APPENDIX 1 - ESTIMATED DAILY INTAKE – *BACILLUS SUBTILIS* R0179

Exponent

Estimated Daily Intake of *Bacillus subtilis* R0179 Based on Proposed Uses in Select Food Categories

Prepared for

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Prepared by

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October 21, 2019

Introduction

At the request of Lallemand, Inc (Lallemand), Exponent®, Inc. (Exponent) estimated the daily intake of *Bacillus subtilis* R0179 by the U.S. population age 2 years and older based on the proposed uses of the organism at a maximum level of 1 x 10^9 colony forming units (CFU) per serving, as consumed, in a total of 11 food categories as defined in 21 CFR §170.3. The estimated daily intake (EDI) from the proposed uses was based on the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2013-2014 and 2015-2016 (NHANES 2013-2016). Estimates of intake will be used to support Lallemand's preparation of a Generally Recognized As Safe (GRAS) determination in the United States (U.S.) for the use of *Bacillus subtilis* R0179 in select categories of foods. The data and methods used to conduct the intake assessment and results are summarized in this report.

Materials and Methods

Intended Uses

Lallemand intends to use *Bacillus subtilis* R0179 as an ingredient in select categories of food at a maximum level of 1 x 10^9 colony forming units (CFU) *Bacillus subtilis* R0179 per serving, as consumed. The intended uses of *Bacillus subtilis* R0179 include addition to the following 11 food categories as defined in 21 CFR §170.3: (n)(1) baked goods and baking mixes; (n)(3) beverage and beverage bases; (n)(4) breakfast cereals; (n)(6) chewing gum; (n)(9) confections and frostings; (n)(10) dairy product analogs; (n)(21) fruit and water ices; (n)(32) nuts and nut products; (n)(33) plant protein products; (n)(35) processed fruits and fruit juices; and (n)(37) snack foods. The proposed food use categories, a description of foods included in each use category, and the serving size of foods in each use category are listed in Table 1 below.

Table 1. Intended Uses of *Bacillus subtilis* R0179

21 CFR §170.3 Food Categories Proposed for Addition of *Bacillus subtilis* R0179			Description of Proposed Uses	Serving Size (as consumed)[a]
1	Baked goods and baking mixes	All ready-to-eat and ready-to-bake products, flours, and mixes requiring preparation before serving	Biscuits, cornbread, scones, hush puppies, crumpets; cakes, lightweight; coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads; croissants; English muffins; pizza crust; soft pretzels; tortillas	55 g
			Breads (excluding sweet quick type), rolls	50 g
			Brownies; grain-based bars	40 g
			Cakes, mediumweight	80 g
			Cookies; crackers that are usually used as snacks; taco shells, hard	30 g
			Crackers that are usually not used as snack, melba toast, hard bread sticks, ice cream cones	15 g
			Croutons	7 g
			French toast, crepes, pancakes, variety mixes; bagels; muffins	110 g
			Pies, cobblers, fruit crisps, turnovers, other pastries; cakes, heavyweight	125 g
			Waffles	85 g

21 CFR §170.3 Food Categories Proposed for Addition of *Bacillus subtilis* R0179			Description of Proposed Uses	Serving Size (as consumed)[a]
3	Beverage and beverage bases	Non-alcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks	Carbonated and noncarbonated beverages, water	360 ml
			Juices, nectars, fruit drinks	240 ml
4	Breakfast cereals	Ready-to-eat and instant and regular hot cereals	Breakfast cereals (hot cereal type), hominy grits	1 cup prepared
			Breakfast cereals, ready-to-eat, weighing less than 20 g per cup	15 g
			Breakfast cereals, ready-to-eat, weighing 20 g or more but less than 43 g per cup; high fiber cereals containing 28 g or more of fiber per 100 g	40 g
			Breakfast cereals, ready-to-eat, weighing 43 g or more per cup	60 g
6	Chewing gum	Chewing gum, all forms	Chewing gum	3 g
9	Confections and frosting	Candy and flavored frostings, marshmallows, baking chocolate, and brown, lump, rock, maple, powdered, and raw sugars	Baking candies	15 g
			Baking decorations (e.g., colored sugars and sprinkles for cookies, cake decorations)	4 g
			Confectioner's sugar, marshmallows	30 g
			Frosting or icing	2 tbsp
			Sugar	8 g
10	Dairy product analogs	Nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products	Cream or cream substitutes, fluid	15 mL
			Cream or cream substitutes, powder	2 g
			Milk-substitute beverages	240 mL
			Non-dairy whipped toppings	2 tbsp
21	Fruit and water ices	All frozen fruit and water ices	Frozen flavored and sweetened ice and pops	2/3 cup
32	Nuts and nut products	Whole or shelled tree nuts, peanuts, coconut, and nut and peanut spreads	Nut butters, pastes, or creams	2 tbsp
			Nuts, mixtures, all types	30 g
33	Plant protein products	"Reconstituted vegetable protein" category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins	Bacon substitutes	15 g
			Cheese, all others	30 g
			Mixed dishes: Measurable with cup	1 cup
			Mixed dishes: Not measurable with cup	140 g
			Substitute for luncheon meat, meat spreads, Canadian bacon, sausages, frankfurters, and seafood	55 g
			Tofu, tempeh	85 g
			Yogurt	170 g

21 CFR §170.3 Food Categories Proposed for Addition of *Bacillus subtilis* R0179			Description of Proposed Uses	Serving Size (as consumed)[a]
35	Processed fruits and fruit juices	All commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom	All other fruits (except those listed as separate categories), fresh, canned or frozen	140 g
			Candied or picked fruit	30 g
			Dried fruit	40 g
			Fruit relish	70 g
			Fruits for garnish or flavor (e.g., maraschino cherries)	4 g
			Juices used as ingredients (e.g., lemon juice, lime juice)	5 mL
			Juices, nectars, fruit drinks	240 mL
37	Snack foods	Chips, pretzels, and other novelty snacks	All varieties, chips, pretzels, popcorn, extruded snacks, fruit and vegetable-based snacks (e.g., fruit chips), grain-based snack mixes	30 g

[a] Serving size correspond to values in Table 2 – Reference Amounts Customarily Consumed per Eating Occasion: General Food Supply as cited in FR Vol 81, No. 103, Friday, May 27, 2016, pp 34000-47. Available at: https://www.govinfo.gov/content/pkg/FR-2016-05-27/pdf/2016-11865.pdf. Serving size as a volume measure (e.g., cup, mL, tbsp) assumed that 1 mL = 1 g.

Consumption Data

The estimated daily intake (EDI) of *Bacillus subtilis* R0179 from the proposed food uses were based on food consumption records collected in the What We Eat in America (WWEIA) component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2013-2014 and 2015-2016 (NHANES 2013-2016). This continuous survey is a complex multistage probability sample designed to be representative of the civilian US population (NCHS 2016, 2018). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection.

NHANES 24-Hour Recall

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first

dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 13,600 individuals age two years and older in the survey period 2013-2016 provided 2 complete days of dietary recalls.

Representative NHANES Foods

Exponent reviewed all foods reported consumed during NHANES 2013-2016 for foods representative of the proposed uses of *Bacillus subtilis* R0179. The USDA Food and Nutrient Database for Dietary Studies (FNDDS) recipes were utilized to identify the weight of ingredients in foods which allowed for the estimation of the foods with proposed uses of *Bacillus subtilis* R0179 that can be consumed as is or as a component in a food (e.g., bread component in sandwiches, tortilla component in burritos, carbonated beverage component in alcoholic mixed drinks, milk substitute component in coffee beverages, peanut butter component in sandwiches, etc.). The FNDDS version 2015-2016 recipes (corresponding to dietary consumption for NHANES 2015-2016) (USDA 2018) were applied to process dietary recall data reported in NHANES 2013-2016 and FNDDS version 2013-2014 recipes (corresponding to dietary consumption for NHANES 2013-2014) (USDA 2016) for foods that were only reported consumed in NHANES 2013-2014.

A summary of the foods included in the analysis is provided in Table 1 and the listing of foods identified as representative of the proposed uses and used to complete the analysis is presented in Appendix A.

Analysis

For each NHANES survey respondent, estimates of all proposed food uses in units of servings were calculated from the diet recall records by dividing the gram weight of each relevant portion of food consumed by its corresponding serving size. Intakes from all proposed use categories

combined were summed for each survey respondent and the 2-day average intake was calculated by dividing the sum by two. Estimates of food servings at the mean and 90th percentile of intake were calculated for the total population age 2 years and older. Estimates of *Bacillus subtilis* R0179 intake from the proposed uses were calculated by multiplying the maximum proposed concentration of *Bacillus subtilis* R0179, i.e., 1 x 10^9 CFU *Bacillus subtilis* R0179 per serving, by the number of servings consumed by each subpopulation.

The 2-day average intakes were estimated using Exponent's Foods Analysis and Residues Evaluation Program (FARE® version 13.04) software and provided on a *per capita* and *per user* basis. All estimates were generated with statistical weights provided by the NCHS for the surveys to adjust for the differential probabilities of selection.

Results

The estimated daily intakes (EDIs) of *Bacillus subtilis* R0179 based on the intended use of up to 1 x 10^9 CFU/serving in a total of 11 broad food categories as defined in 21 CFR §170.3 are presented in Table 2. The EDIs represent potential intakes of *Bacillus subtilis* R0179 per day by the U.S. population age 2 years and older.

During the two days of dietary recall, approximately 100% of the U.S. population age 2 years and older reported consumption of one or more of the foods to which Lallemand intends to add *Bacillus subtilis* R0179. The 2-day average EDI of *Bacillus subtilis* R0179 at the mean and 90th percentile of intake is 5.6 x 10^9 CFU/day and 9.6 x 10^9 CFU/day, respectively.

Table 2. Two-day Average Estimated Daily Intake of *Bacillus subtilis* R0179 by the U.S. Population 2 years and Older Based on Intended Uses in Select Categories of Foods; NHANES 2013-2016

			Bacillus subtilis R0179 (CFU/day)			
			Per Capita		Per User	
Population	N	% Users	Mean	90th Percentile	Mean	90th Percentile
U.S. population 2+ y	13,561	100	5.5E+09	9.6E+09	5.6E+09	9.6E+09

References

National Center for Health Statistics (NCHS). 2016. National Health and Nutrition Examination Survey Data 2013-2014. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available from: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2013.

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U.S. Department of Agriculture, Agricultural Research Service. 2016. USDA Food and Nutrient Database for Dietary Studies 2013-2014. Food Surveys Research. Available at: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds/.

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APPENDIX 3 - TECHNICAL DATA SHEET AND CERTIFICATE OF ANALYSIS

LALLEMAND HEALTH SOLUTIONS — **Technical Data Sheet**

Bacillus subtilis Rosell-179 – 25, powder

Description

Product code	76649-01-00 or 76649-21-00 (Sample)
Appearance	Fine to granular, ivory to beige powder
Ingredients	Lactose, Freeze dried probiotic (*Bacillus subtilis* Rosell-179*), and ascorbic acid (antioxidant) ** Contains milk products and traces of soy products*

Microbial standards

Concentration	≥ 25 x 10^9 CFU/g
Coliforms	< 10 / g
E. coli	Abs / g
Salmonella ssp.	Abs / 25g
Yeast and molds	< 1000 /g

Packaging and storage

Packaging	Bulk powder in 10 kg sealed laminated bag (76649-01-00)
Storage	*B. subtilis* Rosell-179 – 25 powder may be stored for 24 months from the date of manufacture in the unopened original container, in a dry place and at a temperature between 4°C and 8°C.
Shelf Life	*B. subtilis* Rosell-179 – 25, powder is expected to have a residual cell count of at least 25 x 10^9 CFU/g after 24 months when stored at temperatures between 4°C and 8°C in the original sealed packaging.

TDS B. subtilis Rosell-179-25_76649-01-00_2018 08 23

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healthsolutions@lallemand.com

LALLEMAND HEALTH SOLUTIONS

Technical Data Sheet

Bacillus subtilis Rosell-179 – 100, powder

Description

Product code	76475-01-01 or 76475-21-00 (sample)
Appearance	Fine to granular grey-beige powder Strong and specific odour
Ingredients	Lactose, Freeze dried probiotic (*Bacillus subtilis* Rosell-179*), and ascorbic acid (antioxidant) ** Contains milk products and traces of soy products*

Microbial standards

Concentration	≥ 100 x 10^9 CFU/g
Coliforms	< 10 / g
E. coli	Abs / g
Salmonella ssp.	Abs / 25g
Yeast and molds	< 1000 /g

Packaging and storage

Packaging	Bulk powder in 10 kg sealed laminated bag (76475-01-01)
Storage	*B. subtilis* Rosell-179 – 100 powder may be stored for 24 months from the date of manufacture in the unopened original container, in a dry place and at a temperature between 4°C and 8°C
Shelf Life	*B. subtilis* Rosell-179 – 100, powder is expected to have a residual cell count of at least 100 x 10^9 CFU/g after 24 months when stored at 4°C or a residual cell count of at least 80 x 10^9 CFU/g after 24 months when stored at temperatures ≤ 25°C in the original sealed packaging

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Bacillus Subtilis R0179 STA – 25 billion

INSTITUT ROSELL LALLEMAND

CERTIFICATE OF ANALYSIS

PRODUCT:	BACILLUS SUBTILIS R0179 - 25, POWDER
LOT:	LE0938
CODE:	76649-01-00
DATE OF ANALYSIS:	2019/05/21
MANUFACTURING DATE:	2019/05/21
BEST BEFORE:	2021/05/20

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count (at manufacturing)	≥ 25,0 X 10^9/g	40,4 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg /g	Neg /g	NF ISO 7251
Salmonella	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	< 5/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, ivory to beige powder

Signed: [redacted]

Eric Guevara
Supervisor, Quality Control

Date: 2019-06-04

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INSTITUT ROSELL LALLEMAND

CERTIFICATE OF ANALYSIS

PRODUCT:	BACILLUS SUBTILIS R0179 - 25, POWDER
LOT:	LD0643
CODE:	76649-01-00
DATE OF ANALYSIS:	2019/04/17
MANUFACTURING DATE:	2019/04/17
BEST BEFORE:	2021/04/16

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count (at manufacturing)	$\geq$ 25,0 X 10^9/g	36,9 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg /g	Neg /g	NF ISO 7251
Salmonella	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	150/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, ivory to beige powder

Signed: [redacted]

Eric Guevara
Supervisor, Quality Control

Date: 2019-05-17

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INSTITUT ROSELL LALLEMAND

CERTIFICATE OF ANALYSIS

PRODUCT:	BACILLUS SUBTILIS R0179 - 25, POWDER
LOT:	LD0767
CODE:	76649-01-00
DATE OF ANALYSIS:	2019/04/17
MANUFACTURING DATE:	2019/04/17
BEST BEFORE:	2021/04/16

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count (at manufacturing)	$\geq$ 25,0 X 10^9/g	36,9 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg /g	Neg /g	NF ISO 7251
Salmonella	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	150/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, ivory to beige powder

Signed: [redacted]

Eric Guevara
Supervisor, Quality Control

Date: 2019-05-17

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Bacillus Subtilis R0179 STA – 100 billion

INSTITUT ROSELL LALLEMAND

CERTIFICATE OF ANALYSIS

PRODUCT:	B. SUBTILIS R0179 – 100
LOT:	KI1157
CODE:	76475-01-01
DATE OF ANALYSIS:	2018/09/14
MANUFACTURING DATE:	2018/09/14
EXPIRY DATE:	2020/09/13

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count at manufacturing	≥ 100,0 X 10^9/g	148,8 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg/g	Neg/g	NF ISO 7521
Salmonella ssp.	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	75/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, grey - beige powder
Odour:	Strong and specific odour

Signed: [redacted]
Lucie Doyon
Director, Quality Control

Date: 2018-09-24

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CERTIFICATE OF ANALYSIS

PRODUCT:	B. SUBTILIS R0179 – 100
LOT:	LE0905
CODE:	76475-01-01
DATE OF ANALYSIS:	2019/05/21
MANUFACTURING DATE:	2019/05/21
EXPIRY DATE:	2021/05/20

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count at manufacturing	≥ 100,0 X 10^9/g	136,3 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg/g	Neg/g	NF ISO 7521
Salmonella ssp.	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	< 5/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, grey - beige powder
Odour:	Strong and specific odour

Signed: [redacted]

Eric Guevara
Supervisor, Quality Control

Date: 2019-06-03

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INSTITUT ROSELL LALLEMAND

CERTIFICATE OF ANALYSIS

PRODUCT:	B. SUBTILIS R0179 – 100
LOT:	KE0774
CODE:	76475-01-01
DATE OF ANALYSIS:	2018/05/17
MANUFACTURING DATE:	2018/05/17
EXPIRY DATE:	2020/05/16

TEST	SPECIFICATIONS	RESULTS	METHODS
Bacteriological count at manufacturing	≥ 100,0 X 10^9/g	108,8 X 10^9/g	QA118
Coliforms	< 10/g	< 10/g	NF ISO 4831
E. coli	Neg/g	Neg/g	NF ISO 7521
Salmonella ssp.	Neg/25g	Neg/25g	MFHPB-20
Yeast and Molds	< 1000/g	5/g	MFHPB-22

PHYSICAL ASPECT:

Aspect of Powder:	Fine to granular, grey - beige powder
Odour:	Strong and specific odour

Signed: [redacted]

Lucie Doyon
Director, Quality Control

Date: 2018-05-22

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APPENDIX 4 – HEAVY METALS STATEMENT

LALLEMAND HEALTH SOLUTIONS INC.

Mirabel, December 13, 2019

OBJECT : Heavy metals

Lallemand Health Solutions (Institut Rosell) does not perform heavy metals tests on each batch of probiotic formulas. Heavy metals compliance of our products is established based on a matrix approach which includes a compilation of heavy metal specifications and tests results of all our raw materials. Testing is also done on raw materials and bacterial cultures (the active ingredients that we manufacture), and the analyses are selected according to the intended process and end-product. All these data enable us to calculate, based on product formulation, the worst case scenario for heavy metals content when a formula has never been tested directly.

Additionally, we compile data on finished products, from analytical testing that has been done randomly over time or following specific requests or requirements. Simulations using the matrix are reasonably accurate when compared with testing results. Data on finished products can also be used to assess the heavy metals potential level when compared to similar formulas.

This being said, among Lallemand Health Solutions manufacturing sites, many probiotic products have been tested for heavy metals and the results were always within the limits of the corresponding regulations (European and Canadian).

Based on our matrix approach and previous results for heavy metals on all probiotic formulas, Lallemand Health Solutions (Institut Rosell) can provide reasonable assurance that the probiotics formulas *Bacillus subtilis* Rosell®-179 – 25, powder and *Bacillus subtilis* Rosell®-179 – 100, powder are in compliance with the following Regulations:

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LALLEMAND HEALTH SOLUTIONS INC.

Heavy Metals	Specifications USP 2232 (mg/kg)	Specification Regulation (EU)1881/2006 (mg/kg)
Lead (Pb)	0.5	3
Cadmium (Cd)	0.5	1
Arsenic (As)	1.5	N/A
Mercury (Hg)	1.5	0.1

This information is provided in good faith,

Martin Lampron, B.Sc.
Scientific Affairs Specialist
Lallemand Health Solutions Inc.

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Neopharm Labs Inc.

865, boul. Michèle-Bohec, Blainville Québec
Canada J7C 5J6
Phone (450) 435-8864

Certificate of analysis

NCA : R34-140403-034
Req : 19036-00
PO No : 19036-00
Code Client : R34
Date spec :
Version : 1

Printed : 2014-04-17
Received : 2014-04-03
Closed : 2014-04-17

Page : 1 of 1

ROSELL (INSTITUT)
8480, boul. St-Laurent
Montréal, Québec H2P 2M6

Sample : BACILLUS SUBTILIS

Section : 1
Lot No : U120140250 (00071928)
Template : Finished product
Description : Report

Beige powder

TEST	METHOD	SPECIFICATION	RESULT
Arsenic	USP, ICP/MS	Report (µg/g)	0.2 µg/g
Lead	USP, ICP/MS	Report (µg/g)	0.04 µg/g
Mercury	USP, ICP/MS	Report (µg/g)	0.2 µg/g
Cadmium	USP, ICP/MS	Report (µg/g)	0.09 µg/g

Verified by : [redacted]

Approved by : Chemist [redacted]

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Canada J7C 5J6
Phone (450) 435-8864

Certificate of analysis

NCA : R34-140403-035
Req : 19035-00
PO No : 19035-00
Code Client : R34
Date spec :
Version : 1

ROSELL (INSTITUT)
8480, boul. St-Laurent
Montréal, Québec H2P 2M6

Printed : 2014-04-17
Received : 2014-04-03
Closed : 2014-04-17

Page : 1 of 1

Sample : BACILLUS SUBTILIS

Section : 1
Lot No : U120140248 (00071923)
Template : Finished product
Description : Report

Beige powder

TEST	METHOD	SPECIFICATION	RESULT
Arsenic	USP, ICP/MS	Report (µg/g)	0.2 µg/g
Lead	USP, ICP/MS	Report (µg/g)	0.04 µg/g
Mercury	USP, ICP/MS	Report (µg/g)	0.05 µg/g
Cadmium	USP, ICP/MS	Report (µg/g)	0.09 µg/g

Verified by :

Approved by :
Chemist