

DEPARTMENT OF HEALTH AND HUMAN SERVICES Food and Drug Administration GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE	Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016 (See last page for OMB Statement)	
	FDA USE ONLY	
	GRN NUMBER 000762	DATE OF RECEIPT
	ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
	NAME FOR INTERNET	FEB 22 2018
KEYWORDS	OFFICE OF FOOD ADDITIVE SAFETY	

Transmit completed form and attachments electronically via the Electronic Submission Gateway (*see Instructions*); OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety (HFS-200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission (*Check one*)

New Amendment to GRN No. _____ Supplement to GRN No. _____

2. All electronic files included in this submission have been checked and found to be virus free. (*Check box to verify*)

3a. For New Submissions Only: Most recent presubmission meeting (*if any*) with FDA on the subject substance (*yyyy/mm/dd*): _____

3b. For Amendments or Supplements: Is your (*Check one*)
amendment or supplement submitted in response to a communication from FDA? Yes If yes, enter the date of communication (*yyyy/mm/dd*) _____
 No

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Laura Boivin		Position President	
	Company (<i>if applicable</i>) Furnoir grizzly Inc.			
	Mailing Address (<i>number and street</i>) 159 Rue d'Amsterdam			
City Saint-Augustin-de-Desmaures		State or Province Quebec	Zip Code/Postal Code G3A 2V5	Country Canada
Telephone Number (418) 878-8941		Fax Number 418-878-8942	E-Mail Address lb@grizzly.qc.ca	
1b. Agent or Attorney (if applicable)	Name of Contact Person		Position	
	Company (<i>if applicable</i>)			
	Mailing Address (<i>number and street</i>)			
City		State or Province	Zip Code/Postal Code	Country
Telephone Number		Fax Number	E-Mail Address	

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

Carnobacterium divergens M35 viable culture

2. Submission Format: (Check appropriate box(es))

Electronic Submission Gateway

Electronic files on physical media with paper signature page

Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 3

Total number of pages 34

4. Does this submission incorporate any information in FDA's files by reference? (Check one)

Yes (Proceed to Item 5)

No (Proceed to Item 6)

5. The submission incorporates by reference information from a previous submission to FDA as indicated below (Check all that apply)

a) GRAS Notice No. GRN _____

b) GRAS Affirmation Petition No. GRP _____

c) Food Additive Petition No. FAP _____

d) Food Master File No. FMF _____

e) Other or Additional (describe or enter information as above) _____

6. Statutory basis for determination of GRAS status (Check one)

Scientific Procedures (21 CFR 170.30(b))

Experience based on common use in food (21 CFR 170.30(c))

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

Yes (Proceed to Item 8)

No (Proceed to Part IV)

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information (Check all that apply)

Yes, see attached Designation of Confidential Information

Yes, information is designated at the place where it occurs in the submission

No

9. Have you attached a redacted copy of some or all of the submission? (Check one)

Yes, a redacted copy of the complete submission

Yes, a redacted copy of part(s) of the submission

No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance (e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).

The bio-preservative preparation containing *Carnobacterium divergens* M35 cells and its metabolites including the bacteriocin divergicin M35, is intended for use as a food ingredient for inhibition of *Listeria monocytogenes* in **smoked** Coho, sockeye and Atlantic salmon as well as in smoked trout-rainbow.

The bio-preservative can be applied to **fresh** or **frozen** products. The lyophilised bio-preservative shall be re-suspended in water of suitable microbiological quality and applied by **spraying** to obtain a final concentration of **10⁶ cfu per g** of smoked product. The treated product must then be vacuum-packed and stored at 4 °C.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? (Check one)

Yes

No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance ¹	Registry Used (CAS, EC)	Registry No. ²	Biological Source (if applicable)	Substance Category (FOR FDA USE ONLY)
1	Carnobacterium divergens			frozen smoked mussels	
2					
3					

¹ Include chemical name or common name. Put synonyms (whether chemical name, other scientific name, or common name) for each respective item (1 - 3) in Item 3 of Part V (synonyms)

² Registry used e.g., CAS (Chemical Abstracts Service) and EC (Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))

2. Description

Provide additional information to identify the notified substance(s), which may include chemical formula(s), empirical formula(s), structural formula(s), quantitative composition, characteristic properties (such as molecular weight(s)), and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source (e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source), and include any known toxicants that could be in the source.

Carnobacterium divergens is a naturally non genetically modified bacterial strain isolated for froze smoked mussels.

Common Name: The common name of C. divergens M35 is defined as "C. divergens protective culture" or "viable Carnobacterium divergens".

Composition: The Carnobacterium divergens M35 product contains viable C. divergens M35 cells, food grade fermentation medium and maltodextrin, an excipient for freeze drying already approved as Generally Recognized As Safe (GRAS) for use in food manufacture.

Method of Manufacture Viable Carnobacterium divergens M35 will be manufactured by Biena Inc, Saint-Hyacinthe, Quebec, Canada in accordance with current good manufacturing practice (GMP) for foods. Viable Carnobacterium divergens M35 manufacturing procedures and specifications were described in detail in annexe 1.

Basis of GRAS Determination: The determination that Carnobacterium divergens M35 is GRAS is based on literature information regarding the safety of the genus carnobacterium and on scientific procedures, as outlined in annex 1. Based on all these information, there is reasonable certainty that viable a Carnobacterium divergens M35 is GRAS under the intended conditions of use.

3. Synonyms

Provide as available or relevant:

1	Viable Carnobacterium divergens
2	C. divergens protective culture
3	

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE
(check list to help ensure your submission is complete – check all that apply)

- Any additional information about identity not covered in Part V of this form
- Method of Manufacture
- Specifications for food-grade material
- Information about dietary exposure
- Information about any self-limiting levels of use (which may include a statement that the intended use of the notified substance is not-self-limiting)
- Use in food before 1958 (which may include a statement that there is no information about use of the notified substance in food prior to 1958)
- Comprehensive discussion of the basis for the determination of GRAS status
- Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

Yes No

Did you include this other information in the list of attachments?

Yes No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Laura Boivin
(name of notifier)
has concluded that the intended use(s) of Carnobacterium divergens M35 viable culture
(name of notified substance)
described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. Laura Boivin
(name of notifier) agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Laura Boivin
(name of notifier) agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

159 d'Amsterdam St-Augustin-De-Desmaures, Québec, Canada G3A 2V5
(address of notifier or other location)

Laura Boivin
(name of notifier) agrees to send these data and information to FDA if FDA asks to do so.

OR

The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No.

(GRAS Affirmation Petition No.)

3. Signature of Responsible Official,
Agent, or Attorney

(b) (6)

Printed Name and Title

Laura Boivin

Date (mm/dd/yyyy)

20-02-2018

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	submission	page 1 to 5
	stabilized lactic acid protective culture for use as a biopreservative in smoked fish	page 1 to 27
	Health Canada	page 1 - 2

OMB Statement: Public reporting burden for this collection of information is estimated to average 150 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

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**APPLICATION FOR REGULATORY APPROVAL AS:
GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE**

**STABILIZED LACTIC ACID PROTECTIVE CULTURE FOR USE AS A BIO-
PRESERVATIVE IN SMOKED FISH**

Submitted by

Fumoirs Grizzly inc.

to

Office of Food Additive Safety (HFS-200), Center for
Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch
Pkwy., College Park, MD 20740-3835.



February_2018

Part 1: Signed statements and certification.

- 1) **We want to submit** *Carnobacterium divergens* M35 as GRAS notice. GRAS is based on literature information regarding the safety of genus *Carnobacterium* and scientific data generated by a research group at Université Laval, Quebec, Canada through a collaborative research project. Based on all this information, there is reasonable certainty that viable *Carnobacterium divergens* M35 is GRAS under the intended condition of use.
- 2) **Name and address of organization:**
Fumoir Grizzly
159 d'Amsterdam, St-Augustin-De-Desmaures
Canada G3A 2V5
- 3) **SCIENTIFIC NAME OF MICROORGANISM:** *Carnobacterium divergens* M35
PROPOSED NAME: BacM35
- 4) The bio-preservative preparation containing *Carnobacterium divergens* M35 cells and its metabolites including the bacteriocin divergicin M35, is intended for use as a food ingredient for inhibition of *Listeria monocytogenes* in **smoked** Coho, sockeye and Atlantic salmon as well as in smoked trout-rainbow.
The bio-preservative can be applied to **fresh** or **frozen** products. The lyophilised bio-preservative shall be re-suspended in water of suitable microbiological quality and applied by **spraying** to obtain a final concentration of **10⁶ cfu per g** of smoked product. The treated product must then be vacuum-packed and stored at 4 °C.
- 5) The bio-preservative preparation has already been accepted by Health Canada as a New Food Additive, as an Antimicrobial Preservative, in Sliced Ready-to-Eat Cold-Smoked Salmon and Sliced Ready-to-Eat Cold-Smoked Trout - Reference Number: NOP/AVP-0018
- 6) The bio-preservative is not subject to the premarket approval requirements of the Federal Food, Drugs and Cosmetic Act.
- 7) We agree to make the data available for the evaluation committee.
When requested, available data will be sent (hard or electronic copy) to the committee. Fumoir Grizzly may be contacted at the address indicated in point #1, during business hours.
- 8) Any of the data and information in Part 2 through 7 of our GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552
- 9) Based on the information and the best of our knowledge, our GRAS notice is complete, representative and includes unfavourable and favourable pertinent information for the evaluation of safety and GRAS status of use

10)

Signature of responsible official	Printed name and title	Date (mm/dd/yyyy)

11) If necessary, We authorize to send any trade secret to the Food Safety and Inspection Service of the U.S department of Agriculture.

PART 2 : Identity, method of manufacture, specification, and physical or technical effect.

1) SCIENTIFIC NAME OF MICROORGANISM: *Carnobacterium divergens* M35

Numerous isolates of lactic acid bacteria demonstrably inhibitory to the growth of the pathogen *Listeria monocytogenes* have been isolated from ready-to-eat fish and seafood products. Three of these isolates, including *Carnobacterium divergens* M35, have shown higher potential and have therefore been selected as a protective culture candidates for use in foods. Using standard methods of characterization, the biochemical and physiological profiles (e.g. production of D or L lactic acid, ability to grow at low temperatures) of these isolates has been examined in order to establish their presumptive taxonomical classification. Isolate M35 was selected for its significant inhibition activity against *L. monocytogenes*. Furthermore, using targeted primers (Cb1/Cb2) to amplify a specific ribosomal RNA sequence 340 base pairs in length, this isolate has been found to belong to the genus *Carnobacterium* (Figure 1).

Other primers were then used to amplify sequences peculiar to known species of *Carnobacterium* (Barakat et al., 2000), which allowed us to identify a 199-bp amplicon characteristic of the species *divergens*, obtained using the primer pair 27F/cdi (Figure 2). Isolate M35 was thus found to be of the species *Carnobacterium divergens*.

Isolate *C. divergens* M35 was filed at the International Depository Authority of Canada in Winnipeg, Manitoba, under the number **ADI 050404-01**.

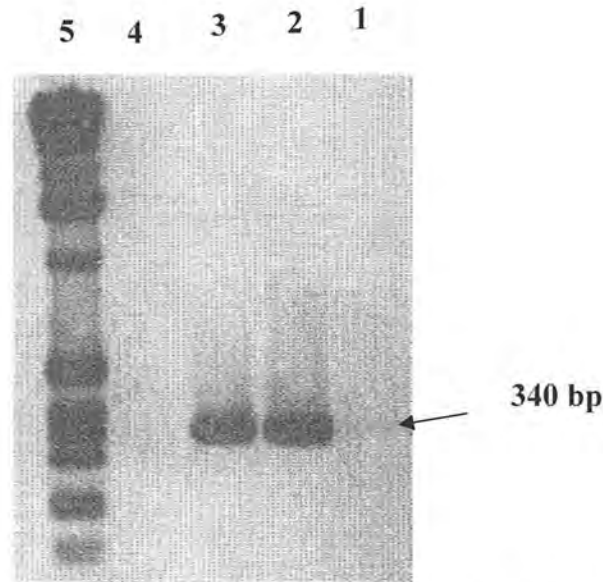


Figure 1. Genotyping of isolate M35 using primer pair Cb1/Cb2r to amplify a sequence of *Carnobacterium* 16S rRNA by PCR. Lane 1 – *Lactobacillus farciminis*; lane 2 – M35; lane 3 – *Carnobacterium piscicola* CS74; lane 4 – negative control; lane 5 – molecular mass marker (up to 1 kb)

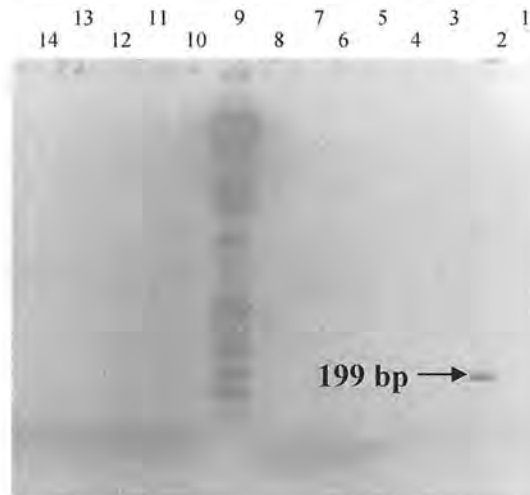


Figure 2. Genotyping of isolate M35 using PCR to amplify 16S rRNA with universal primer 27f and primers Cdi, Cmo, Cpg and Cga specific for *Carnobacterium* species *divergens*, *mobile*, *piscicola/gallinarum* and *gallinarum* respectively (Barakat et al., 2000. Int. J. Food. Micro. 62:83). Lane 1 – *Lb. farciminis* (27f/cdi); lane 2 – M35 (27f/cdi); lane 3 – *C. piscicola* CS74 (27f/cdi); lane 4 – negative control (27f/cdi); lane 5 – *Lb. farciminis* (Cga/Cpg); lane 6 – M35 (Cga/Cpg); lane 7 – *C. piscicola* CS74 (Cga/Cpg); lane 8 – negative control (Cga/Cpg); lane 9 – 1 kb marker; lane 10 – *Lb. farciminis* (27F/Cmo); lane 11 – M35 (27F/Cmo); lane 12 – *C. piscicola* CS74 (27F/Cmo); lane 13 – negative control (27f/Cmo); lane 14 – *Lb. farciminis*

METHOD OF MANUFACTURE

- **Culture medium:** A culture medium used for the manufacture of the proposed *C. divergens*-based bio-preservative may be composed of:

- M19 (Bienna Quebec) 30 g/L
- Glucose: 5 g/L
- Yeast extract: 5 g/L

- **Fermentation conditions:**

- Inoculation volume ratio: 0.5 %
- Incubation temperature: 30 °C
- Agitation: 100 rpm
- pH controlled at 6.0 using NaOH solution (200 g/L)
- CO₂ injected at 0.1 L/L/minute at a pressure of 10 psi
- Fermentation time: about 18 h

Under these culture conditions, an optical density of 1.04, equivalent to about 3×10^9 cfu/mL, is reached within 24 h. Based on agar diffusion and critical dilution methods, the inhibitory activity of the resulting culture broth is about 2.1×10^6 AU/ml, which corresponds to an inhibition zone diameter of 23 mm.

- **Stabilizing the *C. divergens*-M35-based bio-preservative for storage**

Composition of the lyophilisation or spray dried medium:

- Culture broth obtained from the preceding production step
- sucrose: 5 % w/v
- Maltodextrin (Aldrich): 5 % w/v

The mixture is frozen at -25 °C then lyophilised or spray dried under the following conditions:

Lyophilisation:

- Temperature during lyophilisation: 25 °C
- Vacuum conditions: < 30 mTorr

Spray drying:

Temperature during spray drying : 94°C entrance T° and reduced to 80°C drying

The lyophilised product should be stored at 4 °C.

COMPOSITION AND DISTINCTIVE CHARACTERISTICS

A viable and biologically active bacterial culture containing an antimicrobial proteinaceous substance produced naturally during fermentation and inhibitory to the growth of *Listeria monocytogenes*

QUANTITY USED, PROPOSED USES AND PURPOSES THEREOF, DETAILED DIRECTIONS FOR USE, AND RECOMMENDATIONS

The bio-preservative is intended for use on fresh or **frozen smoked salmon**, and on **frozen or cold smoked trout**. Based on prior determination of its cfu/mL content, the lyophilised product shall be re-suspended in water of suitable microbiological quality for application by **spraying** a certain volume in order to obtain a final concentration of **10⁶ cfu per g or per cm²** of smoked product. The treated product must then be vacuum-packed and stored at 4 °C. The supplier guarantees the identity of the bacterial strain and the biological activity of the culture.

DETAILED REPORT ON PRODUCT SAFETY UNDER RECOMMENDED CONDITIONS OF USE

- **Production of biogenic amines:**

The production of biogenic amines in smoked salmon or smoked trout to which *C. divergens* M35 was added was examined weekly. The quantitation of eight amines, namely methylamine, tryptamine, putrescine, spermidine, spermine, tyramine, histamine and cadaverine, was carried out using the method of Vallé et al. (1997). The analyses revealed that other than tryptamine at concentrations of 63 µg/g and 80 µg/g after respectively 15 and 28 days of storage, *C. divergens* M35 did not produce significant quantities of any of these substances (Table 2). The tryptamine concentrations are very low compared to those produced by other species of *Carnobacterium* in salmon (up to 4000 µg/g). Furthermore, this concentration is below the current regulatory limit. It should be noted that *C. divergens* M35 does not produce the toxic amine histamine.

- **Resistance of *C. divergens* M35 to antibiotics**

The antibiotic resistance of *C. divergens* M35 was evaluated in comparison with that of a well-known strain of lactic acid bacteria used routinely in the food industry, namely *Pediococcus acidilactici* PAC 1.0 (commercial strain, Quest). An agar diffusion method based on inhibition zone diameter around paper discs impregnated with antibiotic was used with a variety of culture media, including Mueller-Hinton medium enriched with 2–5 % lysed horse blood, incubated aerobically according to CLSI M-45A specifications (2006 and 2009) as is carried out routinely for testing candidates belonging to other genera of lactic acid bacteria such as *Lactobacillus* and *Pediococcus*. The tested strains were grown first by surface inoculation. A suspension of bacterial growth was then

prepared in 5 mL of physiological saline solution (0.85 %) to obtain a McFarland density of about 0.5 (0.08 – 0.13 DO₆₂₅) for plating on the same medium in Petri plates. The suspension was allowed to adsorb to the agar for 3-5 minutes, and antibiotic discs (five per plate) were then placed on the agar surface and the plates were incubated in the inverted position, for 16–20 h in the case of *Escherichia coli* and *Staphylococcus aureus*, and 20–24 h for the lactic strains.

Table 2: Biogenic amine production (µg/g) in slices of cold-smoked Coho salmon (5 g) in the presence of *C. divergens* M35

Strain	Day	Amine							
		MET	TRP	PUT	SPM	SPD	TYR	HIS	CAD
Control	1	5.94	59.41	1.98	3.96	3.96	ND	ND	ND
	8	7.94	59.52	1.98	3.97	ND	ND	ND	ND
	16	9.77	ND	1.95	3.91	1.95	ND	ND	ND
	22	5.94	ND	1.98	5.94	1.98	ND	ND	ND
	28	5.98	ND	1.99	3.98	ND	ND	ND	ND
M35	1	5.93	27.67	1.98	3.95	1.98	ND	ND	ND
	8	7.98	31.94	2.00	3.99	ND	ND	ND	ND
	16	7.84	21.57	ND	3.92	ND	62.75	ND	ND
	22	7.84	15.69	1.96	3.92	1.96	74.51	ND	ND
	28	15.24	13.33	3.81	3.81	1.90	80.00	ND	ND

Methylamine (MET), tryptamine (TRP), putrescine (PUT), spermine (SPM), spermidine (SPD), tyramine (TYR), histamine (HIS), cadaverine (CAD)
 ND = not detect

Table 3: Zones of inhibition (in mm) of *C. divergens* M35 on various agar media in the presence of different antibiotics

Antibiotic	µg per disc	Medium, hours of incubation							
		MH, 24	MRS7, 24	MRS7, 48	MRS7, blood, 24	MRS7, blood, 24, an	MH, blood, 96*	MH, blood, 96*, an	TSAYE, 24
Ampicillin	10	25.5	28.3	27.9	25.2	27.4	31.0	36.3	26.5
Chloramphenicol	30	28.0	28.7	26.3	28.6	28.0	24.0	23.4	29.0
Ciprofloxacin	5	27.0	23.5	24.6	23.8	23.2	29.8	31.5	28.0
Erythromycin	15	30.3	30.4	31.0	33.5	32.0	32.8	34.0	31.7
Gentamycin	10	18.1	9.7	8.9	20.3	19.7	25.0	29.0	13.4
Methicillin	5	0	10.4	9.1	12.3	13.5	19.2	21.0	11.2
Nitrofurantoin	300	19.0	25.0	23.5	25.8	26.0	24.2	–	21.0
Penicillin G	10 IU	20.0	24.1	21.1	24.1	26.6	30.5	38.7	24.8
Rifampicin	30	34.7	25.9	31.0	37.1	39.6	36.5	–	38.2
Streptomycin	10	0	0	0	9.1	8.7	11.0	11.0	0
Tetracycline	30	33.0	33.9	34.0	33.7	36.0	30.5	29.4	33.3
Vancomycin	5	21.3	18.4	19.5	17.2	17.4	21.8	23.0	20.1
Vancomycin	30	24.9	22.4	24.9	21.4	23.0	25.9	28.8	26.3

an – incubated anaerobically; *Poor growth, not visible until 96 h

MH – Mueller-Hinton agar; MRS7 – Mann-Rogosa-Sharpe agar with pH 7; TSAYE – Tryptic soy agar plus yeast extract

Based solely on the presence of zones of inhibition around the discs, *C. divergens* M35 appears to be sensitive to 11 of the 12 antibiotics tested, under almost all culture conditions tested. Under some conditions, streptomycin did not produce a zone of inhibition, although it did produce a zone 13 mm in diameter under test conditions recommended by the CLSI.

Charteris et al. (2001) have proposed zone inhibition criteria that might be applicable to interpreting the response to some of the antibiotics tested here. Based on these criteria, *C. divergens* M35 appears to be sensitive to all tested antibiotics except streptomycin. We note with interest that *P. acidilactici* appeared to be resistant to methicillin and vancomycin and somewhat resistant to gentamycin

Table 4: Zones of inhibition (in mm) of *P. acidilactici* on various agar media in the presence of different antibiotics

Antibiotic	µg per disc	MH, 24	MRS7, 24	MRS7, blood, 24	MRS7, blood, 24, an	MH cation adj. + blood, 48*	MH, cation adj. + blood 48*, an	TSAYE, 24*
		Ampicillin	10	27.0	20.8	20.5	20.4	27.0
Chloramphenicol	30	35.0	27.0	31.2	32.8	38.0	33.0	31.4
Ciprofloxacin	5	10.0	0	0	Halo	9.0	9.0	10.6
Erythromycin	15	44.7	33.7	33.2	34.1	45.0	42.0	34.6
Gentamycin	10	25.0	11.9	12.6	12.3	34.0	24.0	14.3
Methicillin	5	0	0	0	0	0	0	0
Nitrofurantoin	300	20.0	25.0*	29.1**	27.4**	25.0	20.0	16.0**
Penicillin G	10 IU	33.0	29.3	28.3	28.0	40.0	33.0	27.8
Rifampicin	30	40.0	29.0	33.2	34.2	40.5	39.0	33.7
Streptomycin	10	12.0	0	0	0	13.0	9.0	0
Tetracycline	30	25.0	23.5	21.0	22.0	21.0	22.0	19.6
Vancomycin	5	0	0	0	0	0	0	0
Vancomycin	30	0	0	0	0	0	0	0

an – incubated anaerobically; *Poor growth, not visible until 96 h

MH – Mueller-Hinton agar; MRS7 – Mann-Rogosa-Sharpe agar with pH 7; TSAYE – Tryptic soy agar plus yeast extract

The results are consistent with those obtained by Brillet (2005b) for the sensitivity of *C. divergens* strain V41, which was found sensitive to ampicillin, chloramphenicol, tetracycline, erythromycin, spiramycin, vancomycin, trimethoprim and rifampicin, somewhat sensitive to cefalotin and norfloxacin, and intrinsically resistant to cefotaxim, streptomycin, gentamycin, kanamycin, colistin and nalidixic acid. Based on these results, Brillet concluded that strain V41 does not represent any risk in foods.

Table 5: Antibiotic resistance of *C. divergens* M35 – Interpretation of the results

Antibiotic (µg in disc)	Suggested criteria (zone in mm)			Inhibition zone diameter	Interpretation of result
	R	I	S		
Penicillin G (10)	<19	20-27	>28	31	S
Ampicillin (10)	<12	13-15	>16	31	S
Vancomycin (30)	<14	15-16	>17	36	S
Rifampicin (5)	<14	15-17	>18	37	S
Metronidazole (5)	<14	15-17	>18	Not determined	–
Gentamycin (10)	<12	–	>13	25	S
Streptomycin (10)	<11	12-14	>15	11	R
Tetracycline (30)	<14	15-18	>19	31	S
Chloramphenicol (30)	<13	14-17	>18	24	S
Erythromycin (15)	<13	14-17	>18	33	S

Duffes et al. (1999) established the sensitivity of four strains of *Carnobacterium* (*C. divergens* V41, *C. piscicola* V1, *C. piscicola* SF668 and *C. piscicola* NCDO2762) to antibiotics. Resistance to vefalotin, cefoxitin, moxolactam, cefuroxim, aztreonam, cefotaxim, ceftazidim, cefepim, cepirom, mecillinam, clindamycin, nalidixic acid, colistin, fosfomycin was observed, as was weak resistance to kanamycin, gentamycin, netilmicin, streptomycin, tobramycin and amikacin. However, these strains were sensitive to amoxicillin, ticarcillin, piperacillin, imipenem, erythromycin, pristinamycin, ciprofloxacin, chloramphenicol, tetracycline, minocycline, vancomycin, teicoplanin and rifampicin. These authors mentioned that resistance to cephalosporin, clindamycin, nalidixic acid, fosfomycin and aminoglycosides is intrinsic (non-acquired) and non-transmissible via plasmids. Ringo et al. (2002) found *C. divergens* strain CCUG 30094 to be sensitive to each of 20 antibiotics tested, with the exception of deferroxamine. We note with interest that the preferred treatment of listeriosis uses a combination of gentamycin and ampicillin (Walsch et al. 2001), two antibiotics to which *C. divergens* is sensitive, making it unable to transfer resistance to cells of the genus *Listeria*.

- **Hemolysis**

The method described by Giraffa et al. (1995) was used to evaluate the haemolytic activity of *C. divergens* M35. *C. divergens* (30°C) and *P. acidilactici* (35°C) were incubated simultaneously under aerobic or anaerobic conditions for 24 hours in different media containing defibrinated sheep blood. Zones of alpha or beta hemolysis were noted. Based on the growth obtained, the plates were incubated again under the same conditions.

Table 6: Haemolytic capability of the tested strains

Strain	°C	Medium	Hemolysis
<i>C. divergens</i>	30	TSA + defibrinated sheep blood;	None
		Idem, anaerobic conditions;	None
		MRS7 + defibrinated sheep blood;	*
		Idem, anaerobic conditions	*
<i>P. acidilactici</i>	35	TSA + defibrinated sheep blood;	None
		Idem, anaerobic conditions;	None
		MRS7 + defibrinated sheep blood;	*
		Idem, anaerobic conditions	*

*This medium was greenish coloured at the start and likely of sub-standard quality

Neither *C. divergens* M35 nor *P. acidilactici* displayed haemolytic activity, under aerobic or anaerobic conditions on TSA or MRS7 agars (Table 6). However, the greenish discoloration of MRS7 medium containing sheep blood made it difficult to visualize the type of hemolysis. In addition, *P. acidilactici* apparently produced enough lactic acid on MRS7 to explain the development of large zones of erythrocyte destruction.

- **Allergenic potential**

According to the University of Nebraska database (<http://www.allergenonline.org/index.shtml>) and a document entitled "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology» in the Codex Alimentarius allowed us to confirm that neither *C. divergens* M35 nor the ingredients as a whole that were used in the production and stabilizing of the functional bio-preservative pose any allergenic risk to the consumer.

Part 3: Dietary exposure

1.1 Carnobacterium divergens: a part of the endogenous microbial ecosystem of sea food products

Lactic Acid Bacteria (LAB) including species of the genus Carnobacterium, have been used for centuries for the production of fermented foods (Stoffels et al., 1992; Buchanan and Klawitter, 1992b; Milliere and Lefebvre, 1994; Le Roi et al. 1996, Tahiri et al.). Several published reports have documented the presence of Carnobacterium spp. in food, including meat and meat products, vegetables, fruits, cheeses and seafood, at concentrations from 5.0×10^5 to 1.0×10^9 cfu/g (Larson et al 2006). They are able to grow in foods that contain little free carbohydrate even at refrigerator temperatures (Leroi et al., 1998, Tahiri et al.). Unlike other lactic acid bacteria, the relatively weak acidification by carnobacteria suggest the possibility of adding them directly to cold-smoked salmon and other non-fermented or non-marinated ready-to-eat fish and seafood without risking undesirable changes to the organoleptic and sensory characteristics (Stohr et al., 2001, Cleveland et al. 2001, Leisner et al. 2007, Tahiri et al.).

1.2 Human exposure to carnobacterium and to their bacteriocins

Some of so-called acclimatized strains of Carnobacterium are able to produce various antimicrobial compounds such as bacteriocins (Leisner et al. 2007, Tahiri et al. Le Roi et al.). Bacteriocin-producing Carnobacterium strains have also been isolated directly from various food products including cheese, Yogurt, meat products and fish (Buchanan and Klawitter, 1992; Rieder G et al 2012, Le Roi et al. 1996, Tahiri et al) which give them an undeniable advantage for use in this type of food matrix. This also means that bacteriocins would be naturally found in all these food products.

Production of antimicrobial compounds such as bacteriocins by LAB have attracted a lot of attention because of their bactericidal or bacteriostatic activity against food pathogenic and spoilage bacteria (Ramou et al. , 2015, Cleveland et al. 2001, Fliss et al. chapitre de livre). The production of the bacteriocin divergicin M35 is one of the mechanism involved in the inhibitory activity of C. divergens M35, the strain that is the subject of this application. This bacteriocin as well as the producing strain have been extensively characterized at physicochemical, biological and molecular levels (Tahiri et al, Ben Abbou et al. Naghmouchi et al.).

Since Carnobacterium spp are a part of the endogenous microflora of several foods, it's then obvious that humans have been exposed for centuries to LAB and more specifically those producing bacteriocins such as C. divergens. However, no case of acute or chronic toxicity directly associated with long term exposure to bacteriocin-producing C. divergens or to other Carnobacterium species has been reported until now. In addition, as for many other LAB strains, C. divergens do not seem to be able to survive the different physiological barriers of the digestive tract (gastric acidity, bile salts, etc.), to implant themselves at the colic level or to produce their bacteriocins in situ or to translocate through the digestive barrier. It is also well established that bacteriocins are

only active towards a narrow group of specific micro-organisms, it is then not expected to cause adverse effects to biological diversity when released into the digestive tract.

On the other hands bacteriocins, which have been isolated from several foods such as beef, ham, fish, vegetable and cheese, have probably been consumed for centuries without any reported negative side effects. Indeed, being proteinaceous compounds, the bacteriocins produced in foods are unstable and completely degraded by the proteases and the other enzymes encountered in the mammalian digestive system and are thus unlikely to be toxic or allergenic. This phenomenon has already been clearly described for nisin A, nisin Z and pediocin using in vitro gastrointestinal model (Fernandez et al. Le Lay et al.). The notifier demonstrated scientifically that bacteriocins produced by *C. maltaromaticum* CB1 are readily digestible and unstable in the mammalian digestive system and are thus unlikely to be toxic or allergenic towards animals. It should be emphasised that Nisin, originally isolated in the late 1930s and produced since the 1950s, was approved as an additive for food use in the USA and more than 50 other countries in the late 1960s.

<https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=65>

All these highly suggested that ingestion of bacteriocins would not have any deleterious effect on the human microbiota (Schillinger et al., 1993) and would not induce any allergenic or toxic effect for human.

1.3 Gras status of bacteriocin-producing Carnobacterium

Carnobacterium divergens M35 is a naturally occurring strain isolated from ready-to-eat fish and seafood products in Québec, Canada. The notified strain was filed at the International Depository Authority of Canada in Winnipeg, Manitoba, under the number ADI 050404-01.

The identification of *C. divergens* M35 was based on morphological characteristics, carbohydrate fermentation profile, amplification of specific genes by PCR and 16S rDNA nucleotide sequence determination.

It should be noted that *C. divergens* M35 has recently be recognized as safe and approved as a new additive for biopreservation of smoked fish by Health Canada (<https://www.canada.ca/fr/sante-canada/services/aliments-nutrition/participation-public-partenariats/proposition-permettre-recours-nouvel-additif-alimentaire-carnobacterium-divergens-agent-conservation-antimicrobien-saumon/consultation.html>).

Another *Carnobacterium* strain, *C. maltaromaticum* CB1, was also recognized as GRAS (GRN No. 305) by the U.S. Food and Drug Administration for the use as viable or heat-treated at 1x10⁹ colony forming units/day for preservation of ready-to-eat meat products, meat, poultry and fish products, frozen meals, processed fruit salads and vegetable salads, sauces, and soft cheese and cheese spread-type products

<http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=305>

and Health Canada is approved for use as a microbiological preparation composed of both viable as well as pasteurized, non-viable cells and its metabolites (including carnobacteriocins) in certain ready-to-eat (RTE) meat/poultry products as an intervention against human pathogenic microorganisms such as *Listeria monocytogenes* (http://www.hc-sc.gc.ca/fn-an/legislation/pol/listeria_monocytogenes-addit-eng.php).

1.4 Estimation of total intake of *C. divergens* M35

In our studies, *C. divergens* M35 was inoculated on the surface of smoked salmon at a final concentration of 1×10^6 cfu/g to ensure significant inhibition of *L. monocytogenes*. Since *C. divergens* is a psychrotrophic bacteria able to grow under refrigerated conditions, a final concentration of 1×10^9 ufc/g was reached after 14 days of storage at 4°C. If we consider that in a normal diet, an adult consumes 100g of smoked salmon at once, then the total estimated intake proposed is 1×10^{11} ufc per gram. A significant portion of this ingested *C. divergens* is probably degraded during the passage in the stomach (effect of acidity) and the duodenum (bile salt and proteases). If we consider that the gastro intestinal average survival rate of lactic acid bacteria varies from 3 to 12% (Fernandez et al. Lelay et al.), it is then believed that the highest bacterial concentration reaching the large intestine is around $1,2 \times 10^{10}$ (12% of 1×10^{11} ufc). This is similar to that allowed for *C. maltaromaticum* CB1 for which the daily consumption of *C. maltaromaticum* per capita was estimated at 1.4×10^9 ufc.

2 SAFETY EVALUATION

Several properties have been studied to evaluate the safety of *C. divergens* and the whole results were presented in our original application.

First of all, according to the University of Nebraska database (<http://www.allergenonline.org/index.shtml>) and a document entitled "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" in the Codex Alimentarius, *C. divergens* M35 and the whole ingredients used to produce and stabilize the functional bio-preservative do not pose any allergenic risk to the consumer.

Second, the **antibiotic resistance** of *C. divergens* M35 was evaluated using the agar diffusion method based on inhibition zone diameter around paper discs impregnated with antibiotic was used according to CLSI M-45A specifications (2006 and 2009) as is carried out routinely for testing candidates belonging to other genera of LAB. The results indicate that *C. divergens* M35 was sensitive to 11 of the 12 antibiotics tested, under several culture conditions. This resistance profile is consistent with those obtained with other LAB including those belonging to the genera *Carnobacterium* (Duffes et al. (1999), Brillet et al. 2005b Ringo et al. 2000) , These authors mentioned that resistance to streptomycin and some other antibiotics is intrinsic (non-acquired) and therefore is non-transmissible via plasmids. It is then obvious that there is no risk of transmission of resistance genes by *C. divergens*. Moreover, it is also evident that in the unlikely event of *C. divergens* infection to humans, antibiotic treatments are currently available.

Third, the analysis of **production of biogenic amines** in smoked salmon or smoked trout revealed that *C. divergens* M35 did not induce any of the following amines, namely methylamine, tryptamine, putrescine, spermidine, spermine, tyramine, histamine and cadaverine. The analyses also revealed that tryptamine was produced at concentrations of 63 µg/g and 80 µg/g after respectively 15 and 28 days of storage. These

concentrations are below the current regulatory allowed limit and are very low compared to those produced by other species of *Carnobacterium* in salmon (up to 4000 µg/g).

Fourth, the **haemolytic activity** of *C. divergens* M35 was evaluated in different media containing defibrinated sheep blood. *C. divergens* M35 did not display any haemolytic activity, under both aerobic and anaerobic conditions.

Finally, optimized manufacturing conditions and quality control procedures have been developed and described to ensure a high quality, viable, pure and active preparation of *C. divergens* M35.

Based on all information reported above, it appears that *C. divergens* M35 is not hazardous to humans, and will not produce any significant adverse effects in the general population.

3. General conclusions

In conclusion and considering the fact that:

- *C. divergens* belongs to the big family of LAB which has been largely used for centuries in fermented foods;
- *C. divergens* is naturally present in various foods such as meat, fish and milk products and is one of the endogenous microbial ecosystem of foods;
- Other bacteria within the same genera including *C. maltaromaticum* has already been approved as GRAS;
- No side effect, allergenicity or toxicity have associated with these group of bacteria has been reported over the years;

We can conclude that consumption of *C. divergens* M35 at regular basis and the indicated estimated total intake do not represent any risk for human.

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- <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=> (nisin)

Part 4: Self-limiting levels of use

Figure 3 Shows the evolution of total lactic acid bacteria and endogenous Carnobacteria in cold smoked salmon stored at 4 ° C. The final concentration of the protective culture of *C. divergens* M35 added would then correspond to the maximum concentration found after 15 days of storage. The addition of this concentration allows a significant inhibition of *Listeria monocytogenes* without alternating the intrinsic sensory and organoleptic characteristics of the products

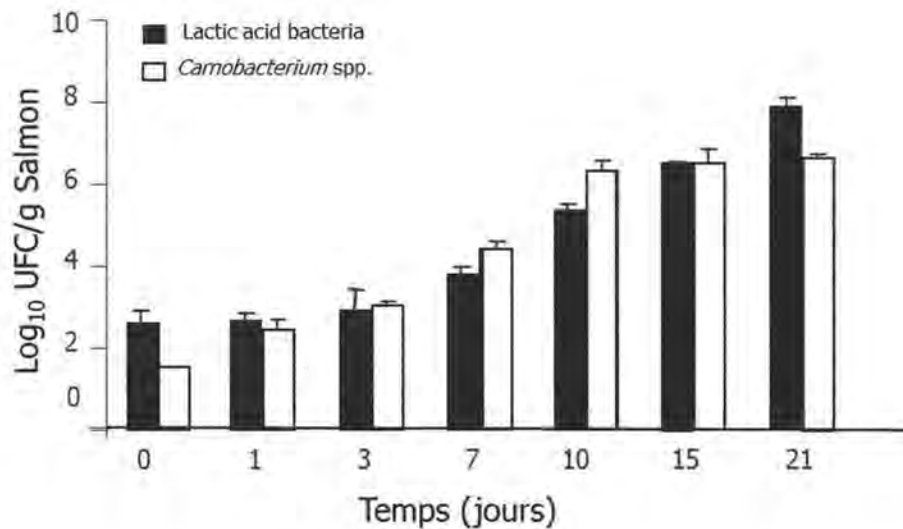


Figure 3: Total lactic acid bacteria and cannobacterium spp counts in smoked salmon stored at 4°C for 21 days

DATA ON THE EFFECTIVENESS OF THE ANTIMICROBIAL BIO-PRESERVATIVE

Cold-smoked Coho salmon fillets (Fumoir Grizzly inc., Saint-Augustin, Québec) divided into 216 slices weighing about 25 g each were distributed into treatment groups as indicated below in table 7.

Table 7. Number of samples (slices) of cold-smoked Coho salmon per treatment group

Bio-preservative added	<i>Listeria monocytogenes</i> added					
	1 cfu per g		10 cfu per g		1,000 cfu per g	
	4 °C	-20 °C	4 °C	-20 °C	4 °C	-20 °C
Storage						
None	24	12	24	12	24	12
10 ⁶ cfu per g	24	12	24	12	24	12

The experimental treatments were inoculation with *L. monocytogenes* at 1, 10 or 1,000 cfu per g of flesh and applying (or not) the bio-preservative at a concentration of about 10⁶ cfu of *C. divergens* M35 per g (suspended at 1.67 x 10⁷ cfu/ml and sprayed onto the slices in two volumes of 750 µL). Forty-eight slices from each group were stored at 4 °C and the 24 remaining slices were stored at -20 °C. All treated samples were left for 10 minutes under the laminar flow hood then vacuum-packed individually in plastic packaging material (bags) provided by Fumoir Grizzly inc. and stored for 20 days. Two samples stored at 4 °C were processed on days 0, 4, 8, 12, 16 and 20 by homogenizing for 10 minutes with 50 mL of 0.5 % peptone water in filter bags in a Lab Blender 400 Stomacher (Seward Medical, London, UK) at maximal speed. Fifty µL of filtered homogenate were used to count *L. monocytogenes* colonies (black with a dark halo) on PALCAM agar (Oxoid) incubated at 37 °C for 72–96 h. Samples stored at -20 °C were thawed at 4 °C and analyzed likewise on days 2, 4 and 7. **Figure 4** shows *L. monocytogenes* viable counts on cold-smoked Coho salmon treated or not with the bio-preservative and stored at 4 °C for 20 days. Inhibition of growth was total for the first 12 days in the case of samples inoculated with 10 cfu/g and treated with the bio-preservative, while counts reached 10³ cfu/g by the fourth day on samples not treated. Although *L. monocytogenes* counts on the treated slices reached 10² cfu/g by the end of the storage period, they were always at least 4 log cycles lower than on untreated slices. The inhibitory effect of the bio-preservative was significant also on slices stored at -20 °C for 20 days, thawed and kept at 4 °C for 7 days. This indicates that the bio-preservative is stable at deep-freeze temperatures and active in the thawed product, preventing proliferation of *L. monocytogenes* for at least a week at proper refrigerator temperature, in the event of the presence of this pathogen in the smoked fish prior to freezing.

These results show clearly that applying a protective bio-ingredient (i.e. bio-preservative) derived from a culture of *C. divergens* M35 suppresses significantly the proliferation of *L. monocytogenes* in cold-smoked Coho salmon. Since the level of *L. monocytogenes* contamination that occurs in the food-processing industry is typically about 10 cfu/g, we suggest that its proliferation on Coho salmon slices stored at 4 °C is completely inhibited for 12 days. Growth of *L. monocytogenes* did occur subsequently, but much less than on slices not treated with the bio-preservative. Comparable results were obtained in other similar studies (Tahiri, 2007). When the initial level of *L. monocytogenes* was higher (10³ cfu/g), the bio-preservative did not provide total inhibition of growth but did slow it considerably, keeping the counts 2 log cycles lower than on untreated slices. It should be noted that contamination with *L. monocytogenes* at levels as high as 10³ cfu/g is rarely if ever seen in the industry.

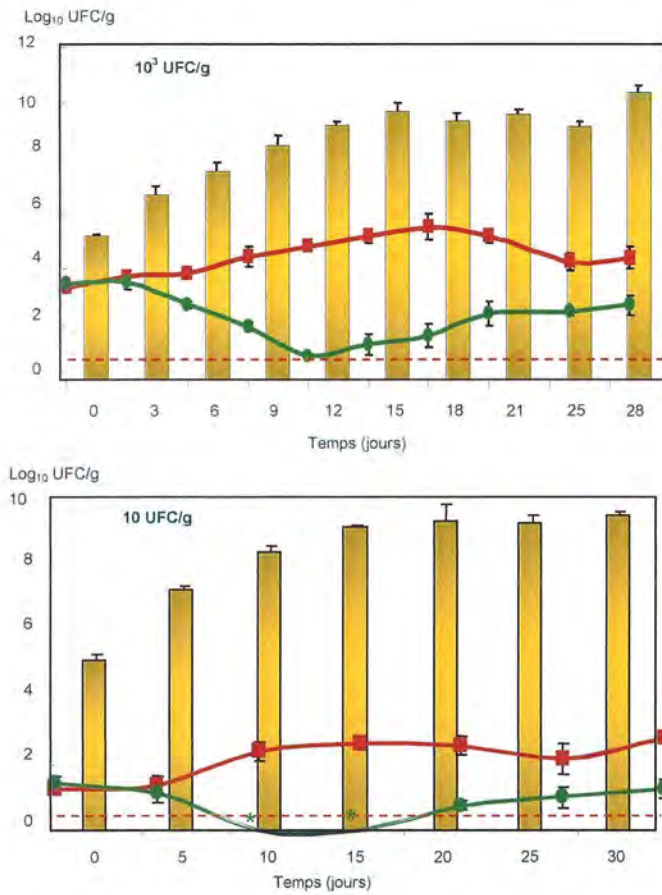


Figure 4: Inhibition of *Listeria monocytogenes* by *C. divergens* M35 bio-preservative applied to cold-smoked salmon. *L. monocytogenes* counts in the absence (red) or presence (green) of the bio-preservative (initial counts were 10³ cfu/g or 10 cfu/g); yellow— *C. divergens* M35 counts.

DATA ON THE QUANTITY OF RESIDUE THAT MIGHT REMAIN ON OR IN THE FOOD PRODUCT

In trials conducted on smoked salmon and as shown in figure 3, we found that *C. divergens* M35 (a psychrotrophe) survived very well at 4 °C, with counts reaching about 5×10^6 cfu/g after 21 days, while total counts (including total lactic acid bacteria) were about 5×10^8 cfu/g. Divergicin M35 was not detected in the samples, possibly due to decomposition by proteases of endogenous lactic bacteria in salmon.

PROPOSED LIMIT FOR RESIDUE ON OR IN THE FOOD PRODUCT

Given the GRAS status of lactic acid bacteria, meaning that they are generally recognized as safe for use in foods, no limit is suggested for the amount of *C. divergens* M35 that should remain on or in cold-smoked salmon or any food in which it is used as a bio-preservative, other than user-determined limits based on cost and sensory characteristics on a case-by-case basis. The final concentration of *C. divergens* did not exceed the concentration naturally found in cold smoked-salmon.

METHOD OF DETECTING AND MONITORING THE BIO-PRESERVATIVE IN THE FOOD PRODUCT

Two methods may be used for the specific detection of *C. divergens* in foods. The first is based on a selective medium developed by Wasney et al. (2001). CTSI agar contains sucrose, manganese sulphate, thallium acetate, inulin, thiamine hydrochloride, vancomycin and nisin. On this medium, *C. divergens* produces highly characteristic pink colonies. Using a top layer of tryptic soy soft agar inoculated with *Listeria ivanovii* (a non-pathogenic organism), M35 colonies that produce divergicin can be counted specifically. The second method is based on amplification of a specific DNA sequence 199 base pairs in length using the primer pair 27F/cdi. The details of this molecular method are described in scientific articles published by Barakat et al. (2000) Tahiri et al. (2004) and Tahiri et al. (2009). These two methods have been successfully used for detection and monitoring *C. divergens* in cold and freeze-dried smoked salmon.

Part 5: Experience based on common use in food before 1958

Please refer to Part 3 (Dietary exposure)

Part 6: Narrative

The present application relies to the regulatory approval of a bioingrédient containing *Carnobacterium divergens* M35 cells and its metabolites including the bacteriocin divergicin M35, as generally recognized as safe (GRAS). This bioingrédient is intended for use as a food ingredient for inhibition of *Listeria monocytogenes* in smoked Coho, sockeye and Atlantic salmon as well as in smoked trout-rainbow.

The GRAS statute claimed is based on literature information regarding the safety of genus *Carnobacterium* and scientific data generated by our research group either in Fumoir Grizzly or at Université Laval, Quebec, Canada through a collaborative research project.

The bacterial strain which is the subject of this application was identified as *Carnobacterium divergens* by using different state of the art biochemical and molecular methods. Its safety has been confirmed by different in vitro assays.

The genus *Carnobacterium* includes several bacterial species that are part of the endogenous microbial ecosystem of several food products. *Carnobacterium* species have been isolated from different food products including fish, milk and meat and have never been associated with food poisoning. More over and According to the University of Nebraska database

(<http://www.allergenonline.org/index.shtml>) allowed us to confirm that neither *C. divergens* M35 nor the ingredients as a whole that were used in the production and stabilizing of the functional bio-preservative pose any allergenic risk to the consumer. Finally, *C. divergens* M35 was shown to be sensitive to all tested antibiotics. It did not show any hemolysis activity and the concentrations of biogenic amines produced in situ were under authorized critical amount.

All these information, taken together, allow as to claim a GRAS statue for *C. divergens* M35 and its metabolites.

Part 7 : list of supporting data and information the GRAS notice

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Update to the List of Permitted Preservatives to Enable the Use of *Carnobacterium divergens* M35 as an Antimicrobial Preservative in Sliced Ready-to-Eat Cold-Smoked Salmon and Trout - Document Reference Number: NOM/ADM-0079

Background

Health Canada received a food additive submission seeking approval for the use of a live culture preparation of the bacterium *Carnobacterium divergens* M35 to limit or inhibit the growth of the foodborne pathogen *Listeria monocytogenes* on sliced ready-to-eat cold-smoked salmon and sliced ready-to-eat cold-smoked trout.

The results of Health Canada's evaluation of available scientific data support the safety and efficacy of *C. divergens* M35 when used as requested by the petitioner.

Health Canada published a proposal on June 7, 2016, which was open for public comment for 75 days. Health Canada received one comment in response to the proposal but no new scientific information was submitted that changed the outcome of the safety evaluation. Since the conclusion of the evaluation remains as described in the proposal, Health Canada has modified the *List of Permitted Preservatives*, effective **October 13, 2016**.

The purpose of this communication is to publically announce the Department's decision in this regard and to provide the appropriate contact information for any inquiries or for those wishing to submit any new scientific information relevant to the safety of this food additive.

Information Document

To obtain an electronic copy of the *Notice of Modification to the List of Permitted Preservatives to Enable the Use of Carnobacterium divergens M35 as an Antimicrobial Preservative in Sliced Ready-to-Eat Cold-Smoked Salmon and Sliced Ready-to-Eat Cold-Smoked Trout - Document Reference Number: NOM/ADM-0079*, please contact our publications office or send an e-mail to publications@hc-sc.gc.ca with the subject heading "hpfb BCS nom-adm-0079-eng".

Contact Information

Health Canada's Food Directorate is committed to reviewing any new scientific information on the safety in use of any food additive, including *Carnobacterium divergens* M35. Anyone wishing to submit new scientific information on the use of this food additive or to submit any inquiries may do so in writing, by regular mail or electronically. If you wish to contact the Food Directorate electronically, please use the word "***Carnobacterium divergens* M35**" in the subject line of your e-mail.

Bureau of Chemical Safety, Food Directorate

Supporting Information

Food Additives

Date modified:

2016-10-13