Ala Tyr Thr Ile Asn Leu Thr Ala 565 570 575

Page 6

- BCS149058WO\_ST25.txt
  Gly Leu Leu Val Ser Ser Arg Ile Thr Val Asn Gly Ser Pro Leu Ala
  580 585 590
- Gly Thr Ile Asn Ser Pro Thr Val Ala Thr Gly Ser Phe Ser Ala Thr 595 600 605
- Ile Ile Ala Ser Leu Pro Ala Gly Ala Ala Val Ser Leu Gln Leu Phe 610 620
- Gly Val Val Ala Leu Ala Thr Leu Ser Thr Ala Thr Pro Gly Ala Thr 625 630 635 640
- Leu Thr Ile Ile Arg Leu Ser 645
- <210> 7
- <211> 34
- <212> PRT
- <213> Bacillus anthracis
- <400> 7
- Met Lys Gln Asn Asp Lys Leu Trp Leu Asp Lys Gly Ile Ile Gly Pro  $1 \hspace{1cm} 10 \hspace{1cm} 15$
- Glu Asn Ile Gly Pro Thr Phe Pro Val Leu Pro Pro Ile His Ile Pro 20 25 30
- Thr Gly
- <210> 8
- <211> 366
- <212> PRT <213> Bacillus anthracis
- <400> 8
- Met Lys Gln Asn Asp Lys Leu Trp Leu Asp Lys Gly Ile Ile Gly Pro  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$
- Glu Asn Ile Gly Pro Thr Phe Pro Val Leu Pro Pro Ile His Ile Pro 20 25 30
- Thr Gly Ile Thr Gly Ala Thr Gly Ala Thr Gly Ile Thr Gly Ala Thr 35 40 45
- Gly Pro Thr Gly Thr Thr Gly Ala Thr Gly Ala Thr Gly Ile Thr Gly 50 60
- Val Thr Gly Ala Thr Gly Ile Thr Gly Val Thr Gly Ala Thr Gly Ile Page 7

65					70			BCS1	L4905	58WO_ 75	_ST25	5.tx1	t		80
Thr	Glу	Val	Thr	Gly 85	Ala	Thr	Gly	Ile	Thr 90	Glу	Val	Thr	Glу	Pro 95	Thr
Gly	Ile	Thr	Gly 100	Ala	Thr	Gly	Pro	Thr 105	Glу	Ile	Thr	Gly	Ala 110	Thr	Gly
Pro	Ala	Gly 115	Ile	Thr	Gly	Val	Thr 120	Gly	Pro	Thr	Gly	Ile 125	Thr	Gly	Ala
Thr	Gly 130	Pro	Thr	Gly	Thr	Thr 135	Gly	Val	Thr	Glу	Pro 140	Thr	Gly	Asp	Thr
Gly 145	Leu	Ala	Gly	Ala	Thr 150	Gly	Pro	Thr	Gly	Ala 155	Thr	Glу	Leu	Ala	Gly 160
Ala	Thr	Glу	Pro	Thr 165	Glу	Asp	Thr	Glу	Ala 170	Thr	Glу	Pro	Thr	Gly 175	Ala
Thr	Gly	Leu	Ala 180	Gly	Ala	Thr	Gly	Pro 185	Thr	Gly	Ala	Thr	Gly 190	Leu	Thr
Gly	Ala	Thr 195	Gly	Ala	Thr	Gly	Ala 200	Thr	Gly	Gly	Gly	Ala 205	Ile	Ile	Pro
Phe	Ala 210	Ser	Gly	Thr	Thr	Pro 215	Ala	Leu	Leu	Val	Asn 220	Ala	Val	Leu	Ala
Asn 225	Thr	Glу	Thr	Leu	Leu 230	Glу	Phe	Glу	Phe	Ser 235	Gln	Pro	Glу	Ile	Ala 240
Pro	Gly	Val	Gly	Gly 245	Thr	Leu	Thr	Ile	Leu 250	Pro	Gly	Val	Val	Gly 255	Asp
Tyr	Ala	Phe	Val 260	Ala	Pro	Arg	Asp	Gly 265	Ile	Ile	Thr	Ser	Leu 270	Ala	Gly
Phe	Phe	Ser 275	Ala	Thr	Ala	Ala	Leu 280	Ala	Pro	Leu	Thr	Pro 285	Val	Gln	Ile
Gln	Met 290	Gln	Ile	Phe	Ile	Ala 295	Pro	Ala	Ala	Ser	Asn 300	Thr	Phe	Thr	Pro
Val 305	Ala	Pro	Pro	Leu	Leu 310	Leu	Thr	Pro	Ala	Leu 315	Pro	Ala	Ile	Ala	Ile 320

Page 8

```
BCS149058WO_ST25.txt
Gly Thr Thr Ala Thr Gly Ile Gln Ala Tyr Asn Val Pro Val Val Ala
Gly Asp Lys Ile Leu Val Tyr Val Ser Leu Thr Gly Ala Ser Pro Ile
                                 345
Ala Ala Val Ala Gly Phe Val Ser Ala Gly Leu Asn Ile Val
<210>
<211>
       30
<212>
       PRT
       Bacillus anthracis
<400> 9
Met Asp Glu Phe Leu Ser Ser Ala Ala Leu Asn Pro Gly Ser Val Gly
Pro Thr Leu Pro Pro Met Gln Pro Phe Gln Phe Arg Thr Gly
<210>
<211> 77
<212>
      PRT
       Bacillus anthracis
<400> 10
Met Asp Glu Phe Leu Ser Ser Ala Ala Leu Asn Pro Gly Ser Val Gly
Pro Thr Leu Pro Pro Met Gln Pro Phe Gln Phe Arg Thr Gly Pro Thr
Gly Ser Thr Gly Ala Lys Gly Ala Ile Gly Asn Thr Glu Pro Tyr Trp
His Thr Gly Pro Pro Gly Ile Val Leu Leu Thr Tyr Asp Phe Lys Ser 50 60
Leu Ile Ile Ser Phe Ala Phe Arg Ile Leu Pro Ile Ser
<210>
       11
<211>
       39
<212>
       PRT
<213>
       Bacillus weihenstephensis
<400>
       11
Met Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln Ala 1 5 10 15
                                        Page 9
```

# BCS149058WO\_ST25.txt

Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Pro Phe Thr Leu Pro Thr Gly 35

- <210> 12
- <211> 299
- <211> 299 <212> PRT
- <213> Bacillus weihenstephensis

<400> 12

Met Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln Ala  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Pro Phe Thr Leu Pro Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly 35 40 45

Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro 50 60

Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr 65 70 75 80

Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly 85 90 95

Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro
100 105 110

Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Glu Thr 115 120 125

Gly Pro Thr Gly Gly Thr Glu Gly Cys Leu Cys Asp Cys Cys Val Leu 130 140

Pro Met Gln Ser Val Leu Gln Gln Leu Ile Gly Glu Thr Val Ile Leu 145 150 155 160

Gly Thr Ile Ala Asp Thr Pro Asn Thr Pro Pro Leu Phe Phe Leu Phe 165 170 175

Thr Ile Thr Ser Val Asn Asp Phe Leu Val Thr Val Thr Asp Gly Thr 180 185 190 Page 10

BCS149058WO\_ST25.txt

Thr Thr Phe Val Val Asn Ile Ser Asp Val Thr Gly Val Gly Phe Leu 195 200 205

Pro Pro Gly Pro Pro Ile Thr Leu Leu Pro Pro Thr Asp Val Gly Cys 210 220

Glu Cys Glu Cys Arg Glu Arg Pro Ile Arg Gln Leu Leu Asp Ala Phe 225 230 235 240

Ile Gly Ser Thr Val Ser Leu Leu Ala Ser Asn Gly Ser Ile Ala Ala 245 250 255

Asp Phe Ser Val Glu Gln Thr Gly Leu Gly Ile Val Leu Gly Thr Leu 260 265 270

Pro Ile Asn Pro Thr Thr Thr Val Arg Phe Ala Ile Ser Thr Cys Lys 275 280 285

Ile Thr Ala Val Asn Ile Thr Pro Ile Thr Met 290 295

<210> 13

<211> 39

<212> PRT

<213> Bacillus weihenstephensis

<400> 13

Met Phe Asp Lys Asn Glu Met Lys Lys Thr Asn Glu Val Leu Gln Ala 1 5 10 15

Asn Ala Leu Asp Pro Asn Ile Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Pro Phe Thr Leu Pro Thr Gly

<210> 14

<211> 289

<212> PRT

<213> Bacillus weihenstephensis

<400> 14

Met Phe Asp Lys Asn Glu Met Lys Lys Thr Asn Glu Val Leu Gln Ala  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asn Ala Leu Asp Pro Asn Ile Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Page 11

BCS149058WO\_ST25.txt

Pro Phe Thr Leu Pro Thr Gly Pro Thr Gly Pro Thr Gly 35 40 45

Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro 50 55 60

Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Leu Thr 65 70 75 80

Gly Pro Thr Gly Pro Thr Gly Leu Thr Gly Pro Thr Gly Leu Thr Gly 85 90 95

Pro Thr Gly Pro Thr Gly Leu Thr Gly Gln Thr Gly Ser Thr Gly Pro 100 105 110

Thr Gly Ala Thr Glu Gly Cys Leu Cys Asp Cys Cys Val Phe Pro Met 115 120 125

Gln Glu Val Leu Arg Gln Leu Val Gly Gln Thr Val Ile Leu Ala Thr 130 135 140

Ile Ala Asp Ala Pro Asn Val Ala Pro Arg Phe Phe Leu Phe Asn Ile 145 150 155 160

Thr Ser Val Asn Asp Phe Leu Val Thr Val Thr Asp Pro Val Ser Asn 165 170 175

Thr Thr Phe Val Val Asn Ile Ser Asp Val Ile Gly Val Gly Phe Ser 180 185 190

Leu Thr Val Pro Pro Leu Thr Leu Leu Pro Pro Ala Asp Leu Gly Cys 195 200 205

Glu Cys Asp Cys Arg Glu Arg Pro Ile Arg Glu Leu Leu Asp Thr Leu 210 220

Ile Gly Ser Thr Val Asn Leu Leu Val Ser Asn Gly Ser Ile Ala Thr 225 230 235 240

Gly Phe Asn Val Glu Gln Thr Ala Leu Gly Ile Val Ile Gly Thr Leu 245 250 255

Pro Ile Pro Ile Asn Pro Pro Pro Pro Thr Leu Phe Arg Phe Ala Ile 260 265 270

Ser Thr Cys Lys Ile Thr Ala Val Asp Ile Thr Pro Thr Pro Thr Ala 275 280 285 Page 12

# BCS149058WO\_ST25.txt

Thr

<210> 15

<211> 49

<212> PRT

<213> Bacillus cereus

<400> 15

Met Ser Arg Lys Asp Lys Phe Asn Arg Ser Arg Met Ser Arg Lys Asp  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile Ser Ile Ser Pro Asp  $20 \hspace{1cm} 25 \hspace{1cm} 30$ 

Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr 35 40 45

Gly

<210> 16

<211> 189

<212> PRT <213> Bacillus cereus

<400> 16

Met Ser Arg Lys Asp Lys Phe Asn Arg Ser Arg Met Ser Arg Lys Asp  $1 \hspace{1.5cm} 10 \hspace{1.5cm} 15$ 

Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile Ser Ile Ser Pro Asp  $20 \hspace{1cm} 25 \hspace{1cm} 30$ 

Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr 35 40 45

Gly Ile Thr Gly Pro Thr Phe Asn Ile Asn Phe Arg Ala Glu Lys Asn 50 55 60

Val Ala Gln Ser Phe Thr Pro Pro Ala Asp Ile Gln Val Ser Tyr Gly 65 70 75 80

Asn Ile Ile Phe Asn Asn Gly Gly Gly Tyr Ser Ser Val Thr Asn Thr 85 90 95

Phe Thr Ala Pro Ile Asn Gly Ile Tyr Leu Phe Ser Ala Ser Ile Gly  $100 \hspace{1cm} 105 \hspace{1cm} 110$ 

Page 13

BCS149058WO\_ST25.txt

Phe Asn Pro Thr Leu Gly Thr Thr Ser Thr Leu Arg Ile Thr Ile Arg 115 120 125

Lys Asn Leu Val Ser Val Ala Ser Gln Thr Gly Thr Ile Thr Thr Gly 130 140

Gly Thr Pro Gln Leu Glu Ile Thr Thr Ile Ile Asp Leu Leu Ala Ser 145 150 155 160

Gln Thr Ile Asp Ile Gln Phe Ser Ala Ala Glu Ser Gly Thr Leu Thr  $165 \hspace{1cm} 170 \hspace{1cm} 175$ 

Val Gly Ser Ser Asn Phe Phe Ser Gly Ala Leu Leu Pro 180 185

<210> 17

<211> 33

<212> PRT

<213> Bacillus cereus

<400> 17

Met Asn Glu Glu Tyr Ser Ile Leu His Gly Pro Ala Leu Glu Pro Asn 1 5 10 15

Leu Ile Gly Pro Thr Leu Pro Ser Ile Pro Pro Phe Thr Phe Pro Thr 20 25 30

Gly

<210> 18

<211> 84

<212> PRT

<213> Bacillus cereus

<400> 18

Met Asn Glu Glu Tyr Ser Ile Leu His Gly Pro Ala Leu Glu Pro Asn 1 10 15

Leu Ile Gly Pro Thr Leu Pro Ser Ile Pro Pro Phe Thr Phe Pro Thr 20 25 30

Gly Pro Thr Gly Ile Thr Gly Pro Thr Gly Ala Thr Gly Phe Thr Gly 35 40 45

Ile Gly Ile Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Ile Gly 50 60

Page 14

BCS149058WO\_ST25.txt
Ile Thr Gly Pro Thr Gly Ala Thr Gly Pro Thr Gly Ile Gly Ile Thr
65 70 75 80

Gly Pro Thr Gly

<210> 19

<211> 39 <212> PRT

<213> Bacillus cereus

<400> 19

Met Lys Asn Arg Asp Asn Asn Arg Lys Gln Asn Ser Leu Ser Ser Asn  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Phe Arg Ile Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly 35

<210> 20

<211> 1056

<212> PRT

<213> Bacillus cereus

<400> 20

Met Lys Asn Arg Asp Asn Asn Arg Lys Gln Asn Ser Leu Ser Ser Asn  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Phe Arg Ile Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly Ile Thr Gly Pro Thr Gly Pro Gln Gly 35 40 45

Pro Thr Gly Pro Gln Gly Pro Arg Gly Leu Gln Gly Pro Met Gly Glu 50 60

Met Gly Pro Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Ser Val 65 70 75 80

Gly Pro Ile Gly Ala Thr Gly Pro Glu Gly Gln Gln Gly Pro Gln Gly 85 90 95

Leu Arg Gly Pro Gln Gly Glu Thr Gly Ala Thr Gly Pro Gly Gly Val 100 105 110

Gln Gly Leu Gln Gly Pro Ile Gly Pro Thr Gly Ala Thr Gly Ala Gln Page 15

Gly Ile Gln Gly Ile Gln Gly Leu Gln Gly Pro Ile Gly Ala Thr Gly 130 140 Pro Glu Gly Ser Gln Gly Ile Gln Gly Val Gln Gly Leu Pro Gly Ala 145 150 155 160 Thr Gly Pro Gln Gly Ile Gln Gly Ala Gln Gly Ile Gln Gly Thr Pro 165 170 175 Gly Pro Ser Gly Asn Thr Gly Ala Thr Gly Ala Thr Gly 180 185 190 Gln Gly Ile Thr Gly Pro Thr Gly Ile Thr Gly Pro Thr Gly Ile Thr 195 200 205 Gly Pro Ser Gly Gly Pro Pro Gly Pro Thr Gly Pro Thr Gly Ala Thr 210 215 220 Gly Pro Gly Gly Gly Pro Ser Gly Ser Thr Gly Ala Thr Gly Ala Thr 225 230 235 240 Gly Asn Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Ala Thr Gly 245 250 255 Ser Thr Gly Pro Thr Gly Ser Thr Gly Ala Gln Gly Leu Gln Gly Ile 260 265 270 Gln Gly Ile Gln Gly Pro Ile Gly Pro Thr Gly Pro Glu Gly Ser Gln 275 280 285 Gly Ile Gln Gly Ile Pro Gly Pro Thr Gly Val Thr Gly Glu Gln Gly 290 295 300 Ile Gln Gly Val Gln Gly Ile Gln Gly Ala Thr Gly Ala Thr Gly Asp 315 310 315Gln Gly Pro Gln Gly Ile Gln Gly Val Ile Gly Pro Gln Gly Val Thr 325 330 335 Gly Ala Thr Gly Asp Gln Gly Pro Gln Gly Ile Gln Gly Val Pro Gly 340 345 350 Pro Ser Gly Glu Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro 355 360 365

Page 16

BCS149058WO\_ST25.txt Met Gly Asp Ile Gly Pro Thr Gly Pro Glu Gly Pro Glu Gly Leu Gln 370 380 Gly Pro Gln Gly Ile Gln Gly Val Pro Gly Pro Val Gly Ala Thr Gly 385 390 395 400 Pro Glu Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Pro Val Gly Ala 405 410 415 Thr Gly Pro Gln Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Val Gln Gly Ile Thr Gly Ala Thr Gly Val Gln Gly Ala Thr Gly Ile Gln Gly
435 440 445 Ile Gln Gly Glu Ile Gly Ala Thr Gly Pro Glu Gly Pro Gln Gly Val 450 Gln Gly Ala Gln Gly Ala Ile Gly Pro Thr Gly Pro Met Gly Pro Gln 465 470 475 480 Gly Val Gln Gly Val Gln Gly Ile Gln Gly Ala Thr Gly Ala Gln Gly Val Gln Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Pro Thr Gly Ala
500 510 Thr Gly Asp Met Gly Ala Thr Gly Ala Thr Gly Glu Gly Thr Thr Gly 515 520 525 Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Ser Gly Gly 530 540 Pro Ala Gly Pro Thr Gly Pro Ser Gly Pro Ala Gly Val Thr Gly Pro Ser Gly Gly Pro Pro Gly Pro Thr Gly Ala Thr Gly Ala 565 570 575 Thr Gly Val Thr Gly Asp Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Ala Thr Gly Glu Thr Gly Ala Thr Gly Val Thr Gly Leu Gln Gly Pro Gln Gly Ile Gln Gly Val Gln Gly Glu Ile Gly Pro Thr Gly Pro

Page 17

BCS149058WO\_ST25.txt

Page 18

Gln Gly Val Gln Gly Pro Gln Gly Ile Gln Gly Val Thr Gly Ala Thr 625 630 635 640 630 Gly Asp Gln Gly Pro Gln Gly Ile Gln Gly Pro Gln Gly Asp Ile Gly 645 650 655 Pro Thr Gly Pro Gln Gly Ile Gln Gly Pro Gln Gly Ser Gln Gly Ile 660 665 670 Gln Gly Ala Thr Gly Gly Thr Gly Ala Gln Gly Pro Gln Gly Ile Gln 675 680 685 Gly Pro Gln Gly Asp Ile Gly Leu Thr Gly Ser Gln Gly Pro Thr Gly 690 700 Ile Gln Gly Ile Gln Gly Glu Ile Gly Pro Thr Gly Pro Glu Gly Pro 705 710 715 720 Glu Gly Leu Gln Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Pro Val 725 730 735 Gly Ala Thr Gly Pro Glu Gly Pro Gln Gly Ile Gln Gly 740 745 750 Val Gln Gly Ala Thr Gly Pro Gln Gly Pro Gln Gly Ile Gln Gly Ile 755 760 765 Gln Gly Val Gln Gly Ile Thr Gly Ala Thr Gly Ala Gln Gly Ala Thr 770 775 780 Gly Ile Gln Gly Ile Gln Gly Glu Ile Gly Ala Thr Gly Pro Glu Gly 785 790 795 800 Pro Gln Gly Val Gln Gly Ile Gln Gly Ala Ile Gly Pro Thr Gly Pro 805 810 815 Met Gly Ala Gln Gly Val Gln Gly Ile Gln Gly Ile Gln Gly Ala Thr 820 825 830 Gly Ala Gln Gly Val Gln Gly Pro Gln Gly Ile Gln Gly Val Gln Gly 835 840 845 Pro Thr Gly Ala Thr Gly Glu Thr Gly Ala Thr Gly Ala Thr Gly Glu 850 855 860 Gly Thr Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly 865 870 875

BCS149058WO\_ST25.txt

Pro Ser Gly Gly Pro Ala Gly Pro Thr Gly Pro Thr Gly Pro Ser Gly 885 890 895

Pro Ala Gly Val Thr Gly Pro Ser Gly Gly Pro Pro Gly Pro Thr Gly 900 905 910

Ala Thr Gly Ala Thr Gly Val Thr Gly Asp Thr Gly Ala Thr Gly Ser 915 920 925

Thr Gly Val Thr Gly Ala Thr Gly Ala Thr Gly Val Thr 930 935 940

Gly Leu Gln Gly Pro Gln Gly Ile Gln Gly Val Gln Gly Glu Ile Gly 945 950 955 960

Pro Thr Gly Pro Gln Gly Ile Gln Gly Pro Gln Gly Ile Gln Gly Val 965 970 975

Thr Gly Ala Thr Gly Ala Gln Gly Pro Gln Gly Ile Gln Gly Pro Gln 980 985 990

Gly Asp Ile Gly Pro Thr Gly Ser Gln Gly Ile Gln Gly Pro Gln Gly 995 1000 1005

Pro Gln Gly Ile Gln Gly Ala Thr Gly Ala Thr Gly Ala Gln Gly 1010 1015 1020

Pro Gln Gly Ile Gln Gly Pro Gln Gly Glu Ile Gly Pro Thr Gly 1025 1030 1035

Pro Gln Gly Pro Gln Gly Ile Gln Gly Pro Gln Gly Ile Gln Gly 1040 1045 1050

Pro Thr Gly 1055

<210> 21

<211> 39 <212> PRT

<213> Bacillus weihenstephensis

<400> 21

Met Ser Asp Lys His Gln Met Lys Lys Ile Ser Glu Val Leu Gln Ala 1 5 10 15

His Ala Leu Asp Pro Asn Leu Ile Gly Pro Pro Leu Pro Pro Ile Thr 20 25 30 Page 19

# BCS149058WO\_ST25.txt

Pro Phe Thr Phe Pro Thr Gly 35

<210> 22

<211> 365 <212> PRT

<213> Bacillus weihenstephensis

<400> 22

Met Ser Asp Lys His Gln Met Lys Lys Ile Ser Glu Val Leu Gln Ala  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

His Ala Leu Asp Pro Asn Leu Ile Gly Pro Pro Leu Pro Pro Ile Thr 20 25 30

Pro Phe Thr Phe Pro Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly 35 40 45

Ser Thr Gly Pro Thr Gly Ser Thr Gly Asn Thr Gly Pro Thr Gly Pro 50 60

Thr Gly Pro Pro Val Gly Thr Asn Leu Asp Thr Ile Tyr Val Thr Asn 65 70 75 80

Asp Ile Ser Asn Asn Val Ser Ala Ile Asp Gly Asn Thr Asn Thr Val

Leu Thr Thr Ile Pro Val Gly Thr Asn Pro Val Gly Val Gly Val Asn 100 105 110

Ser Ser Thr Asn Leu Ile Tyr Val Val Asn Asn Gly Ser Asp Asn Ile 115 120

Ser Val Ile Asn Gly Ser Thr Asn Thr Val Val Ala Thr Ile Pro Val 130 135 140

Gly Thr Gln Pro Phe Gly Val Gly Val Asn Pro Ser Thr Asn Leu Ile 145 150 155 160

Tyr Val Ala Asn Arg Thr Ser Asn Asn Val Ser Val Ile Lys Gly Gly 165 170 175

Thr Asn Thr Val Leu Thr Thr Ile Pro Val Gly Thr Asn Pro Val Gly
180 185 190

Val Gly Val Asn Ser Ser Thr Asn Leu Ile Tyr Val Thr Asn Glu Ile 195 200 205 Page 20

BCS149058WO\_ST25.txt

Pro	Asn	Ser	٧a٦	Ser	٧a٦	Ile	Lys	Gly	Gly	Thr	Asn	Thr	٧a٦	٧a٦	Ala
	210					215	-	-	-		220				

Page 21

<sup>&</sup>lt;210> 23

<sup>&</sup>lt;211> 30

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Bacillus weihenstephensis

<sup>&</sup>lt;400> 23

Met Asp Glu Phe Leu Ser Phe Ala Ala Leu Asn Pro Gly Ser Ile Gly  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Pro Thr Leu Pro Pro Val Pro Pro Phe Gln Phe Pro Thr Gly

<sup>&</sup>lt;210> 24

<sup>&</sup>lt;211> 160

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Bacillus weihenstephensis

<sup>&</sup>lt;400> 24

BCS149058WO\_ST25.txt

Met Asp Glu Phe Leu Ser Phe Ala Ala Leu Asn Pro Gly Ser Ile Gly  $1 \hspace{1cm} 10 \hspace{1cm} 15$ 

Pro Thr Leu Pro Pro Val Pro Pro Phe Gln Phe Pro Thr Gly Pro Thr 20 25 30

Gly Ser Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly 40 45

Pro Thr Gly Phe Asn Leu Pro Ala Gly Pro Ala Ser Ile Thr Leu Thr 50 60

Ser Asn Glu Thr Thr Ala Cys Val Ser Thr Gln Gly Asn Asn Thr Leu 70 75 80

Phe Phe Ser Gly Gln Val Leu Val Asn Gly Ser Pro Thr Pro Gly Val 85 90 95

Val Val Ser Phe Ser Phe Ser Asn Pro Ser Leu Ala Phe Met Val Pro 100 105 110

Leu Ala Val Ile Thr Asn Ala Ser Gly Asn Phe Thr Ala Val Phe Leu 115 120 125

Ala Ala Asn Gly Pro Gly Thr Val Thr Val Thr Ala Ser Leu Leu Asp 130 135 140

Ser Pro Gly Thr Met Ala Ser Val Thr Ile Thr Ile Val Asn Cys Pro 145 150 155 160

<210> 25

<211> 30

<212> PRT

<213> Bacillus weihenstephensis

<400> 25

Met Asp Glu Phe Leu Ser Ser Thr Ala Leu Asn Pro Cys Ser Ile Gly
1 10 15

Pro Thr Leu Pro Pro Met Gln Pro Phe Gln Phe Pro Thr Gly 20 25 30

<210> 26

<211> 69

<212> PRT

<213> Bacillus weihenstephensis

<400> 26

- $$\operatorname{BCS149058W0\_ST25.txt}$$  Met Asp Glu Phe Leu Ser Ser Thr Ala Leu Asn Pro Cys Ser Ile Gly 1 5 10 15
- Pro Thr Leu Pro Pro Met Gln Pro Phe Gln Phe Pro Thr Gly Pro Thr  $20 \\ 25 \\ 30$
- Gly Ser Thr Gly Thr Thr Gly Pro Thr Gly Ser Ile Gly Pro Thr Gly 40 45
- Asn Thr Gly Leu Thr Gly Asn Thr Gly Pro Thr Gly Ile Thr Gly Pro 50 60
- Thr Gly Asp Thr Gly
- <210> 27
- <211> 36
- <212> PRT
- <213> Bacillus weihenstephensis
- <400> 27
- Met Lys Glu Arg Asp Arg Gln Asn Ser Leu Asn Ser Asn Phe Arg Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$
- Ser Pro Asn Leu Ile Gly Pro Thr Phe Pro Pro Val Pro Thr Gly Phe 20 25 30
- Thr Gly Ile Gly 35
- <210> 28
- <211> 934
- <212> PRT
- <213> Bacillus weihenstephensis
- <400> 28
- Met Lys Glu Arg Asp Arg Gln Asn Ser Leu Asn Ser Asn Phe Arg Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$
- Ser Pro Asn Leu Ile Gly Pro Thr Phe Pro Pro Val Pro Thr Gly Phe 20 25 30
- Thr Gly Ile Gly Ile Thr Gly Pro Thr Gly Pro Gln Gly Pro Thr Gly 35 40 45
- Pro Gln Gly Pro Arg Gly Phe Gln Gly Pro Met Gly Glu Met Gly Pro 50 55 60
- Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro Ala Gly Gln Met Page 23

BCS149058WO\_ST25.txt 75 70 65 80 Gly Ala Thr Gly Pro Glu Gly Gln Gln Gly Pro Gln Gly Leu Arg Gly 85 90 95 Pro Gln Gly Glu Thr Gly Ala Thr Gly Pro Gln Gly Val Gln Gly Leu 100 105 110 Gln Gly Pro Ile Gly Pro Thr Gly Ala Thr Gly Ala Gln Gly Ile Gln 115 120 125 Gly Ile Gln Gly Leu Gln Gly Pro Ile Gly Ala Thr Gly Pro Glu Gly 130 140 Pro Gln Gly Ile Gln Gly Val Gln Gly Val Pro Gly Ala Thr Gly Ser 145 150 155 160 Gln Gly Ile Gln Gly Ala Gln Gly Ile Gln Gly Pro Gln Gly Pro Ser 165 170 175 Gly Asn Thr Gly Ala Thr Gly Val Thr Gly Gln Gly Ile Ser Gly Pro 180 185 190 Thr Gly Ile Thr Gly Pro Thr Gly Ile Thr Gly Pro Ser Gly Gly Pro 195 200 205 Pro Gly Pro Thr Gly Ala Thr Gly Ala Thr Gly Pro Gly Gly Gly Pro 210 220 Ser Gly Ser Thr Gly Ala Thr Gly Ala Thr Gly Asn Thr Gly Val Thr 225 230 235 240 Gly Ser Ala Gly Val Thr Gly Asn Thr Gly Ser Thr Gly 245 250 255 Glu Thr Gly Ala Gln Gly Leu Gln Gly Ile Gln Gly Val Gln Gly Pro 260 265 270 Ile Gly Pro Thr Gly Pro Glu Gly Pro Gln Gly Ile Gln Gly Ile Pro 275 280 285 Gly Pro Thr Gly Val Thr Gly Glu Gln Gly Ile Gln Gly Val Gln Gly 290 295 300 Ile Gln Gly Ile Thr Gly Ala Thr Gly Asp Gln Gly Pro Gln Gly Ile 305 310 315 320

Gln Gly Ala Ile Gly Pro Gln Gly Ile Thr Gly Ala Thr Gly Asp Gln 325 330 335 Gly Pro Gln Gly Ile Gln Gly Val Pro Gly Pro Thr Gly Asp Thr Gly 340 345 350 Ser Gln Gly Val Gln Gly Ile Gln Gly Pro Met Gly Asp Ile Gly Pro 355 360 365 Thr Gly Pro Glu Gly Pro Glu Gly Leu Gln Gly Pro Gln Gly Ile Gln 370 375 380 Gly Val Pro Gly Pro Ala Gly Ala Thr Gly Pro Glu Gly Pro Gln Gly 385 390 395 400 Ile Gln Gly Ile Gln Gly Pro Ile Gly Val Thr Gly Pro Glu Gly Pro 405 410 415Gln Gly Ile Gln Gly Ile Gln Gly Ile Thr Gly Ala Thr 420 425 430 Gly Ala Gln Gly Ala Thr Gly Val Gln Gly Val Gln Gly Asn Ile Gly 435 440 445 Ala Thr Gly Pro Glu Gly Pro Gln Gly Val Gln Gly Thr Gln Gly Asp 450 455 460 Ile Gly Pro Thr Gly Pro Met Gly Pro Gln Gly Val Gln Gly Ile Gln 465 470 475 480 Gly Ile Gln Gly Pro Thr Gly Ala Gln Gly Val Gln Gly Pro Gln Gly 485 490 495 Ile Gln Gly Ile Gln Gly Pro Thr Gly Val Thr Gly Asp Thr Gly Thr 500 510 Thr Gly Ala Thr Gly Glu Gly Thr Thr Gly Ala Thr Gly Val Thr Gly 515 520 525 Pro Ser Gly Val Thr Gly Pro Ser Gly Gly Pro Ala Gly Pro Thr Gly Pro Thr Gly Pro Ser Gly Pro Thr Gly Leu Thr Gly Pro Ser Gly Gly 545 550 555 560 Pro Pro Gly Pro Thr Gly Ala Thr Gly Val Thr Gly Gly Val Gly Asp 565 570 575

Page 25

BCS149058WO\_ST25.txt

Page 26

Thr Gly Ala Thr Gly Ser Thr Gly Val Thr Gly Ala Thr Gly Val Thr 580 585 590 Gly Ala Thr Gly Ala Thr Gly Leu Gln Gly Pro Gln Gly Ile Gln Gly 595 600 605 Val Gln Gly Asp Ile Gly Pro Thr Gly Pro Gln Gly Val Gln Gly Pro 610 620 Gln Gly Ile Gln Gly Ile Thr Gly Ala Thr Gly Asp Gln Gly Pro Gln 625 635 640 Gly Ile Gln Gly Pro Gln Gly Ile Gln Gly Pro Thr Gly Pro Gln Gly
645 650 655 Ile Gln Gly Gln Gly Pro Gln Gly Ile Gln Gly Ala Thr Gly Ala 660 665 670 Thr Gly Ala Gln Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Val Gln 675 680 685 Gly Pro Thr Gly Pro Gln Gly Pro Thr Gly Ile Gln Gly Val Gln Gly 690 700 Glu Ile Gly Pro Thr Gly Pro Gln Gly Val Gln Gly Leu Gln Gly Pro 705 710 715 720 Gln Gly Pro Thr Gly Asp Thr Gly Pro Thr Gly Pro Gln Gly Pro Gln 725 730 735 Gly Ile Gln Gly Pro Thr Gly Ala Thr Gly Ala Thr Gly Ser Gln Gly 740 745 750 Ile Gln Gly Pro Thr Gly Ala Thr Gly Ala Thr Gly Ser Gln Gly Ile 755 760 765 Gln Gly Pro Thr Gly Ala Thr Gly Ala Thr Gly Ala Thr 770 775 780 Gly Ala Thr Gly Ala Thr Gly Val Thr Gly Val Ser Thr 785 790 795 800 Thr Ala Thr Tyr Ser Phe Ala Asn Asn Thr Ser Gly Ser Ala Ile Ser 805 810 815 Val Leu Leu Gly Gly Thr Asn Ile Pro Leu Pro Asn Asn Gln Asn Ile 820 825 830

BCS149058WO\_ST25.txt

Gly Pro Gly Ile Thr Val Ser Gly Gly Asn Thr Val Phe Thr Val Thr

Asn Ala Gly Asn Tyr Tyr Ile Ala Tyr Thr Ile Asn Ile Thr Ala Ala

Leu Leu Val Ser Ser Arg Ile Thr Val Asn Gly Ser Pro Leu Ala Gly

Thr Ile Asn Ser Pro Ala Val Ala Thr Gly Ser Phe Asn Ala Thr Ile

Ile Ser Asn Leu Ala Ala Gly Ser Ala Ile Ser Leu Gln Leu Phe Gly

Leu Leu Ala Val Ala Thr Leu Ser Thr Thr Pro Gly Ala Thr Leu 915 920 925

Thr Ile Ile Arg Leu Ser 930

<210> 29

<211> 39

<212> PRT <213> Bacillus mycoides

<400>

Val Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln Ala 1 5 10 15

Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Pro Phe Thr Leu Pro Thr Gly

30 <210>

<211> 287

<212> PRT

<213> Bacillus mycoides

<400>

Val Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln Ala 1 5 10 15

Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro 20 25 30

Page 27

BCS149058WO\_ST25.txt

Pro Phe Thr Leu Pro Thr Gly Pro Thr Gly Gly Thr Gly Pro Thr Gly 35 40 45

Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro 50 60

Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr 65 70 75 80

Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly 85 90 95

Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro 100 105 110

Thr Gly Val Thr Gly Pro Thr Gly Gly Thr Glu Gly Cys Leu Cys Asp 115 120 125

Cys Cys Val Leu Pro Met Gln Ser Val Leu Gln Gln Leu Ile Gly Glu 130 135 140

Thr Val Ile Leu Gly Thr Ile Ala Asp Thr Pro Asn Thr Pro Pro Leu 145 150 155 160

Phe Phe Leu Phe Thr Ile Thr Ser Val Asn Asp Phe Leu Val Thr Val 165 170 175

Thr Asp Gly Thr Thr Thr Phe Val Val Asn Ile Ser Asp Val Thr Gly 180 185 190

Val Gly Phe Leu Pro Pro Gly Pro Pro Ile Thr Leu Leu Pro Pro Thr 195 200 205

Asp Val Gly Cys Glu Cys Glu Cys Arg Glu Arg Pro Ile Arg Gln Leu 210 215 220

Leu Asp Ala Phe Ile Gly Ser Thr Val Ser Leu Leu Ala Ser Asn Gly 225 230 235 240

Ser Ile Ala Ala Asp Phe Ser Val Glu Gln Thr Gly Leu Gly Ile Val 245 250 255

Leu Gly Thr Leu Pro Ile Asn Pro Thr Thr Thr Val Arg Phe Ala Ile 260 265 270

Ser Thr Cys Lys Ile Thr Ala Val Asn Ile Thr Pro Ile Thr Met 275 280 285 Page 28

# BCS149058WO\_ST25.txt

<210> 31 <211> 30 <212> PRT <213> Bacillus mycoides <400> 31 Met Asp Glu Phe Leu Tyr Phe Ala Ala Leu Asn Pro Gly Ser Ile Gly  $1 \hspace{1cm} 10 \hspace{1cm} 15$ Pro Thr Leu Pro Pro Val Gln Pro Phe Gln Phe Pro Thr Gly 20 25 30 <210> 32 <211> 190 <212> PRT <213> Bacillus mycoides <400> 32 Met Asp Glu Phe Leu Tyr Phe Ala Ala Leu Asn Pro Gly Ser Ile Gly  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Pro Thr Leu Pro Pro Val Gln Pro Phe Gln Phe Pro Thr Gly Pro Thr Gly Ser Thr Gly Ala Thr Gly Ser Thr Gly Ser Thr Gly Pro Thr Gly Ser Thr Gly Ser Thr Gly Ser Thr Gly Pro 50 55 60 Thr Gly Pro Thr Gly Pro Thr Gly Ser Thr Gly Pro Thr Gly Pro Thr 65 70 75 80 Gly Phe Asn Leu Pro Ala Gly Pro Ala Ser Ile Thr Leu Thr Ser Asn Glu Thr Thr Ala Cys Val Ser Thr Gln Gly Asn Asn Thr Leu Phe Phe Ser Gly Gln Val Leu Val Asn Gly Ser Pro Thr Pro Gly Val Val Val

Ser Phe Ser Phe Ser Asn Pro Ser Leu Ala Phe Met Val Pro Leu Ala

Val Ile Thr Asn Ala Ser Gly Asn Phe Thr Ala Val Phe Leu Ala Ala

Page 29

BCS149058WO\_ST25.txt

Asn Gly Pro Gly Thr Val Thr Val Thr Ala Ser Leu Leu Asp Ser Pro 165 170 175

Gly Thr Met Ala Ser Val Thr Ile Thr Ile Val Asn Cys Pro 180 185 190

<210> 33

<211> 21

<212> PRT

<213> Bacillus mycoides

<400> 33

Met Asp Ser Lys Asn Ile Gly Pro Thr Phe Pro Pro Leu Pro Ser Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asn Phe Pro Thr Gly 20

<210> 34

<211> 335

<212> PRT

<213> Bacillus mycoides

<400> 34

Met Asp Ser Lys Asn Ile Gly Pro Thr Phe Pro Pro Leu Pro Ser Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asn Phe Pro Thr Gly Val Thr Gly Glu Thr Gly Ala Thr Gly Glu Thr 20 25 30

Gly Ala Thr Gly Ala Thr Gly Glu Thr Gly Ala Thr Gly Glu Thr Gly 35 40 45

Glu Thr Gly Ala Thr Gly Ala Thr Gly Ala Thr Gly Glu 50 55 60

Thr Gly Ala Thr Gly Ala Thr Gly Ala Thr Gly Ala Ala Gly Ala Thr 65 70 75 80

Gly Glu Thr Gly Ala Thr Gly Glu Thr Gly Ala Thr Gly Glu Thr Gly 85 90 95

Ala Thr Gly Glu Thr Gly Ala Thr Gly Val Thr Gly Glu Thr Gly Ala 100 105 110

Thr Gly Glu Thr Gly Ala Ala Gly Glu Thr Gly Ile Thr Gly Val Thr 115 120 125

BCS149058WO\_ST25.txt Gly Pro Thr Gly Glu Thr Gly Ala Thr Gly Glu Thr Gly Ala Thr Gly 130 140 Ala Thr Gly Ile Thr Gly Ala Thr Gly Ile Thr Gly Val Ala Gly Ala 145 150 155 160 Thr Gly Glu Thr Gly Ala Ala Gly Glu Thr Gly Pro Thr Gly Ala Thr Gly Ala Ile Gly Ala Ile Gly Ala Thr Gly Ala Thr Gly Ile Thr Gly Val Thr Gly Ala Thr Gly Glu Thr Gly Ala Ala Gly Ala Thr Gly Ile Thr Gly Val Thr Gly Ala Thr Gly Glu Thr Gly Ala Ala Gly Ala Thr Gly Ile Thr Gly Ala Thr Gly Ile Thr Gly Val Ala Gly Ala Thr Gly Ile Thr Gly Pro Thr Gly Ile Pro Gly Thr Ile Pro Thr Thr Asn Leu Leu Tyr Phe Thr Phe Ser Asp Gly Glu Lys Leu Ile Tyr Thr Asn Ala 265 Asp Gly Ile Ala Gln Tyr Gly Thr Thr Gln Ile Leu Ser Pro Ser Glu Val Ser Tyr Ile Asn Leu Phe Ile Asn Gly Ile Leu Gln Pro Gln Pro 295 Phe Tyr Glu Val Thr Ala Gly Gln Leu Thr Leu Leu Asp Asp Glu Pro Pro Ser Gln Gly Ser Ser Ile Ile Leu Gln Phe Ile Ile Asn <210> <211> 22 <212> PRT Bacillus thuringiensis <400> Met Ile Gly Pro Glu Asn Ile Gly Pro Thr Phe Pro Ile Leu Pro Pro 1 5 10 15

BCS149058WO\_ST25.txt

Ile Tyr Ile Pro Thr Gly 20

<210> 36

<211> 234 <212> PRT

<213> Bacillus thuringiensis

<400> 36

Met Ile Gly Pro Glu Asn Ile Gly Pro Thr Phe Pro Ile Leu Pro Pro  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Ile Tyr Ile Pro Thr Gly Glu Thr Gly Pro Thr Gly Ile Thr Gly Ala 20 25 30

Thr Gly Glu Thr Gly Pro Thr Gly Ile Thr Gly Pro Thr Gly Ile Thr 35 40 45

Gly Ala Thr Gly Glu Thr Gly Ser Thr Gly Ile Thr Gly Ala Thr Gly 50 60

Glu Thr Gly Ser Thr Gly Ile Thr Gly Pro Ile Gly Ile Thr Gly Ala 65 70 75 80

Thr Gly Glu Thr Gly Pro Ile Gly Ile Thr Gly Ala Thr Gly Glu Thr 85 90 95

Gly Pro Thr Gly Ile Thr Gly Ser Thr Gly Ile Thr Gly Leu Thr Gly 100 105 110

Val Thr Gly Leu Thr Gly Glu Thr Gly Pro Ile Gly Ile Thr Gly Pro 115 120 125

Thr Gly Ile Thr Gly Pro Thr Gly Val Thr Gly Ala Thr Gly Pro Thr 130 140

Gly Gly Ile Gly Pro Ile Thr Thr Asn Leu Leu Tyr Tyr Thr Phe 145 150 155 160

Ala Asp Gly Glu Lys Leu Ile Tyr Thr Asp Thr Asp Gly Ile Pro Gln 165 170 175

Tyr Gly Thr Thr Asn Ile Leu Ser Pro Ser Glu Val Ser Tyr Ile Asn 180 185 190

Leu Phe Val Asn Gly Ile Leu Gln Pro Gln Pro Leu Tyr Glu Val Ser 195 200 205

BCS149058WO_ST25.txt Thr Gly Lys Leu Thr Leu Leu Asp Thr Gln Pro Pro Ser Gln Gly Ser 210 215 220	
Ser Ile Ile Leu Gln Phe Ile Ile Ile Asn 225 230	
<210> 37 <211> 23 <212> DNA <213> Artificial sequence	
<220> <223> Primer	
<400> 37 ggatccatgg ctgaacacaa tcc	23
<210> 38 <211> 24 <212> DNA <213> Artificial sequence	
<220> <223> Primer	
<400> 38 ggatccttaa ttcgtattct ggcc	24
<210> 39 <211> 21 <212> DNA <213> Artificial sequence	
<220> <223> Primer	
<400> 39 ggatccatga aacggtcaat c	21
<210> 40 <211> 24 <212> DNA <213> Artificial sequence	
<220> <223> Primer	
<400> 40 ggatccttac taatttggtt ctgt	24
<210> 41 <211> 21 <212> DNA <213> Artificial sequence	
<220> <223> Primer	

# BCS149058WO\_ST25.txt

<400> 41 ggatccatgc taccaaaagc c											
<210> 42 <211> 24 <212> DNA <213> Artificial sequence											
<220> <223> Primer											
<400> 42 ggatccttag tccgcaggcg tagc											
<210> 43 <211> 35 <212> PRT <213> Bacillus cereus											
<400> 43											
Met Ser Asn Asn Ile Pro Ser Pro Phe Phe Asn Asn Phe Asn 1 5 10 15											
Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Ile Pro Pro Leu Thr Leu 20 25 30											
Pro Thr Gly 35											
<210> 44 <211> 222 <212> PRT <213> Bacillus cereus											
<400> 44											
Met Ser Asn Asn Ile Pro Ser Pro Phe Phe Asn Asn Phe Asn 1 5 10 15											
Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Ile Pro Pro Leu Thr Leu 20 25 30											
Pro Thr Gly Pro Thr Gly Ser Thr Gly Ala Thr Gly Ala Thr Gly Pro 35 40 45											
Thr Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly Pro Thr Gly Ala Thr 50 60											
Gly Ala Thr Gly Ser Thr Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly 65 70 75 80											

BCS149058WO\_ST25.txt Thr Phe Ser Ser Ala Asn Ala Ser Ile Val Thr Pro Ala Pro Gln Thr Val Asn Asn Leu Ala Pro Ile Gln Phe Thr Ala Pro Val Leu Ile Ser Lys Asn Val Thr Phe Asn Gly Ile Asp Thr Phe Thr Ile Gln Ile Pro Gly Asn Tyr Phe Phe Ile Gly Ala Val Met Thr Ser Asn Asn Gln Ala 130 135 140 Gly Pro Val Ala Val Gly Val Gly Phe Asn Gly Ile Pro Val Pro Ser Leu Asp Gly Ala Asn Tyr Gly Thr Pro Thr Gly Gln Glu Val Val Cys 165 Phe Gly Phe Ser Gly Gln Ile Pro Ala Gly Thr Thr Ile Asn Leu Tyr Asn Ile Ser Asp Lys Thr Ile Ser Ile Gly Gly Ala Thr Ala Ala Gly Ser Ser Ile Val Ala Ala Arg Leu Ser Phe Phe Arg Ile Ser 210 215 220 210 <210> 45 <211> 41 <212> PRT <213> Bacillus cereus <400> 45 Met Phe Ser Glu Lys Lys Arg Lys Asp Leu Ile Pro Asp Asn Phe Leu
1 10 15 Ser Ala Pro Ala Leu Asp Pro Asn Leu Ile Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr Gly <210> 46 <211> 293 <212> PRT <213> Bacillus cereus <400> 46 Met Phe Ser Glu Lys Lys Arg Lys Asp Leu Ile Pro Asp Asn Phe Leu Page 35

BCS149058WO\_ST25.txt 15 1 5 Ser Ala Pro Ala Leu Asp Pro Asn Leu Ile Gly Pro Thr Phe Pro Pro 20 25 30 Ile Pro Ser Phe Thr Leu Pro Thr Gly Ser Thr Gly Pro Thr Gly Pro 35 40 45 Thr Gly Asp Thr Gly Pro Thr Gly Pro Thr Ala Thr Ile Cys Ile Arg 50 60 Thr Asp Pro Asp Asn Gly Cys Ser Val Ala Glu Gly Ser Gly Thr Val 65 70 75 80 Ala Ser Gly Phe Ala Ser His Ala Glu Ala Cys Asn Thr Gln Ala Ile  $85 \hspace{1cm} 90 \hspace{1cm} 95$ Gly Asp Cys Ser His Ala Glu Gly Gln Phe Ala Thr Ala Ser Gly Thr  $100 \hspace{1cm} 105 \hspace{1cm} 110$ Ala Ser His Ala Glu Gly Phe Gln Thr Thr Ala Ser Gly Phe Ala Ser 115 120 125 His Thr Glu Gly Ser Gly Thr Thr Ala Asp Ala Asn Phe Ser His Thr 130 135 140 Glu Gly Ile Asn Thr Ile Val Asp Val Leu His Pro Gly Ser His Ile Met Gly Lys Asn Gly Thr Thr Arg Ser Ser Phe Ser Trp His Leu Ala 165 170 175 Asn Gly Leu Ala Val Gly Pro Ser Leu Asn Ser Ala Val Ile Glu Gly Val Thr Gly Asn Leu Tyr Leu Asp Gly Val Val Ile Ser Pro Asn Ala 195 200 205 Ala Asp Tyr Ala Glu Met Phe Glu Thr Ile Asp Gly Asn Leu Ile Asp 210 220 Val Gly Tyr Phe Val Thr Leu Tyr Gly Glu Lys Ile Arg Lys Ala Asn 235 230 235 Ala Asn Asp Asp Tyr Ile Leu Gly Val Val Ser Ala Thr Pro Ala Met 245 250 255

Page 36

BCS149058WO\_ST25.txt Ile Ala Asp Ala Ser Asp Leu Arg Trp His Asn Leu Phe Val Arg Asp 260 265 270 Glu Trp Gly Arg Thr Gln Tyr His Glu Val Val Pro Glu Lys Lys 275 280 285 Met Ala Met Glu Glu <210> 47 <211> 49 <212> PRT <213> Bacillus cereus <400> 47 Met Thr Arg Lys Asp Lys Phe Asn Arg Ser Arg Ile Ser Arg Arg Asp 10 15Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile Leu Ile Ser Pro Asp  $20 \hspace{1.5cm} 25 \hspace{1.5cm} 30$ Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr 35 40 45Gly <210> 48 <211> 83 <212> PRT <213> Bacillus cereus <400> 48 Met Thr Arg Lys Asp Lys Phe Asn Arg Ser Arg Ile Ser Arg Arg Asp  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile Leu Ile Ser Pro Asp 20 25 30 Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr 35 40 45 Gly Val Thr Gly Pro Thr Gly Asn Thr Gly Pro Thr Gly Ile Thr Gly 50 60 Pro Thr Gly Asp Thr Gly Pro Thr Gly Asp Thr Gly Pro Thr Gly Ile 65 70 75 80 Thr Gly Pro

# BCS149058WO\_ST25.txt

- <210> 49 <211> 38 <212> PRT <213> Bacillus cereus <400> Met Ser Arg Lys Asp Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Ser Ile Ser Pro Asp Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr Gly 35 <210> 50 <211> 163 <212> PRT <213> Bacillus cereus <400> Met Ser Arg Lys Asp Arg Phe Asn Ser Pro Lys Ile Lys Ser Glu Ile  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Ser Ile Ser Pro Asp Leu Val Gly Pro Thr Phe Pro Pro Ile Pro Ser Phe Thr Leu Pro Thr Gly Ile Thr Gly Pro Thr Gly Asn Thr Gly Pro Thr Gly Asp Thr Gly Pro Thr Gly Pro Thr Phe Asn Ile Asn Phe Arg 50 55 60 Ala Glu Lys Asn Gly Ala Gln Ser Phe Thr Pro Pro Ala Asp Ile Gln 65 70 75 80 Val Ser Tyr Gly Asn Ile Ile Phe Asn Asn Gly Gly Gly Tyr Ser Ser 85 90 95
- Val Thr Asn Thr Phe Thr Ala Pro Ile Asn Gly Ile Tyr Leu Phe Ser 100 105 110
- Ala Asn Ile Gly Phe Asn Pro Thr Leu Gly Thr Thr Ser Thr Leu Arg 115 120 125
- Ile Thr Ile Arg Lys Asn Leu Val Ser Val Ala Ser Gln Thr Ile Asp 130 135 140 Page 38

# BCS149058WO\_ST25.txt

Ile Gln Phe Ser Ala Ala Glu Ser Gly Thr Leu Thr Val Gly Ser Ser 145 150 155 160

Asn Phe Phe

- <210> 51
- <211> 39
- <212> PRT
- <213> Bacillus cereus

<400> 51

Met Lys Glu Arg Asp Asn Lys Gly Lys Gln His Ser Leu Asn Ser Asn  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Phe Arg Ile Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly 35

- <210> 52
- <211> 323
- <212> PRT
- <213> Bacillus cereus

<400> 52

Met Lys Glu Arg Asp Asn Lys Gly Lys Gln His Ser Leu Asn Ser Asn  $1 \hspace{1cm} 15$ 

Phe Arg Ile Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly Ile Thr Gly Pro Thr Gly Pro Gln Gly 35 40 45

Pro Thr Gly Pro Gln Gly Pro Arg Gly Phe Gln Gly Pro Met Gly Glu 50 55 60

Met Gly Pro Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro Ala 65 70 75 80

Gly Gln Met Gly Ala Thr Gly Pro Glu Gly Gln Gln Gly Pro Glu Gly 85 90 95

Leu Arg Gly Pro Val Gly Ala Thr Gly Ala Thr Gly Leu Gln Gly Val  $100 \,$   $105 \,$   $110 \,$ 

Page 39

BCS149058WO\_ST25.txt

Gln Gly Ile Gln Gly Pro Ile Gly Ser Thr Gly Ala Thr Gly Ala Gln 115 120 125

Gly Ile Gln Gly Ile Gln Gly Leu Gln Gly Pro Ile Gly Ala Thr Gly 130 140

Pro Glu Gly Pro Gln Gly Ile Gln Gly Val Gln Gly Leu Pro Gly Ala 145 150 155 160

Thr Gly Pro Gln Gly Val Gln Gly Val Ile Gly Pro Gln
165 170 175

Gly Pro Ser Gly Ser Thr Gly Gly Thr Gly Ala Thr Gly Gln Gly Val 180 185 190

Thr Gly Pro Thr Gly Ile Thr Gly Ser Thr Gly Val Thr Gly Pro Ser 195 200 205

Gly Gly Pro Pro Gly Pro Thr Gly Pro Thr Gly Ala Thr Gly Pro Gly 210 215 220

Gly Gly Pro Ser Gly Ser Thr Gly Val Thr Gly Ser Thr Gly Asn Thr 225 230 235 240

Gly Ala Thr Gly Ser Pro Gly Val Thr Gly Ala Thr Gly Pro Thr Gly 245 250 255

Ser Thr Gly Ala Thr Gly Ile Gln Gly Ser Gln Gly Ile Gln Gly Ile 260 270

Gln Gly Ile Gln Gly Pro Leu Gly Pro Thr Gly Pro Glu Gly Pro Gln 275 280 285

Gly Ile Gln Gly Ile Pro Gly Pro Thr Gly Ile Thr Gly Glu Gln Gly 290 295 300

Ile Gln Gly Val Gln Gly Ile Gln Gly Ile Thr Gly Ala Thr Gly Asp 315 310 315

Gln Gly Thr

<210> 53

<211> 39

<212> PRT <213> Bacillus cereus

<400> 53

Page 40

BCS149058WO\_ST25.txt

Met Arg Glu Arg Asp Asn Lys Arg Gln Gln His Ser Leu Asn Pro Asn 1 5 10 15

Phe Arg Ile Ser Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly 35

<210> 54

<211> 436

<212> PRT

<213> Bacillus cereus

<400> 54

Met Arg Glu Arg Asp Asn Lys Arg Gln Gln His Ser Leu Asn Pro Asn 10 15

Phe Arg Ile Ser Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro 20 25 30

Thr Gly Phe Thr Gly Ile Gly Ile Thr Gly Pro Thr Gly Pro Gln Gly 35 40 45

Pro Thr Gly Pro Gln Gly Pro Arg Gly Phe Gln Gly Pro Met Gly Glu 50 55 60

Met Gly Pro Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro Val 65 70 75 80

Gly Pro Ile Gly Ala Thr Gly Pro Glu Gly Gln Gln Gly Pro Gln Gly 85 90 95

Leu Arg Gly Pro Gln Gly Glu Thr Gly Ala Thr Gly Pro Gly Gly Val  $100 \,$   $105 \,$   $110 \,$ 

Gln Gly Leu Gln Gly Pro Ile Gly Pro Thr Gly Ala Thr Gly Ala Gln
115 120 125

Gly Val Gln Gly Ile Gln Gly Leu Gln Gly Pro Ile Gly Ala Thr Gly

Pro Glu Gly Pro Gln Gly Ile Gln Gly Val Gln Gly Leu Pro Gly Ala 145 150 155 160

Thr Gly Ser Gln Gly Ile Gln Gly Val Gln Gly Ile Gln Gly Pro Gln 165 170 175

Page 41

BCS149058WO\_ST25.txt

Gly Pro Ser Gly Asn Thr Gly Ala Thr Gly Ala Thr Gly Gln Gly Ile 180 185 190

Thr Gly Pro Thr Gly Ile Thr Gly Pro Thr Gly Ile Thr Gly Pro Ser 195 200 205

Gly Gly Pro Pro Gly Pro Thr Gly Pro Thr Gly Ala Thr Gly Pro Gly 210 215 220

Gly Gly Pro Ser Gly Ser Thr Gly Ala Thr Gly Ala Thr Gly Asn Thr 225 230 235 240

Gly Ala Thr Gly Asn Thr Gly Ile Thr Gly Ala Thr Gly Ser Thr Gly 245 250 255

Pro Thr Gly Ser Thr Gly Ala Gln Gly Leu Gln Gly Ile Gln Gly Ile 260 265 270

Gln Gly Pro Ile Gly Pro Thr Gly Pro Glu Gly Pro Gln Gly Ile Gln 275 280 285

Gly Ile Pro Gly Pro Thr Gly Val Thr Gly Glu Gln Gly Ile Gln Gly 290 295 300

Val Gln Gly Ile Gln Gly Ile Thr Gly Ala Thr Gly Asp Gln Gly Pro 305 310 315 320

Gln Gly Ile Gln Gly Val Ile Gly Ala Gln Gly Val Thr Gly Ala Thr 325 330 335

Gly Asp Gln Gly Pro Gln Gly Ile Gln Gly Val Pro Gly Pro Ser Gly 340 345 350

Ala Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro Met Gly Asp 365

Ile Gly Pro Thr Gly Pro Glu Gly Pro Glu Gly Leu Gln Gly Pro Gln 370 375 380

Gly Ile Gln Gly Val Pro Gly Pro Val Gly Ala Thr Gly Pro Glu Gly 385 390 395 400

Pro Gln Gly Ile Gln Gly Ile Gln Gly Val Gln Gly Ala Thr Gly Pro 405 410 415

Gln Gly Pro Gln Gly Ile Gln Gly Ile Gln Gly Val Gln Gly Ile Thr 420 425 430 Page 42

# BCS149058WO\_ST25.txt

- Gly Ala Thr Gly 435
- <210> 55
- <211> 36
- <212> PRT
- <213> Bacillus thuringiensis
- <400> 55

Met Lys Asn Arg Asp Asn Lys Gly Lys Gln Gln Ser Asn Phe Arg Ile 1 10 15

Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro Thr Gly Phe 20 25 30

Thr Gly Ile Gly 35

- <210> 56
- <211> 470
- <212> PRT
- <213> Bacillus thuringiensis
- <400> 56

Met Lys Asn Arg Asp Asn Lys Gly Lys Gln Gln Ser Asn Phe Arg Ile 1 5 10 15

Pro Pro Glu Leu Ile Gly Pro Thr Phe Pro Pro Val Pro Thr Gly Phe 20 25 30

Thr Gly Ile Gly Ile Thr Gly Pro Thr Gly Pro Gln Gly Pro Thr Gly 35 40 45

Pro Gln Gly Pro Arg Gly Phe Gln Gly Pro Met Gly Glu Met Gly Pro 50 55 60

Thr Gly Pro Gln Gly Val Gln Gly Ile Gln Gly Pro Val Gly Pro Ile 65 70 75 80

Gly Ala Thr Gly Pro Glu Gly Gln Gln Gly Ala Gln Gly Leu Arg Gly 85 90 95

Pro Gln Gly Glu Thr Gly Ala Thr Gly Pro Gln Gly Val Gln Gly Leu
100 105 110

Gln Gly Pro Ile Gly Pro Thr Gly Ala Thr Gly Ala Gln Gly Ile Gln
115 120 125

Page 43

BCS149058WO\_ST25.txt

Gly Ile Gln Gly Leu Gln Gly Pro Ile Gly Ala Thr Gly Pro Glu Gly 130 140

Pro Gln Gly Ile Gln Gly Val Gln Gly Leu Pro Gly Ala Thr Gly Pro 145 155 160

Gln Gly Ile Gln Gly Ala Gln Gly Ile Gln Gly Thr Gln Gly Pro Ser 165 170 175

Gly Asn Thr Gly Ala Thr Gly Ala Thr Gly Gln Gly Leu Thr Gly Pro 180 185 190

Thr Gly Ile Thr Gly Pro Thr Gly Ile Thr Gly Pro Ser Gly Gly Pro 195 200 205

Pro Gly Pro Thr Gly Pro Thr Gly Ala Thr Gly Pro Gly Gly Pro 210 215 220

Ser Gly Ser Thr Gly Ala Thr Gly Ala Thr Gly Asp Thr Gly Ala Thr 225 230 235 240

Gly Ser Thr Gly Val Thr Gly Ala Thr Gly Ala Gln Gly Pro Gln Gly 245 250 255

Val Gln Gly Ile Gln Gly Pro Thr Gly Ala Thr Gly Ala 260 265 270

Thr Gly Pro Gln Gly Ile Gln Gly Pro Gln Gly Ile Gln Gly Pro Thr 275 280 285

Gly Ala Thr Gly Ala Thr Gly Ser Gln Gly Pro Thr Gly Asn Thr Gly 290 295 300

Pro Thr Gly Ser Gln Gly Ile Gln Gly Pro Thr Gly Pro Thr Gly Ala 305 310 315 320

Gly Ala Thr Gly Ala Thr Gly Ala Thr Gly Val Ser Thr 325 330 335

Thr Ala Thr Tyr Ala Phe Ala Asn Asn Thr Ser Gly Ser Ile Ile Ser 340 345 350

Val Leu Leu Gly Gly Thr Asn Ile Pro Leu Pro Asn Asn Gln Asn Ile 355 360 365

Gly Pro Gly Ile Thr Val Ser Gly Gly Asn Thr Val Phe Thr Val Ala 370 375 380 Page 44

# BCS149058WO\_ST25.txt

Asn Ala Gly Asn Tyr Tyr Ile Ala Tyr Thr Ile Asn Leu Thr Ala Gly 385 390 400

Leu Leu Val Ser Ser Arg Ile Thr Val Asn Gly Ser Pro Leu Ala Gly 405 410 415

Thr Ile Asn Ser Pro Ala Val Ala Ala Gly Ser Phe Ser Ala Thr Ile 420 425 430

Ile Ala Asn Leu Pro Ala Gly Ala Ala Val Ser Leu Gln Leu Phe Gly 435 440 445

Val Ile Ala Leu Ala Thr Leu Ser Thr Ala Thr Pro Gly Ala Thr Leu 450 460

Thr Ile Ile Arg Leu Ser 465 470

<210> 57

<211> 136

<212> PRT

<213> Bacillus mycoides

<400> 57

Met Lys Phe Ser Lys Lys Ser Thr Val Asp Ser Ser Ile Val Gly Lys  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Arg Val Val Ser Lys Val Asn Ile Leu Arg Phe Tyr Asp Ala Arg Ser 20 25 30

Cys Gln Asp Lys Asp Val Asp Gly Phe Val Asp Val Gly Glu Leu Phe 35 40 45

Thr Ile Phe Arg Lys Leu Asn Met Glu Gly Ser Val Gln Phe Lys Ala 50 55 60

His Asn Ser Ile Gly Lys Thr Tyr Tyr Ile Thr Ile Asn Glu Val Tyr 65 70 75 80

Val Phe Val Thr Val Leu Gln Tyr Ser Thr Leu Ile Gly Gly Ser 85 90 95

Tyr Val Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln 100 105 110

Ala Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile 115 120 125 Page 45

# BCS149058WO\_ST25.txt

Pro Pro Phe Thr Leu Pro Thr Gly 130 135

<210> 58

<211> 384 <212> PRT

<213> Bacillus mycoides

<400> 58

Met Lys Phe Ser Lys Lys Ser Thr Val Asp Ser Ser Ile Val Gly Lys  $1 \hspace{1cm} 10 \hspace{1cm} 15$ 

Arg Val Val Ser Lys Val Asn Ile Leu Arg Phe Tyr Asp Ala Arg Ser 20 25 30

Cys Gln Asp Lys Asp Val Asp Gly Phe Val Asp Val Gly Glu Leu Phe 35 40

Thr Ile Phe Arg Lys Leu Asn Met Glu Gly Ser Val Gln Phe Lys Ala 50 55 60

His Asn Ser Ile Gly Lys Thr Tyr Tyr Ile Thr Ile Asn Glu Val Tyr 65 70 75 80

Val Phe Val Thr Val Leu Leu Gln Tyr Ser Thr Leu Ile Gly Gly Ser 85 90 95

Tyr Val Phe Asp Lys Asn Glu Ile Gln Lys Ile Asn Gly Ile Leu Gln
100 105 110

Ala Asn Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile 115 120 125

Pro Pro Phe Thr Leu Pro Thr Gly Pro Thr Gly Gly Thr Gly Pro Thr 130 135 140

Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly 145 150 155 160

Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val 165 170 175

Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr

Gly Val Thr Gly Pro Thr Gly Val Thr Gly Pro Thr Gly Val Thr Gly 195 200 205

Page 46

BCS149058WO\_ST25.txt

Pro Thr	Gly	٧a٦	Thr	Gly	Pro	Thr	Gly	Gly	Thr	Glu	Gly	Cys	Leu	Cys
210	-			-	215		-	_		220	-	-		-

Leu Leu Asp Ala Phe Ile Gly Ser Thr Val Ser Leu Leu Ala Ser Asn 325 330 335

Val Leu Gly Thr Leu Pro Ile Asn Pro Thr Thr Val Arg Phe Ala 355 360 365

Ile Ser Thr Cys Lys Ile Thr Ala Val Asn Ile Thr Pro Ile Thr Met 370 380

<sup>&</sup>lt;210> 59

<sup>&</sup>lt;211> 196

<sup>&</sup>lt;212> PRT

<sup>&</sup>lt;213> Bacillus anthracis

<sup>&</sup>lt;400> 59

Met Ser Asn Asn Asn Tyr Ser Asn Gly Leu Asn Pro Asp Glu Ser Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Ser Ala Ser Ala Phe Asp Pro Asn Leu Val Gly Pro Thr Leu Pro Pro 20 25 30

BCS149058WO\_ST25.txt

Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly 50 55 60

Pro Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Thr Thr Gly Pro 65 70 75 80

Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr 85 90 95

Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro
100 105 110

Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Thr Thr Gly Pro Thr 115 120 125

Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly 130 140

Thr Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro 145 150 155 160

Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly 165 170 175

Pro Thr Gly Ala Thr Gly Leu Thr Gly Pro Thr Gly Pro 180 185 190

Ser Gly Leu Gly 195

<210> 60

<211> 17

<212> PRT

<213> Bacillus anthracis

<400> 60

Met Ala Phe Asp Pro Asn Leu Val Gly Pro Thr Leu Pro Pro Ile Pro 1  $\phantom{000}$   $\phantom{000}$ 

Pro

<210> 61

<211> 17

<212> PRT

<213> Bacillus anthracis

<400> 61

Page 48

```
BCS149058WO_ST25.txt
Met Ala Leu Glu Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro
Pro
<210> 62
<211>
       17
<212>
       PRT
<213>
        Bacillus weihenstephensis
<400>
Met Ala Leu Asn Pro Asn Leu Ile Gly Pro Thr Leu Pro Pro Ile Pro 1 5 10 15
Pro
        63
<210>
<211>
       17
<212>
       PRT
<213>
        Bacillus weihenstephensis
<400> 63
Met Ala Leu Asp Pro Asn Ile Ile Gly Pro Thr Leu Pro Pro Ile Pro 1 	 5 	 10 	 15
Pro
<210>
        64
       17
<211>
<212>
        PRT
<213>
        Bacillus cereus
<400> 64
Met Ala Leu Glu Pro Asn Leu Ile Gly Pro Thr Leu Pro Ser Ile Pro 1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15
Pro
<210>
        65
<211>
        17
<212>
        PRT
        Bacillus weihenstephensis
<213>
<400> 65
Met Ala Leu Asp Pro Asn Leu Ile Gly Pro Pro Leu Pro Pro Ile Thr
                                            Page 49
```

# BCS149058WO\_ST25.txt

```
Pro
<210> 66
<211>
        17
<212>
<213>
        Bacillus weihenstephensis
<400> 66
Met Ala Leu Asn Pro Gly Ser Ile Gly Pro Thr Leu Pro Pro Val Pro 1 \ 5 \ 10 \ 15
Pro
        67
17
<210>
<211>
<212>
        PRT
<213>
        Bacillus weihenstephensis
<400> 67
Met Ala Leu Asn Pro Cys Ser Ile Gly Pro Thr Leu Pro Pro Met Gln 1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15
Pro
<210> 68
<211> 17
<212>
        PRT
<213> Bacillus mycoides
<400> 68
Met Ala Leu Asn Pro Gly Ser Ile Gly Pro Thr Leu Pro Pro Val Gln 10 15
Pro
<210> 69
<211> 17
<212>
        PRT
<213>
        Bacillus anthracis
<400>
Met Ala Leu Asn Pro Gly Ser Val Gly Pro Thr Leu Pro Pro Met Gln 1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15
Pro
```

# BCS149058WO\_ST25.txt

Page 51

<210> 70 17 <211> <212> PRT <213> Bacillus cereus <400> Met Ala Leu Asp Pro Asn Leu Ile Gly Pro Thr Phe Pro Pro Ile Pro 1 5 10 15 ser <210> 71 <211> 799 <212> PRT <213> Bacillus mycoides <400> 71 Met Lys Arg Lys Thr Pro Phe Lys Val Phe Ser Ser Leu Ala Ile Thr  $1 \hspace{1cm} 10 \hspace{1cm} 15$ Thr Met Leu Gly Cys Thr Phe Ala Leu Gly Thr Ser Val Ala Tyr Ala 20 25 30 Glu Thr Thr Ser Gln Ser Lys Gly Ser Ile Ser Thr Thr Pro Ile Asp Asn Asn Leu Ile Gln Glu Glu Arg Leu Ala Glu Ala Leu Lys Glu Arg 50 60 Gly Thr Ile Asp Gln Ser Ala Ser Lys Glu Glu Thr Gln Lys Ala Val 65 70 75 80 Glu Gln Tyr Ile Glu Lys Lys Lys Gly Asp Gln Pro Asn Lys Glu Ile 85 90 95 Leu Pro Asp Asp Pro Ala Lys Glu Ala Ser Asp Phe Val Lys Lys Val 100 105 110Lys Glu Lys Lys Met Glu Glu Lys Glu Lys Val Lys Ser Val Glu
115 120 125 Asn Ala Ser Ser Glu Gln Thr Pro Ser Gln Asn Lys Lys Gln Leu Asn 130 140 Gly Lys Val Pro Thr Ser Pro Ala Lys Gln Ala Pro Tyr Asn Gly Ala 145 150 155 160

### BCS149058WO\_ST25.txt

Val Arg Thr Asp Lys Val Leu Val Leu Val Glu Phe Ser Asp Tyr 165 170 175 Lys His Asn Asn Ile Glu Gln Ser Pro Gly Tyr Met Tyr Ala Asn Asp Phe Ser Arg Glu His Tyr Gln Lys Met Leu Phe Gly Asn Glu Pro Phe 195 200 205 Thr Leu Phe Asp Gly Ser Lys Val Lys Thr Phe Lys Gln Tyr Tyr Glu 210 220 Glu Gln Ser Gly Gly Ser Tyr Thr Thr Asp Gly Tyr Val Thr Glu Trp 225 230 235 240 Leu Thr Val Pro Gly Lys Ala Ala Asp Tyr Gly Ala Asp Gly Lys Thr 245 250 255 Gly His Asp Asn Lys Gly Pro Lys Gly Ala Arg Asp Leu Val Lys Glu 260 265 270 Ala Leu Lys Ala Ala Ala Glu Lys Gly Leu Asp Leu Ser Gln Phe Asp 275 280 285 Gln Phe Asp Arg Tyr Asp Thr Asn Gly Asp Gly Asn Gln Asn Glu Pro Asp Gly Val Ile Asp His Leu Met Val Ile His Ala Gly Val Gly Gln Glu Ala Gly Gly Lys Leu Gly Asp Ala Ile Trp Ser His Arg 325 330 335 Ser Lys Leu Ala Gln Asp Pro Val Ala Ile Glu Gly Thr Lys Ser Lys 340 345 350 Val Ser Tyr Trp Asp Gly Lys Val Ala Ala His Asp Tyr Thr Ile Glu 355 360 365 Pro Glu Asp Gly Ala Val Gly Val Phe Ala His Glu Phe Gly His Asp 370 380 Leu Gly Leu Pro Asp Glu Tyr Asp Thr Asn Tyr Thr Gly Ala Gly Ser 385 390 Pro Val Glu Ala Trp Ser Leu Met Ser Gly Gly Ser Trp Thr Gly Arg

Page 52

BCS149058WO\_ST25.txt 405 410 415

Ile Ala Gly Thr Glu Pro Thr Ser Phe Ser Pro Gln Asn Lys Asp Phe 420 430 Leu Gln Lys Asn Met Asp Gly Asn Trp Ala Lys Ile Val Glu Val Asp 435 440 445 Tyr Asp Lys Ile Lys Arg Gly Val Gly Phe Pro Thr Tyr Ile Asp Gln 450 460 Ser Val Thr Lys Ser Asn Arg Pro Gly Leu Val Arg Val Asn Leu Pro 465 470 475 480 Glu Lys Ser Val Glu Thr Ile Lys Thr Gly Phe Gly Lys His Ala Tyr 485 490 495 Tyr Ser Thr Arg Gly Asp Asp Met His Thr Thr Leu Glu Thr Pro Leu 500 510 Phe Asp Leu Thr Lys Ala Ala Asn Ala Lys Phe Asp Tyr Lys Ala Asn 515 520 525 Tyr Glu Leu Glu Ala Glu Cys Asp Phe Ile Glu Val His Ala Val Thr 530 540 Glu Asp Gly Thr Lys Thr Leu Ile Asp Lys Leu Gly Asp Lys Val Val 545 550 560 Lys Gly Asp Gln Asp Thr Thr Glu Gly Lys Trp Ile Asp Lys Ser Tyr 565 570 575 Asp Leu Ser Gln Phe Lys Gly Lys Lys Val Lys Leu Gln Phe Asp Tyr 580 585 590 Ile Thr Asp Pro Ala Leu Thr Tyr Lys Gly Phe Ala Met Asp Asn Val 595 600 605 Asn Val Thr Val Asp Gly Lys Val Val Phe Ser Asp Asp Ala Glu Gly 610 620 Gln Ala Lys Met Lys Leu Asn Gly Phe Val Val Ser Asp Gly Thr Glu Lys Lys Pro His Tyr Tyr Leu Glu Trp Arg Asn Tyr Ala Gly Ser 645 650 655

Page 53

BCS149058Wo\_ST25.txt Asp Glu Gly Leu Lys Val Gly Arg Gly Pro Val Tyr Asn Thr Gly Leu Val Val Trp Tyr Ala Asp Asp Ser Phe Lys Asp Asn Trp Val Gly Arg 675 680 685 His Pro Gly Glu Gly Phe Leu Gly Val Val Asp Ser His Pro Glu Ala Val Val Gly Asn Leu Asn Gly Lys Pro Val Tyr Gly Asn Thr Gly Leu 705 710 715 720 Gln Ile Ala Asp Ala Ala Phe Ser Leu Asp Gln Thr Pro Ala Trp Asn Val Asn Ser Phe Thr Arg Gly Gln Phe Asn Tyr Pro Gly Leu Pro Gly Val Ala Thr Phe Asp Asp Ser Lys Val Tyr Ser Asn Thr Gln Ile Pro 755 760 765 Asp Ala Gly Arg Lys Val Pro Gln Leu Gly Leu Lys Phe Gln Val Val 770 780 Gly Gln Ala Asp Asp Lys Ser Ala Gly Ala Ile Trp Ile Arg Arg 785 790 795 <210> 72 <211> 152 <212> PRT <213> Bacillus anthracis <400> 72 Met Ser Cys Asn Glu Asn Lys His His Gly Ser Ser His Cys Val Val 1 5 10 15Asp Val Val Lys Phe Ile Asn Glu Leu Gln Asp Cys Ser Thr Thr 20 25 30 Cys Gly Ser Gly Cys Glu Ile Pro Phe Leu Gly Ala His Asn Thr Ala 35 40 45 Ser Val Ala Asn Thr Arg Pro Phe Ile Leu Tyr Thr Lys Ala Gly Ala Pro Phe Glu Ala Phe Ala Pro Ser Ala Asn Leu Thr Ser Cys Arg Ser 65 70 75 80

Page 54

BCS149058WO\_ST25.txt Pro Ile Phe Arg Val Glu Ser Val Asp Asp Ser Cys Ala Val Leu 85 90 95 Arg Val Leu Ser Val Val Leu Gly Asp Ser Ser Pro Val Pro Pro Thr 100 105 110 Asp Asp Pro Ile Cys Thr Phe Leu Ala Val Pro Asn Ala Arg Leu Val Ser Thr Ser Thr Cys Ile Thr Val Asp Leu Ser Cys Phe Cys Ala Ile Gln Cys Leu Arg Asp Val Thr Ile <210> 73 167 <211> <212> PRT <213> Bacillus anthracis <400> Met Phe Ser Ser Asp Cys Glu Phe Thr Lys Ile Asp Cys Glu Ala Lys  $1 \hspace{1cm} 10 \hspace{1cm} 15$ Pro Ala Ser Thr Leu Pro Ala Phe Gly Phe Ala Phe Asn Ala Ser Ala 20 25 30 Pro Gln Phe Ala Ser Leu Phe Thr Pro Leu Leu Leu Pro Ser Val Ser Pro Asn Pro Asn Ile Thr Val Pro Val Ile Asn Asp Thr Val Ser Val 50 60 Gly Asp Gly Ile Arg Ile Leu Arg Ala Gly Ile Tyr Gln Ile Ser Tyr 65 70 75 80 Thr Leu Thr Ile Ser Leu Asp Asn Ser Pro Val Ala Pro Glu Ala Gly 85 90 95 Arg Phe Phe Leu Ser Leu Gly Thr Pro Ala Asn Ile Ile Pro Gly Ser Gly Thr Ala Val Arg Ser Asn Val Ile Gly Thr Gly Glu Val Asp Val Ser Ser Gly Val Ile Leu Ile Asn Leu Asn Pro Gly Asp Leu Ile Arg 130 140

BCS149058WO\_ST25.txt

Ile Val Pro Val Glu Leu Ile Gly Thr Val Asp Ile Arg Ala Ala Ala
145 150 155 160

Leu Thr Val Ala Gln Ile Ser

<210> 74 <211> 156

<212> PRT <213> Bacillus anthracis

<400> 74

Met Ser Cys Asn Cys Asn Glu Asp His His His Asp Cys Asp Phe  $1 \hspace{1cm} 10 \hspace{1cm} 15$ 

Asn Cys Val Ser Asn Val Val Arg Phe Ile His Glu Leu Gln Glu Cys 20 25 30

Ala Thr Thr Cys Gly Ser Gly Cys Glu Val Pro Phe Leu Gly Ala 35 40 45

His Asn Ser Ala Ser Val Ala Asn Thr Arg Pro Phe Ile Leu Tyr Thr 50 60

Lys Ala Gly Ala Pro Phe Glu Ala Phe Ala Pro Ser Ala Asn Leu Thr 65 70 75 80

Ser Cys Arg Ser Pro Ile Phe Arg Val Glu Ser Ile Asp Asp Asp 85 90 95

Cys Ala Val Leu Arg Val Leu Ser Val Val Leu Gly Asp Thr Ser Pro 100 105 110

Val Pro Pro Thr Asp Asp Pro Ile Cys Thr Phe Leu Ala Val Pro Asn 115 120 125

Ala Arg Leu Ile Ser Thr Asn Thr Cys Leu Thr Val Asp Leu Ser Cys 130 135 140

Phe Cys Ala Ile Gln Cys Leu Arg Asp Val Thr Ile 145 150 155

<210> 75

<211> 182

<212> PRT

<213> Bacillus anthracis

<400> 75

Met Glu Val Gly Gly Thr Ser Val Lys Asn Lys Asn Lys Ser Ser Thr Page 56

BCS149058WO\_ST25.txt 5 1 15 Val Gly Lys Pro Leu Leu Tyr Ile Ala Gln Val Ser Leu Glu Leu Ala 20 25 30 Ala Pro Lys Thr Lys Arg Ile Ile Leu Thr Asn Phe Glu Asn Glu Asp 35 40 45 Arg Lys Glu Glu Ser Asn Arg Asn Glu Asn Val Val Ser Ser Ala Val 50 55 60 Glu Glu Val Ile Glu Gln Glu Gln Gln Gln Glu Gln Glu Gln Glu 65 70 75 80 Gln Glu Glu Gln Val Glu Glu Lys Thr Glu Glu Glu Glu Gln Val Gln Glu Gln Glu Pro Val Arg Thr Val Pro Tyr Asn Lys Ser Phe Lys Asp Met Asn Asn Glu Glu Lys Ile His Phe Leu Leu Asn Arg Pro His Tyr Ile Pro Lys Val Arg Cys Arg Ile Lys Thr Ala Thr Ile Ser Tyr 130 135 140 Val Gly Ser Ile Ile Ser Tyr Arg Asn Gly Ile Val Ala Ile Met Pro 145 150 155 160 Pro Asn Ser Met Arg Asp Ile Arg Leu Ser Ile Glu Glu Ile Lys Ser 165 170 175 Ile Asp Met Ala Gly Phe 76 174 <210> <211> PRT Bacillus anthracis <213> <400> Met Lys Glu Arg Ser Glu Asn Met Arg Ser Ser Ser Arg Lys Leu Thr  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Asn Phe Asn Cys Arg Ala Gln Ala Pro Ser Thr Leu Pro Ala Leu Gly 20 25 30

Phe Ala Phe Asn Ala Thr Ser Pro Gln Phe Ala Thr Leu Phe Thr Pro

Page 57

BCS149058WO\_ST25.txt 35 40 45

Leu Leu Leu Pro Ser Thr Gly Pro Asn Pro Asn Ile Thr Val Pro Val 50 60

Ile Asn Asp Thr Ile Ser Thr Gly Thr Gly Ile Arg Ile Gln Val Ala 65 70 75 80

Gly Ile Tyr Gln Ile Ser Tyr Thr Leu Thr Ile Ser Leu Asp Asn Val 85 90 95

Pro Val Thr Pro Glu Ala Ala Arg Phe Phe Leu Thr Leu Asn Ser Ser 100 105 110

Thr Asn Ile Ile Ala Gly Ser Gly Thr Ala Val Arg Ser Asn Ile Ile 115 120 125

Gly Thr Gly Glu Val Asp Val Ser Ser Gly Val Ile Leu Ile Asn Leu 130 135 140

Asn Pro Gly Asp Leu Ile Gln Ile Val Pro Val Glu Val Ile Gly Thr 145 150 155 160

Val Asp Ile Arg Ser Ala Ala Leu Thr Val Ala Gln Ile Arg 165 170

<210> 77

<211> 796

PRT

<212>

<213> Bacillus thuringiensis

<400> 77

Met Ser Lys Lys Pro Phe Lys Val Leu Ser Ser Ile Ala Leu Thr Ala 1 5 10 15

Val Leu Gly Leu Ser Phe Gly Ala Gly Thr Gln Ser Ala Tyr Ala Glu 20 25 30

Thr Pro Val Asn Lys Thr Ala Thr Ser Pro Val Asp Asp His Leu Ile 35 40 45

Pro Glu Glu Arg Leu Ala Asp Ala Leu Lys Lys Arg Gly Val Ile Asp 50 60

Ser Lys Ala Ser Glu Thr Glu Thr Lys Lys Ala Val Glu Lys Tyr Val 65 70 75 80

Glu Asn Lys Lys Gly Glu Asn Pro Gly Lys Glu Ala Ala Asn Gly Asp Page 58

BCS149058WO\_ST25.txt 85 90 95

Gln Leu Thr Lys Asp Ala Ser Asp Phe Leu Lys Lys Val Lys Asp Ala 100 105 110Lys Ala Asp Thr Lys Glu Lys Leu Asn Gln Pro Ala Thr Gly Thr Pro 115 120 125 Ala Ala Thr Gly Pro Val Lys Gly Gly Leu Asn Gly Lys Val Pro Thr 130 140 Ser Pro Ala Lys Gln Lys Asp Tyr Asn Gly Glu Val Arg Lys Asp Lys 145 150 155 160 Val Leu Val Leu Leu Val Glu Tyr Ala Asp Phe Lys His Asn Asn Ile 165 170 175 Asp Lys Glu Pro Gly Tyr Met Tyr Ser Asn Asp Phe Asn Lys Glu His 180 185 190 Tyr Glu Lys Met Leu Phe Gly Asn Glu Pro Phe Thr Leu Asp Asp Gly 195 200 205 Ser Lys Ile Glu Thr Phe Lys Gln Tyr Tyr Glu Glu Gln Ser Gly Gly 210 220 Ser Tyr Thr Val Asp Gly Thr Val Thr Lys Trp Leu Thr Val Pro Gly 235 230 240 Lys Ala Ala Asp Tyr Gly Ala Asp Ala Pro Gly Gly His Asp Asn 245 250 255 Lys Gly Pro Lys Gly Pro Arg Asp Leu Val Lys Asp Ala Leu Lys Ala 260 265 270 Ala Val Asp Ser Gly Ile Asp Leu Ser Glu Phe Asp Gln Phe Asp Gln 275 280 285 Tyr Asp Val Asn Gly Asp Gly Asn Lys Asn Gln Pro Asp Gly Leu Ile 290 295 300 Asp His Leu Met Ile Ile His Ala Gly Val Gly Gln Glu Ala Gly Gly 305 310 315 320 Gly Lys Leu Gly Asp Asp Ala Ile Trp Ser His Arg Trp Thr Val Gly 325 330 335

Page 59

BCS149058WO\_ST25.txt Pro Lys Pro Phe Pro Ile Glu Gly Thr Gln Ala Lys Val Pro Tyr Trp 345 Gly Gly Lys Met Ala Ala Phe Asp Tyr Thr Ile Glu Pro Glu Asp Gly 355 360 365 Ala Val Gly Val Phe Ala His Glu Tyr Gly His Asp Leu Gly Leu Pro Asp Glu Tyr Asp Thr Gln Tyr Ser Gly Gln Gly Glu Pro Ile Glu Ala Trp Ser Ile Met Ser Gly Gly Ser Trp Ala Gly Lys Ile Ala Gly Thr 405 410 415Thr Pro Thr Ser Phe Ser Pro Gln Asn Lys Glu Phe Phe Gln Lys Thr 420 Ile Gly Gly Asn Trp Ala Asn Ile Val Glu Val Asp Tyr Glu Lys Leu 435 440 445 440 Asn Lys Gly Ile Gly Leu Ala Thr Tyr Leu Asp Gln Ser Val Thr Lys 450 460 Ser Ala Arg Pro Gly Met Ile Arg Val Asn Leu Pro Asp Lys Asp Val Lys Thr Ile Glu Pro Ala Phe Gly Lys Gln Tyr Tyr Ser Thr Lys Gly Asp Asp Leu His Thr Lys Met Glu Thr Pro Leu Phe Asp Leu Thr 505 Asn Ala Thr Ser Ala Lys Phe Asp Phe Lys Ser Leu Tyr Glu Ile Glu Ala Gly Tyr Asp Phe Leu Glu Val His Ala Val Thr Glu Asp Gly Lys 530 Gln Thr Leu Ile Glu Arg Leu Gly Glu Lys Ala Asn Ser Gly Asn Ala 545 Asp Ser Thr Asn Gly Lys Trp Ile Asp Lys Ser Tyr Asp Leu Ser Gln 565 570 575 Phe Lys Gly Lys Lys Val Lys Leu Thr Phe Asp Tyr Ile Thr Asp Gly 580 585 590

Page 60

BCS149058WO\_ST25.txt

Gly Leu Ala Leu Asn Gly Phe Ala Leu Asp Asn Ala Ser Leu Thr Val
595 600 605

Asp Gly Lys Val Val Phe Ser Asp Asp Ala Glu Gly Thr Pro Gln Leu 610 620

Lys Leu Asp Gly Phe Val Val Ser Asn Gly Thr Glu Lys Lys Lys His 625 630 635 640

Asn Tyr Tyr Val Glu Trp Arg Asn Tyr Ala Gly Ala Asp Asn Ala Leu 645 650 655

Lys Phe Ala Arg Gly Pro Val Phe Asn Thr Gly Met Val Val Trp Tyr 660 665 670

Ala Asp Ser Ala Tyr Thr Asp Asn Trp Val Gly Val His Pro Gly His 675 680 685

Gly Phe Leu Gly Val Val Asp Ser His Pro Glu Ala Ile Val Gly Thr 690 700

Leu Asn Gly Lys Pro Thr Val Lys Ser Ser Thr Arg Phe Gln Ile Ala 705 710 715 720

Asp Ala Ala Phe Ser Phe Asp Lys Thr Pro Ala Trp Lys Val Val Ser 725 730 735

Pro Thr Arg Gly Thr Phe Thr Tyr Asp Gly Leu Ala Gly Val Pro Lys 740 745 750

Phe Asp Asp Ser Lys Thr Tyr Ile Asn Gln Gln Ile Pro Asp Ala Gly 765 765

Arg Ile Leu Pro Lys Leu Gly Leu Lys Phe Glu Val Val Gly Gln Ala 770 775 780

Asp Asp Asn Ser Ala Gly Ala Val Arg Leu Tyr Arg 785 790 795

<210> 78

<211> 430

<212> PRT

<213> Bacillus cereus

<400> 78

Met Lys His Asn Asp Cys Phe Asp His Asn Asn Cys Asn Pro Ile Val  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Page 61

BCS149058WO\_ST25.txt

Phe Ser Ala Asp Cys Cys Lys Asn Pro Gln Ser Val Pro Ile Thr Arg 20 25 30

Glu Gln Leu Ser Gln Leu Ile Thr Leu Leu Asn Ser Leu Val Ser Ala 35 40 45

Ile Ser Ala Phe Phe Ala Asn Pro Ser Asn Ala Asn Arg Leu Val Leu 50 60

Leu Asp Leu Phe Asn Gln Phe Leu Ile Phe Leu Asn Ser Leu Leu Pro 65 70 75 80

Ser Pro Glu Val Asn Phe Leu Lys Gln Leu Thr Gln Ser Ile Ile Val 85 90 95

Leu Leu Gln Ser Pro Ala Pro Asn Leu Gly Gln Leu Ser Thr Leu Leu 100 105 110

Gln Gln Phe Tyr Ser Ala Leu Ala Gln Phe Phe Ala Leu Asp Leu 115 120 125

Ile Pro Ile Ser Cys Asn Ser Asn Val Asp Ser Ala Thr Leu Gln Leu 130 140

Leu Phe Asn Leu Leu Ile Gln Leu Ile Asn Ala Thr Pro Gly Ala Thr 145 150 155 160

Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Ala Gly 165 170 175

Thr Gly Ala Gly Pro Thr Gly Ala Thr Gly Ala Thr Gly 180 185 190

Pro Thr Gly Ala Thr Gly Pro Ala Gly Thr Gly Gly Ala Thr Gly Ala 195 200 205

Thr Gly Ala Thr Gly Val Thr Gly Ala Thr Gly Ala Thr 210 220

Gly Pro Thr Gly Pro Thr Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly 225 235 240

Ala Thr Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly Pro Thr Gly Ala 245 250 255

Thr Gly Leu Thr Gly Ala Thr Gly Ala Ala Gly Gly Ala Ile Ile 260 265 270 Page 62

## BCS149058WO\_ST25.txt

- Pro Phe Ala Ser Gly Thr Thr Pro Ser Ala Leu Val Asn Ala Leu Val 275 280 285
- Ala Asn Thr Gly Thr Leu Leu Gly Phe Gly Phe Ser Gln Pro Gly Val 290 295 300
- Ala Leu Thr Gly Gly Thr Ser Ile Thr Leu Ala Leu Gly Val Gly Asp 305 310 315 320
- Tyr Ala Phe Val Ala Pro Arg Ala Gly Thr Ile Thr Ser Leu Ala Gly 325 330 335
- Phe Phe Ser Ala Thr Ala Ala Leu Ala Pro Ile Ser Pro Val Gln Val 340 345 350
- Gln Ile Gln Ile Leu Thr Ala Pro Ala Ala Ser Asn Thr Phe Thr Val 355 360 365
- Gln Gly Ala Pro Leu Leu Thr Pro Ala Phe Ala Ala Ile Ala Ile 370 375 380
- Gly Ser Thr Ala Ser Gly Ile Ile Ala Glu Ala Ile Pro Val Ala Ala 385 390 395 400
- Gly Asp Lys Ile Leu Leu Tyr Val Ser Leu Thr Ala Ala Ser Pro Ile 405 410 415
- Ala Ala Val Ala Gly Phe Val Ser Ala Gly Ile Asn Ile Val 420 425 430
- <210> 79
- <211> 437
- <212> PRT
- <213> Bacillus cereus
- <400> 79
- Met Lys His Asn Asp Cys Phe Gly His Asn Asn Cys Asn Asn Pro Ile 1 5 10 15
- Val Phe Thr Pro Asp Cys Cys Asn Asn Pro Gln Thr Val Pro Ile Thr 20 25 30
- Ser Glu Gln Leu Gly Arg Leu Ile Thr Leu Leu Asn Ser Leu Ile Ala
- Ala Ile Ala Ala Phe Phe Ala Asn Pro Ser Asp Ala Asn Arg Leu Ala 50 55 60 Page 63

### BCS149058WO\_ST25.txt

Leu Leu Asn Leu Phe Thr Gln Leu Leu Asn Leu Leu Asn Glu Leu Ala Pro Ser Pro Glu Gly Asn Phe Leu Lys Gln Leu Ile Gln Ser Ile Ile Asn Leu Leu Gln Ser Pro Asn Pro Asn Leu Gly Gln Leu Leu Ser Leu Leu Gln Gln Phe Tyr Ser Ala Leu Ala Pro Phe Phe Ser Leu Ile 115 Leu Asp Pro Ala Ser Leu Gln Leu Leu Leu Asn Leu Leu Ala Gln Leu 135 Ile Gly Val Thr Pro Gly Gly Gly Ala Thr Gly Pro Thr Gly Pro Thr 145 150 160 Gly Pro Gly Gly Gly Ala Thr Gly Pro Thr Gly Pro Gly 165 170 175 Gly Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly Pro Thr Gly Asp Thr Gly Leu Ala Gly Ala Thr Gly Ala Thr Gly Pro Thr Gly Asp Thr Gly 195 200 205 Val Ala Gly Pro Ala Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Leu 210 215 220 Ala Gly Ala Thr Gly Pro Thr Gly Pro Thr Gly Asp Thr Gly Leu Ala 225 230 235 240 Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly Leu Ala Gly Ala Thr Gly Pro Thr Gly Ala Thr Gly Leu Thr Gly Ala Thr Gly Ala Thr Gly Ala 260 265 270 Ala Gly Gly Ala Ile Ile Pro Phe Ala Ser Gly Thr Thr Pro Ala 275 280 285 Ala Leu Val Asn Ala Leu Ile Ala Asn Thr Gly Thr Leu Leu Gly Phe 290 Gly Phe Ser Gln Pro Gly Ile Gly Leu Ala Gly Gly Thr Ser Ile Thr

Page 64

BCS149058WO\_ST25.txt 305 310 320 Leu Ala Leu Gly Val Gly Asp Tyr Ala Phe Val Ala Pro Arg Asp Gly 325 330 335 Val Ile Thr Ser Leu Ala Gly Phe Phe Ser Ala Thr Ala Ala Leu Ser Pro Leu Ser Pro Val Gln Val Gln Ile Gln Ile Leu Thr Ala Pro Ala Ala Ser Asn Thr Phe Thr Val Gln Gly Ala Pro Leu Leu Leu Thr Pro Ala Phe Ala Ala Ile Ala Ile Gly Ser Thr Ala Ser Gly Ile Ile Pro Glu Ala Ile Pro Val Val Ala Gly Asp Lys Ile Leu Leu Tyr Val Ser 405 410 415 Leu Thr Ala Ala Ser Pro Ile Ala Ala Val Ala Gly Phe Val Ser Ala 420 425 430 Gly Ile Asn Ile Val 435 <210> <211> 119 <212> PRT Bacillus anthracis <213> <400> Met Leu Phe Thr Ser Trp Leu Leu Phe Phe Ile Phe Ala Leu Ala Ala  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Phe Arg Leu Thr Arg Leu Ile Val Tyr Asp Lys Ile Thr Gly Phe Leu 20 25 30 Arg Arg Pro Phe Ile Asp Glu Leu Glu Ile Thr Glu Pro Asp Gly Ser Val Ser Thr Phe Thr Lys Val Lys Gly Lys Gly Leu Arg Lys Trp Ile 50 55 60 Gly Glu Leu Leu Ser Cys Tyr Trp Cys Thr Gly Val Trp Val Ser Ala 65 70 75 80 Phe Leu Leu Val Leu Tyr Asn Trp Ile Pro Ile Val Ala Glu Pro Leu Page 65

BCS149058WO\_ST25.txt 85 90 95

Leu Ala Leu Leu Ala Ile Ala Gly Ala Ala Ile Ile Glu Thr Ile  $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110$ 

Thr Gly Tyr Phe Met Gly Glu 115

<210> 81

<211> 61

<212> PRT

<213> Bacillus anthracis

<400> 81

Met Phe Ala Val Ser Asn Asn Pro Arg Gln Asn Ser Tyr Asp Leu Gln 1 5 10 15

Gln Trp Tyr His Met Gln Gln Gln His Gln Ala Gln Gln Gln Ala Tyr 20 25 30

Gln Glu Gln Leu Gln Gln Gln Gly Phe Val Lys Lys Gly Cys Asn 35 40 45

Cys Gly Lys Lys Ser Thr Ile Lys His Tyr Glu Glu 50 60

<210> 82

<211> 481

<212> PRT

<213> Bacillus anthracis

<400> 82

Met Ser Arg Tyr Asp Asp Ser Gln Asn Lys Phe Ser Lys Pro Cys Phe 1 5 10 15

Pro Ser Ser Ala Gly Arg Ile Pro Asn Thr Pro Ser Ile Pro Val Thr 20 25 30

Lys Ala Gln Leu Arg Thr Phe Arg Ala Ile Ile Ile Asp Leu Thr Lys  $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$ 

Ile Ile Pro Lys Leu Phe Ala Asn Pro Ser Pro Gln Asn Ile Glu Asp 50 55 60

Leu Ile Asp Thr Leu Asn Leu Leu Ser Lys Phe Ile Cys Ser Leu Asp 65 70 75 80

Ala Ala Ser Ser Leu Lys Ala Gln Gly Leu Ala Ile Ile Lys Asn Leu 85 90 95 Page 66

## BCS149058WO\_ST25.txt

Ile Thr Ile Leu Lys Asn Pro Thr Phe Val Ala Ser Ala Val Phe Ile Glu Leu Gln Asn Leu Ile Asn Tyr Leu Leu Ser Ile Thr Lys Leu Phe Arg Ile Asp Pro Cys Thr Leu Gln Glu Leu Leu Lys Leu Ile Ala Ala Leu Gln Thr Ala Leu Val Asn Ser Ala Ser Phe Ile Gln Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Thr Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Val Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Val Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Ala Gln Gly Pro Ala Gly Ala Thr Gly 210 215 220 Pro Gln Gly Ala Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Ile Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln 245 250 255 Gly Val Gln Gly Pro Thr Gly Ala Thr Gly Ile Gly Val Thr Gly Pro Thr Gly Pro Ser Gly Gly Pro Ala Gly Ala Thr Gly Pro Gln Gly Pro Gln Gly Asn Thr Gly Ala Thr Gly Pro Gln Gly Ile Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Ala Gln Gly Pro Ala Gly Ala Thr Gly Ala Thr Gly Pro Gln Gly Val Gln Gly Pro Thr Gly Ala

Thr Gly Ile Gly Val Thr Gly Pro Thr Gly Pro Ser Gly Pro Ser Phe

Page 67

BCS149058WO\_ST25.txt 340 345 350

Pro Val Ala Thr Ile Val Val Thr Asn Asn Ile Gln Gln Thr Val Leu 355 360 365

Gln Phe Asn Asn Phe Ile Phe Asn Thr Ala Ile Asn Val Asn Asn Ile 370 380

Ile Phe Asn Gly Thr Asp Thr Val Thr Val Ile Asn Ala Gly Ile Tyr 385 390 395 400

Val Ile Ser Val Ser Ile Ser Thr Thr Ala Pro Gly Cys Ala Pro Leu 405 410 415

Gly Val Gly Ile Ser Ile Asn Gly Ala Val Ala Thr Asp Asn Phe Ser 420 425 430

Ser Asn Leu Ile Gly Asp Ser Leu Ser Phe Thr Thr Ile Glu Thr Leu 435 440 445

Thr Ala Gly Ala Asn Ile Ser Val Gln Ser Thr Leu Asn Glu Ile Thr 450 455 460

Ile Pro Ala Thr Gly Asn Thr Asn Ile Arg Leu Thr Val Phe Arg Ile 465 470 475 480

Ala

<210> 83

<211> 275

<212> PRT

<213> Bacillus thuringiensis

<400> 83

Met Lys Met Lys Arg Gly Ile Thr Thr Leu Leu Ser Val Ala Val Leu  $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ 

Ser Thr Ser Leu Val Ala Cys Ser Gly Ile Thr Glu Lys Thr Val Ala 20 25 30

Lys Glu Glu Lys Val Lys Leu Thr Asp Gln Gln Leu Met Ala Asp Leu 35 40 45

Trp Tyr Gln Thr Ala Gly Glu Met Lys Ala Leu Tyr Tyr Gln Gly Tyr 50 55 60

Asn Ile Gly Gln Leu Lys Leu Asp Ala Val Leu Ala Lys Gly Thr Glu Page 68

BCS149058WO\_ST25.txt 70 65 80 Lys Lys Pro Ala Ile Val Leu Asp Leu Asp Glu Thr Val Leu Asp Asn 85 90 95 Ser Pro His Gln Ala Met Ser Val Lys Thr Gly Lys Gly Tyr Pro Tyr 100 105 110 Lys Trp Asp Asp Trp Ile Asn Lys Ala Glu Ala Glu Ala Leu Pro Gly 115 120 Ala Ile Asp Phe Leu Lys Tyr Thr Glu Ser Lys Gly Val Asp Ile Tyr 130 135 140 Tyr Ile Ser Asn Arg Lys Thr Asn Gln Leu Asp Ala Thr Ile Lys Asn 145 150 155 160 Leu Glu Arg Val Gly Ala Pro Gln Ala Thr Lys Glu His Ile Leu Leu 165 170 175 Gln Asp Pro Lys Glu Lys Gly Lys Glu Lys Arg Arg Glu Leu Val Ser 180 185 190 Gln Thr His Asp Ile Val Leu Phe Phe Gly Asp Asn Leu Ser Asp Phe 195 200 205 Thr Gly Phe Asp Gly Lys Ser Val Lys Asp Arg Asn Gln Ala Val Ala 210 220 Asp Ser Lys Ala Gln Phe Gly Glu Lys Phe Ile Ile Phe Pro Asn Pro 225 230 235 240 Met Tyr Gly Asp Trp Glu Gly Ala Leu Tyr Asp Tyr Asp Phe Lys Lys 245 250 255 Ser Asp Ala Glu Lys Asp Lys Ile Arg Arg Asp Asn Leu Lys Ser Phe 260 265 270 Asp Thr Lys 275 <210> 84 795 <211> <212> <213> Bacillus thuringiensis <400> Met Lys Lys Lys Lys Leu Lys Pro Leu Ala Val Leu Thr Thr Ala

Page 69

BCS149058WO\_ST25.txt 10 5 15 1 Ala Val Leu Ser Ser Thr Phe Ala Phe Gly Gly His Ala Ala Tyr Ala 20 25 30 Glu Thr Pro Thr Ser Ser Leu Pro Ile Asp Glu His Leu Ile Pro Glu 35 40 45 Glu Arg Leu Ala Glu Ala Leu Lys Gln Arg Gly Val Ile Asp Gln Ser 50 60 Ala Ser Gln Ala Glu Thr Ser Lys Ala Val Glu Lys Tyr Val Glu Lys 65 70 75 80 Lys Lys Gly Glu Asn Pro Gly Lys Glu Ile Leu Thr Gly Asp Ser Leu 85 90 95 Thr Gln Glu Ala Ser Asp Phe Met Lys Lys Val Lys Asp Ala Lys Met  $100 \hspace{1cm} 105 \hspace{1cm} 110$ Arg Glu Asn Glu Gln Ala Gln Gln Pro Glu Val Gly Pro Val Ala Gly
115 120 125 Gln Gly Ala Ala Leu Asn Pro Gly Lys Leu Asn Gly Lys Val Pro Thr 130 135 140 Thr Ser Ala Lys Gln Glu Glu Tyr Asn Gly Ala Val Arg Lys Asp Lys 145 150 155 160 Val Leu Val Leu Val Glu Phe Ser Asp Phe Lys His Asn Asn Ile 165 170 175 Asp Gln Glu Pro Gly Tyr Met Tyr Ser Lys Asp Phe Asn Arg Glu His 180 185 190 Tyr Gln Lys Met Leu Phe Gly Asp Glu Pro Phe Thr Leu Phe Asp Gly 200 205 Ser Lys Ile Asn Thr Phe Lys Gln Tyr Tyr Glu Glu Gln Ser Gly Gly 210 220 Ser Tyr Thr Val Asp Gly Thr Val Thr Glu Trp Leu Thr Val Pro Gly 225 230 235 Lys Ala Ser Asp Tyr Gly Ala Asp Ala Gly Thr Gly His Asp Asn Lys 245 250 255

Page 70

BCS149058WO\_ST25.txt Gly Pro Leu Gly Pro Lys Asp Leu Val Lys Glu Ala Leu Lys Ala Ala 260 270 Val Ala Lys Gly Ile Asn Leu Ala Asp Phe Asp Gln Tyr Asp Gln Tyr 275 280 285 Asp Gln Asn Gly Asn Gly Asn Lys Asn Glu Pro Asp Gly Ile Ile Asp His Leu Met Val Val His Ala Gly Val Gly Gln Glu Ala Gly Gly Gly Lys Leu Lys Asp Asp Ala Ile Trp Ser His Arg Ser Lys Leu Gly Ser Lys Pro Tyr Ala Ile Asp Gly Thr Lys Ser Ser Val Ser Asn Trp Gly Gly Lys Met Ala Ala Tyr Asp Tyr Thr Ile Glu Pro Glu Asp Gly Ala 355 360 365Val Gly Val Phe Ala His Glu Tyr Gly His Asp Leu Gly Leu Pro Asp 370 375 380 Glu Tyr Asp Thr Lys Tyr Ser Gly Gln Gly Glu Pro Val Glu Ser Trp 385 390 395 400 Ser Ile Met Ser Gly Gly Ser Trp Ala Gly Lys Ile Ala Gly Thr Glu 405 410 415 Pro Thr Ser Phe Ser Pro Gln Asn Lys Glu Phe Phe Gln Lys Asn Met  $420 \hspace{1.5cm} 425 \hspace{1.5cm} 430$ Lys Gly Asn Trp Ala Asn Ile Leu Glu Val Asp Tyr Asp Lys Leu Ser 435 440 445 Lys Gly Ile Gly Val Ala Thr Tyr Val Asp Gln Ser Thr Thr Lys Ser 450 460 Lys Arg Pro Gly Ile Val Arg Val Asn Leu Pro Asp Lys Asp Ile Lys Asn Ile Glu Ser Ala Phe Gly Lys Lys Phe Tyr Tyr Ser Thr Lys Gly 485 490 495 Asn Asp Ile His Thr Thr Leu Glu Thr Pro Val Phe Asp Leu Thr Asn

Page 71

BCS149058WO\_ST25.txt

Ala Lys Asp Ala Lys Phe Asp Tyr Lys Ala Phe Tyr Glu Leu Glu Ala 515 520 525

Lys Tyr Asp Phe Leu Asp Val Tyr Ala Ile Ala Glu Asp Gly Thr Lys 530 540

Thr Arg Ile Asp Arg Met Gly Glu Lys Asp Ile Lys Gly Gly Ala Asp 545 550 555 560

Thr Thr Asp Gly Lys Trp Val Asp Lys Ser Tyr Asp Leu Ser Gln Phe 565 570 575

Lys Gly Lys Lys Val Lys Leu Gln Phe Glu Tyr Leu Thr Asp Ile Ala 580 585 590

Val Ala Tyr Lys Gly Phe Ala Leu Asp Asn Ala Ala Leu Thr Val Asp 595 600 605

Gly Lys Val Val Phe Ser Asp Asp Ala Glu Gly Gln Pro Ala Met Thr 610 620

Leu Lys Gly Phe Thr Val Ser Asn Gly Phe Glu Gln Lys Lys His Asn 625 635 640

Tyr Tyr Val Glu Trp Arg Asn Tyr Ala Gly Ser Asp Thr Ala Leu Gln 645 650 655

Tyr Ala Arg Gly Pro Val Phe Asn Thr Gly Met Val Val Trp Tyr Ala 660 665 670

Asp Gln Ser Phe Thr Asp Asn Trp Val Gly Val His Pro Gly Glu Gly 675 680 685

Phe Leu Gly Val Val Asp Ser His Pro Glu Ala Ile Val Gly Thr Leu 690 695 700

Asn Gly Gln Pro Thr Val Lys Ser Ser Thr Arg Tyr Gln Ile Ala Asp 705 710 715 720

Ala Ala Phe Ser Phe Asp Gln Thr Pro Ala Trp Lys Val Asn Ser Pro 725 730 735

Thr Arg Gly Ile Phe Asp Tyr Lys Gly Leu Pro Gly Val Ala Lys Phe 740 745 750

Asp Asp Ser Lys Gln Tyr Ile Asn Ser Val Ile Pro Asp Ala Gly Arg 755 760 765 Page 72

# BCS149058WO\_ST25.txt

Lys Leu Pro Lys Leu Gly Leu Lys Phe Glu Val Val Gly Gln Ala Glu 770 775 780	
Asp Lys Ser Ala Gly Ala Val Trp Leu His Arg 785 790 795	
<210> 85 <211> 169 <212> DNA <213> Bacillus anthracis	
<400> 85 taatcaccct cttccaaatc aatcatatgt tatacatata ctaaactttc catttttta	60
aattgttcaa gtagtttaag atttcttttc aataattcaa atgtccgtgt cattttcttt	120
cggttttgca tctactatat aatgaacgct ttatggaggt gaatttatg	169
<210> 86 <211> 303 <212> DNA <213> Bacillus anthracis	
<400> 86 atttattca ttcaatttt cctatttagt acctaccgca ctcacaaaaa gcacctctca	60
ttaatttata ttatagtcat tgaaatctaa tttaatgaaa tcatcatact atatgtttta	120
taagaagtaa aggtaccata cttaattaat acatatctat acacttcaat atcacagcat	180
gcagttgaat tatatccaac tttcatttca aattaaataa gtgcctccgc tattgtgaat	240
gtcatttact ctccctacta catttaataa ttatgacaag caatcatagg aggttactac	300
atg	303
<210> 87 <211> 173 <212> DNA <213> Bacillus anthracis	
<400> 87 aattacataa caagaactac attagggagc aagcagtcta gcgaaagcta actgcttttt	60
tattaaataa ctattttatt aaatttcata tatacaatcg cttgtccatt tcatttggct	120
ctacccacgc atttactatt agtaatatga atttttcaga ggtggatttt att	173
<210> 88 <211> 124	
<212> DNA <213> Bacillus weihenstephensis	
<400> 88 ctatgattta agatacacaa tagcaaaaga gaaacatatt atataacgat aaatgaaact	60

Page 73

BCS149058WO_ST25.txt tatgtatatg tatggtaact gtatatatta ctacaataca gtatactcat aggaggtagg	120
tatg	124
<210> 89 <211> 376 <212> DNA <213> Bacillus weihenstephensis	
<400> 89 ggtaggtaga tttgaaatat gatgaagaaa aggaataact aaaaggagtc gatatccgac	60
tccttttagt tataaataat gtggaattag agtataattt tatataggta tattgtatta	120
gatgaacgct ttatccttta attgtgatta atgatggatt gtaagagaag gggcttacag	180
tcctttttt atggtgttct ataagccttt ttaaaagggg taccacccca cacccaaaaa	240
cagggggggt tataactaca tattggatgt tttgtaacgt acaagaatcg gtattaatta	300
ccctgtaaat aagttatgtg tatataaggt aactttatat attctcctac aataaaataa	360
aggaggtaat aaagtg	376
<210> 90 <211> 225 <212> DNA <213> Bacillus thuringiensis	
<400> 90 aacccttaat gcattggtta aacattgtaa agtctaaagc atggataatg ggcgagaagt	60
aagtagattg ttaacaccct gggtcaaaaa ttgatattta gtaaaattag ttgcactttg	120
tgcatttttt cataagatga gtcatatgtt ttaaattgta gtaatgaaaa acagtattat	180
atcataatga attggtatct taataaaaga gatggaggta actta	225
<210> 91 <211> 125 <212> DNA <213> Bacillus thuringiensis	
<400> 91 taattccacc ttcccttatc ctctttcgcc tatttaaaaa aaggtcttga gattgtgacc	60
aaatctcctc aactccaata tcttattaat gtaaatacaa acaagaagat aaggagtgac	120
attaa	125
<210> 92 <211> 144 <212> DNA <213> Bacillus thuringiensis	
<400> 92 aggatgtctt tttttatatt gtattatgta catccctact atataaattc cctgctttta	60
tcgtaagaat taacgtaata tcaaccatat cccgttcata ttgtagtagt gtatgtcaga Page 74	120

# BCS149058WO\_ST25.txt

actcacgaga aggagtgaac ataa	144
<210> 93 <211> 126 <212> DNA <213> Bacillus thuringiensis	
<400> 93 ttaatgtcac tccttatctt cttgtttgta tttacattaa taagatattg gagttgagga	60
gatttggtca caatctcaag acctttttt taaataggcg aaagaggata agggaaggtg	120
gaatta	126
<210> 94 <211> 103 <212> DNA <213> Bacillus thuringiensis	
<400> 94 atatatttc ataatacgag aaaaagcgga gtttaaaaga atgagggaac ggaaataaag	60
agttgttcat atagtaaata gacagaattg acagtagagg aga	103
<210> 95 <211> 169 <212> DNA <213> Bacillus thuringiensis	
<400> 95 aaactaaata atgagctaag catggattgg gtggcagaat tatctgccac ccaatccatg	60
cttaacgagt attattatgt aaatttctta aaattgggaa cttgtctaga acatagaacc	120
tgtccttttc attaactgaa agtagaaaca gataaaggag tgaaaaaca	169
<210> 96 <211> 111 <212> DNA <213> Bacillus thuringiensis	
<400> 96 attcactaca acggggatga gtttgatgcg gatacatatg agaagtaccg gaaagtgttt	60
gtagaacatt acaaagatat attatctcca tcataaagga gagatgcaaa g	111
<210> 97 <211> 273 <212> DNA <213> Bacillus anthracis	
<400> 97 cgcgcaccac ttcgtcgtac aacaacgcaa gaagaagttg gggatacagc agtattctta	60
ttcagtgatt tagcacgcgg cgtaacagga gaaaacattc acgttgattc agggtatcat	120
atcttaggat aaatataata ttaattttaa aggacaatct ctacatgttg agattgtcct Page 75	180

# BCS149058WO\_ST25.txt

ttttatttgt tcttagaaag aacgattttt aacgaaagtt cttaccacgt tatgaatata 240
agtataatag tacacgattt attcagctac gta 27
<210> 98 <211> 303 <212> DNA <213> Bacillus anthracis
<400> 98 tgaagtatct agagctaatt tacgcaaagg aatctcagga caacactttc gcaacaccta 60
tattttaaat ttaataaaaa aagagactcc ggagtcagaa attataaagc tagctgggtt 120
caaatcaaaa atttcactaa aacgatatta tcaatacgca gaaaatggaa aaaacgcctt 180
atcataaggc gtttttcca tttttcttc aaacaaacga ttttactatg accatttaac 240
taatttttgc atctactatg atgagtttca ttcacattct cattagaaag gagagattta 300
atg 30:
<210> 99 <211> 240 <212> DNA <213> Bacillus anthracis
<400> 99 tatatcatat gtaaaattag ttcttattcc cacatatcat atagaatcgc catattatac 60
atgcagaaaa ctaagtatgg tattattctt aaattgttta gcaccttcta atattacaga 120
tagaatccgt cattttcaac agtgaacatg gatttcttct gaacacaact ctttttcttt 180
ccttatttcc aaaaagaaaa gcagcccatt ttaaaatacg gctgcttgta atgtacatta 240
<210> 100 <211> 267 <212> DNA <213> Bacillus thuringiensis
<400> 100 tatcacataa ctctttattt ttaatatttc gacataaagt gaaactttaa tcagtggggg 60
ctttgttcat cccccactg attattaatt gaaccaaggg ataaaaagat agagggtctg 120
accagaaaac tggagggcat gattctataa caaaaagctt aatgtttata gaattatgtc 180
tttttatata gggagggtag taaacagaga tttggacaaa aatgcaccga tttatctgaa 240
ttttaagttt tataaagggg agaaatg 26
<210> 101 <211> 124 <212> DNA <213> Bacillus thuringiensis <400> 101

Page 76

attttttact	tagcagtaaa	actgatatca	BCS149058WO_ gttttactgc		ttaaattcaa	60
tcattaaatc	ttccttttct	acatagtcat	aatgttgtat	gacattccgt	aggaggcact	120
tata						124
<210> 102 <211> 170 <212> DNA <213> Bac	illus thurin	ngiensis				
<400> 102 acataaattc	acctccataa	agcgttcatt	atatagtaga	tgcaaaaccg	aaagaaaatg	60
acacggacat	ttgaattatt	gaaaagaaat	cttaaactac	ttgaacaatt	taaaaaaatg	120
gaaagtttag	tatatgtata	acatatgatt	gatttggaag	agggtgatta		170
<210> 103 <211> 212 <212> DNA <213> Bac	illus thurin	ngiensis				
<400> 103 ttctattttc	caacataaca	tgctacgatt	aaatggtttt	ttgcaaatgc	cttcttggga	60
agaaggatta	gagcgttttt	ttatagaaac	caaaagtcat	taacaatttt	aagttaatga	120
cttttttgtt	tgcctttaag	aggttttatg	ttactataat	tatagtatca	ggtactaata	180
acaagtataa	gtatttctgg	gaggatatat	ca			212
<210> 104 <211> 1500 <212> DNA <213> Bac	) illus subti <sup>-</sup>	lis				
<400> 104 atgaaacggt	caatctcgat	ttttattacg	tgtttattga	ttacgttatt	gacaatgggc	60
ggcatgatag	cttcgccggc	atcagcagca	gggacaaaaa	cgccagtagc	caagaatggc	120
cagcttagca	taaaaggtac	acagctcgtt	aaccgagacg	gtaaagcggt	acagctgaag	180
gggatcagtt	cacacggatt	gcaatggtat	ggagaatatg	tcaataaaga	cagcttaaaa	240
tggctgagag	atgattgggg	tatcaccgtt	ttccgtgcag	cgatgtatac	ggcagatggc	300
ggttatattg	acaacccgtc	cgtgaaaaat	aaagtaaaag	aagcggttga	agcggcaaaa	360
gagcttggga	tatatgtcat	cattgactgg	catatcttaa	atgacggtaa	tccaaaccaa	420
aataaagaga	aggcaaaaga	attcttcaag	gaaatgtcaa	gcctttacgg	aaacacgcca	480
aacgtcattt	atgaaattgc	aaacgaacca	aacggtgatg	tgaactggaa	gcgtgatatt	540
aaaccatatg	cggaagaagt	gatttcagtt	atccgcaaaa	atgatccaga	caacatcatc	600
attgtcggaa	ccggtacatg	gagccaggat	gtgaatgatg	ctgccgatga	ccagctaaaa	660

Page 77

gatgcaaacg ttatgtacgc		SCS149058WO_ tatgccggca		atttttacgg	720
gataaagcaa actatgcact	cagcaaagga	gcacctattt	ttgtgacaga	gtggggaaca	780
agcgacgcgt ctggcaatgg	cggtgtattc	cttgatcaat	cgagggaatg	gctgaaatat	840
ctcgacagca agaccattag	ctgggtgaac	tggaatcttt	ctgataagca	ggaatcatcc	900
tcagctttaa agccgggggc	atctaaaaca	ggcggctggc	ggttgtcaga	tttatctgct	960
tcaggaacat tcgttagaga	aaacattctc	ggcaccaaag	attcgacgaa	ggacattcct	1020
gaaacgccat caaaagataa	acccacacag	gaaaatggta	tttctgtaca	gtacagagca	1080
ggggatggga gtatgaacag	caaccaaatc	cgtccgcagc	ttcaaataaa	aaataacggc	1140
aataccacgg ttgatttaaa	agatgtcact	gcccgttact	ggtataaagc	gaaaaacaaa	1200
ggccaaaact ttgactgtga	ctacgcgcag	attggatgcg	gcaatgtgac	acacaagttt	1260
gtgacgttgc ataaaccaaa	gcaaggtgca	gatacctatc	tggaacttgg	atttaaaaac	1320
ggaacgttgg caccgggagc	aagcacaggg	aatattcagc	tccgtcttca	caatgatgac	1380
tggagcaatt atgcacaaag	cggcgattat	tcctttttca	aatcaaatac	gtttaaaaca	1440
acgaaaaaaa tcacattata	tgatcaagga	aaactgattt	ggggaacaga	accaaattag	1500
<210> 105					
<pre>&lt;211&gt; 852 &lt;212&gt; DNA &lt;213&gt; Bacillus thurin</pre>	giensis				
<212> DNA		gctattacat	tggttgctcc	attacaaagt	60
<212> DNA <213> Bacillus thurin <400> 105	tttagcggca				60 120
<212> DNA <213> Bacillus thurin <400> 105 atgaaaaaga aagtacttgc	tttagcggca tgatggggga	cagagatttg	gagttattcc	gcgctggtct	
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa</pre>	tttagcggca tgatggggga aggcgtgaat	cagagatttg tctcatttat	gagttattcc ggattgtaaa	gcgctggtct tcgtgcaatt	120
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta	cagagatttg tctcatttat aaacaagatc	gagttattcc ggattgtaaa gagttgcact	gcgctggtct tcgtgcaatt attaaatgaa	120 180
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat	cagagatttg tctcatttat aaacaagatc gctgctgact	gagttattcc ggattgtaaa gagttgcact atgaaaatcc	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat	120 180 240
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac	cagagatttg tctcatttat aaacaagatc gctgctgact cctgacaatg	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat	120 180 240 300
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa	cagagatttg tctcatttat aaacaagatc gctgctgact cctgacaatg tatttaaat	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa	120 180 240 300 360
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca gcaaagcagg caaaggaaac</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa attcttctat	cagagatttg tctcatttat aaacaagatc gctgctgact cctgacaatg tattttaaat ttaggattat	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga ctcttcatta	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa tctaggggat	120 180 240 300 360 420
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca gcaaagcagg caaaggaaac aataaagata tgcaacaagc</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa attcttctat agcaaacttt	cagagatttg tctcatttat aaacaagatc gctgctgact cctgacaatg tatttaaat ttaggattat acaaaccttt	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga ctcttcatta cgtatccaca	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa tctaggggat agggttccat	120 180 240 300 360 420 480
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca gcaaagcagg caaaggaaac aataaagata tgcaacaagc gtaaaccaac cgatgcatgc</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa attcttctat agcaaacttt	cagagatttg tctcatttat aaacaagatc gctgctgact cctgacaatg tatttaaat ttaggattat acaaaccttt aaagataact	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga ctcttcatta cgtatccaca ataaagtaac	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa tctaggggat agggttccat ggatggaaat	120 180 240 300 360 420 480 540
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca gcaaagcagg caaaggaaac aataaagata tgcaacaagc gtaaaccaac cgatgcatgc tctaaatatg aaaactttgt</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa attcttctat agcaaacttt agatacgata tacgaatcca	cagagattg tctcatttat aaacaagatc gctgctgact cctgacaatg tattttaaat ttaggattat acaaaccttt aaagataact gaagattgga	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga ctcttcatta cgtatccaca ataaagtaac ttcatggagc	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa tctaggggat agggttccat ggatggaaat ggcagtagtt	120 180 240 300 360 420 480 540 600
<pre>&lt;212&gt; DNA &lt;213&gt; Bacillus thurin &lt;400&gt; 105 atgaaaaaga aagtacttgc gttgcatttg ctcatgaaaa gctgaagata aacataaaga gatattatgt ctcgtaatac tggcgtactg agttagagaa aatagcacat ttgcttcaca gcaaagcagg caaaggaaac aataaagata tgcaacaagc gtaaaccaac cgatgcatgc tctaaatatg aaaactttgt ggatattgga actggaaagg</pre>	tttagcggca tgatggggga aggcgtgaat aacacttgta cggtatttat tttctatgac tggagctaaa attcttctat agcaaacttt agatacgata tacgaatcca cattgtaaat	cagagattg tctcatttat aaacaagatc gctgctgact cctgacaatg tatttaaat ttaggattat acaaaccttt aaagataact gaagattgga gataatacga	gagttattcc ggattgtaaa gagttgcact atgaaaatcc ggaaaactta tagctggtga ctcttcatta cgtatccaca ataaagtaac ttcatggagc aagattggtt	gcgctggtct tcgtgcaatt attaaatgaa ttattatgat tattccgtat gtcttacaaa tctaggggat agggttccat ggatggaaat ggcagtagtt cgtgagagct	120 180 240 300 360 420 480 540 600 660

Page 78

BCS149058WO_ST25.txt ggagatcgtt aa	852
<210> 106 <211> 729 <212> DNA <213> Bacillus subtilis	
<400> 106	
gcgggactga ataaagatca aaagcgccgg gcggaacagc tgacaagtat ctttgaaaac	60
ggcacaacgg agatccaata tggatatgta gagcgattgg atgacgggcg aggctataca	120
tgcggacggg caggctttac aacggctacc ggggatgcat tggaagtagt ggaagtatac	180
acaaaggcag ttccgaataa caaactgaaa aagtatctgc ctgaattgcg ccgtctggcc	240
aaggaagaaa gcgatgatac aagcaatctc aagggattcg cttctgcctg gaagtcgctt	300
gcaaatgata aggaatttcg cgccgctcaa gacaaagtaa atgaccattt gtattatcag	360
cctgccatga aacgatcgga taatgccgga ctaaaaacag cattggcaag agctgtgatg	420
tacgatacgg ttattcagca tggcgatggt gatgaccctg actctttta tgccttgatt	480
aaacgtacga acaaaaaagc gggcggatca cctaaagacg gaatagacga gaagaagtgg	540
ttgaataaat tcttggacgt acgctatgac gatctgatga atccggccaa tcatgacacc	600
cgtgacgaat ggagagaatc agttgcccgt gtggacgtgc ttcgctctat cgccaaggag	660
aacaactata atctaaacgg accgattcat gttcgttcaa acgagtacgg taattttgta	720
atcaaataa	729
	, 23
<210> 107 <211> 499 <212> PRT <213> Bacillus subtilis	
<400> 107	
Met Lys Arg Ser Ile Ser Ile Phe Ile Thr Cys Leu Leu Ile Thr Leu 1 10 15	
Leu Thr Met Gly Gly Met Ile Ala Ser Pro Ala Ser Ala Ala Gly Thr 20 25 30	
Lys Thr Pro Val Ala Lys Asn Gly Gln Leu Ser Ile Lys Gly Thr Gln 35 40 45	
Leu Val Asn Arg Asp Gly Lys Ala Val Gln Leu Lys Gly Ile Ser Ser 50 55 60	
His Gly Leu Gln Trp Tyr Gly Glu Tyr Val Asn Lys Asp Ser Leu Lys 65 70 75 80	

Page 79

BCS149058WO\_ST25.txt Trp Leu Arg Asp Asp Trp Gly Ile Thr Val Phe Arg Ala Ala Met Tyr 85 90 95 Thr Ala Asp Gly Gly Tyr Ile Asp Asn Pro Ser Val Lys Asn Lys Val Lys Glu Ala Val Glu Ala Ala Lys Glu Leu Gly Ile Tyr Val Ile Ile Asp Trp His Ile Leu Asn Asp Gly Asn Pro Asn Gln Asn Lys Glu Lys Ala Lys Glu Phe Phe Lys Glu Met Ser Ser Leu Tyr Gly Asn Thr Pro Asn Val Ile Tyr Glu Ile Ala Asn Glu Pro Asn Gly Asp Val Asn Trp Lys Arg Asp Ile Lys Pro Tyr Ala Glu Glu Val Ile Ser Val Ile Arg Lys Asn Asp Pro Asp Asn Ile Ile Ile Val Gly Thr Gly Trp Ser 195 200 205 Gln Asp Val Asn Asp Ala Ala Asp Asp Gln Leu Lys Asp Ala Asn Val 210 215 220 Met Tyr Ala Leu His Phe Tyr Ala Gly Thr His Gly Gln Phe Leu Arg Asp Lys Ala Asn Tyr Ala Leu Ser Lys Gly Ala Pro Ile Phe Val Thr 245 250 255 Glu Trp Gly Thr Ser Asp Ala Ser Gly Asn Gly Gly Val Phe Leu Asp 260 265 270 Gln Ser Arg Glu Trp Leu Lys Tyr Leu Asp Ser Lys Thr Ile Ser Trp 275 280 285 Val Asn Trp Asn Leu Ser Asp Lys Gln Glu Ser Ser Ser Ala Leu Lys 290 Pro Gly Ala Ser Lys Thr Gly Gly Trp Arg Leu Ser Asp Leu Ser Ala Ser Gly Thr Phe Val Arg Glu Asn Ile Leu Gly Thr Lys Asp Ser Thr

Page 80

BCS149058WO\_ST25.txt

Lys Asp Ile Pro Glu Thr Pro Ser Lys Asp Lys Pro Thr Gln Glu Asn 340 345 350

Gly Ile Ser Val Gln Tyr Arg Ala Gly Asp Gly Ser Met Asn Ser Asn 355 360 365

Gln Ile Arg Pro Gln Leu Gln Ile Lys Asn Asn Gly Asn Thr Thr Val 370 375 380

Asp Leu Lys Asp Val Thr Ala Arg Tyr Trp Tyr Lys Ala Lys Asn Lys 385 390 395 400

Gly Gln Asn Phe Asp Cys Asp Tyr Ala Gln Ile Gly Cys Gly Asn Val 405 410 415

Thr His Lys Phe Val Thr Leu His Lys Pro Lys Gln Gly Ala Asp Thr 420 425 430

Tyr Leu Glu Leu Gly Phe Lys Asn Gly Thr Leu Ala Pro Gly Ala Ser 435 440 445

Thr Gly Asn Ile Gln Leu Arg Leu His Asn Asp Asp Trp Ser Asn Tyr 450 455 460

Ala Gln Ser Gly Asp Tyr Ser Phe Phe Lys Ser Asn Thr Phe Lys Thr 465 470 475 480

Thr Lys Lys Ile Thr Leu Tyr Asp Gln Gly Lys Leu Ile Trp Gly Thr 485 490 495

Glu Pro Asn

<210> 108

<211> 283

PRT

<212>

<213> Bacillus thuringiensis

<400> 108

Met Lys Lys Lys Val Leu Ala Leu Ala Ala Ala Ile Thr Leu Val Ala 1 5 10 15

Pro Leu Gln Ser Val Ala Phe Ala His Glu Asn Asp Gly Gln Arg 20 25 30

Phe Gly Val Ile Pro Arg Trp Ser Ala Glu Asp Lys His Lys Glu Gly 35 40 45

Page 81

BCS149058WO\_ST25.txt

Val Asn Ser His Leu Trp Ile Val Asn Arg Ala Ile Asp Ile Met Ser 50 60

Arg Asn Thr Thr Leu Val Lys Gln Asp Arg Val Ala Leu Leu Asn Glu 65 70 75 80

Trp Arg Thr Glu Leu Glu Asn Gly Ile Tyr Ala Ala Asp Tyr Glu Asn 85 90 95

Pro Tyr Tyr Asp Asn Ser Thr Phe Ala Ser His Phe Tyr Asp Pro Asp 100 105 110

Asn Gly Lys Thr Tyr Ile Pro Tyr Ala Lys Gln Ala Lys Glu Thr Gly 115 120

Ala Lys Tyr Phe Lys Leu Ala Gly Glu Ser Tyr Lys Asn Lys Asp Met 130 140

Gln Gln Ala Phe Phe Tyr Leu Gly Leu Ser Leu His Tyr Leu Gly Asp 145 150 155 160

Val Asn Gln Pro Met His Ala Ala Asn Phe Thr Asn Leu Ser Tyr Pro 165 170 175

Gln Gly Phe His Ser Lys Tyr Glu Asn Phe Val Asp Thr Ile Lys Asp 180 185 190

Asn Tyr Lys Val Thr Asp Gly Asn Gly Tyr Trp Asn Trp Lys Gly Thr 195 200 205

Asn Pro Glu Asp Trp Ile His Gly Ala Ala Val Val Ala Lys Gln Asp 210 215 220

Tyr Ala Gly Ile Val Asn Asp Asn Thr Lys Asp Trp Phe Val Arg Ala 225 230 235 240

Ala Val Ser Gln Glu Tyr Ala Asp Lys Trp Arg Ala Glu Val Thr Pro 245 250 255

Met Thr Gly Lys Arg Leu Met Asp Ala Gln Arg Val Thr Ala Gly Tyr 260 265 270

Ile Gln Leu Trp Phe Asp Thr Tyr Gly Asp Arg 275 280

<210> 109 <211> 244

Page 82

BCS149058WO\_ST25.txt

<212> PRT

<213> Bacillus subtilis

<400> 109

Leu Glu Ala Gly Leu Asn Lys Asp Gln Lys Arg Arg Ala Glu Gln Leu 1 5 10 15

Thr Ser Ile Phe Glu Asn Gly Thr Thr Glu Ile Gln Tyr Gly Tyr Val 20 25 30

Glu Arg Leu Asp Asp Gly Arg Gly Tyr Thr Cys Gly Arg Ala Gly Phe 35 40 45

Thr Thr Ala Thr Gly Asp Ala Leu Glu Val Val Glu Val Tyr Thr Lys 50 60

Ala Val Pro Asn Asn Lys Leu Lys Lys Tyr Leu Pro Glu Leu Arg Arg 65 70 75 80

Leu Ala Lys Glu Glu Ser Asp Asp Thr Ser Asn Leu Lys Gly Phe Ala 85 90 95

Ser Ala Trp Lys Ser Leu Ala Asn Asp Lys Glu Phe Arg Ala Ala Gln 100 105 110

Asp Lys Val Asn Asp His Leu Tyr Tyr Gln Pro Ala Met Lys Arg Ser 115 120 125

Asp Asn Ala Gly Leu Lys Thr Ala Leu Ala Arg Ala Val Met Tyr Asp 130 140

Thr Val Ile Gln His Gly Asp Gly Asp Asp Pro Asp Ser Phe Tyr Ala 145 150 155 160

Leu Ile Lys Arg Thr Asn Lys Lys Ala Gly Gly Ser Pro Lys Asp Gly 165 170 175

Ile Asp Glu Lys Lys Trp Leu Asn Lys Phe Leu Asp Val Arg Tyr Asp 180 185 190

Asp Leu Met Asn Pro Ala Asn His Asp Thr Arg Asp Glu Trp Arg Glu 195 200 205

Ser Val Ala Arg Val Asp Val Leu Arg Ser Ile Ala Lys Glu Asn Asn 210 215 220

Tyr Asn Leu Asn Gly Pro Ile His Val Arg Ser Asn Glu Tyr Gly Asn 225 230 235 240 Page 83

BCS149058WO\_ST25.txt

Phe Val Ile Lys

**RECORDED: 06/12/2018** 

Page 84