Preliminary study on enumeration medium of presumptive Bacillus cereus

Preliminary Report

Réf. Proposition :	**SBM** n° **10.845.3**

Boulevard du 13 Juin 1944
BP 2 - 14310 VILLERS BOCAGE
tél. 02 31 25 43 00
fax 02 31 77 49 43
www.adria-normandie.com

Siège social
130, rue Louise Michel
CS 82109
50009 SAINT LO CEDEX
tél. 02 33 06 71 71
fax 02 33 06 71 81

groupe
Laboratoires
groupe
Innovation produit
groupe
Sécurité des aliments
et Innovation procédés
02 31 25 43 00

département
Formation conseil
département
Veille documentaire
02 33 06 71 71

département
Évaluation sensorielle
Études consommateurs
02 31 25 43 03

I. INTRODUCTION

The company CHROMagar requests ADRIA NORMANDIE to study an enumeration medium of presumptive Bacillus cereus.

II. TECHNICAL APPROACH

The proposed technical approach is a study of the selectivity of the enumeration medium of presumptive CHROMagar B.cereus as well as an application on said medium on potentially naturally or artificially contaminated samples.

II. 1 Study of the selectivity

The purpose of this step is to study the selectivity of CHROMagar B. cereus. To do this, inclusivity and exclusivity of the medium were studied. For inclusivity, 30 pure strains of B. cereus were isolated on the medium to ensure that they have distinctive morphological characteristics. These strains were selected from the collection of ADRIA Normandie to present the most biodiversity in terms of genetic (presence of 7 groups) and origin (products). For exclusivity, 20 pure strains of genus or species close to to *B. cereus* were isolated on the medium to ensure that they do not have the distinctive morphological characteristics of *B. cereus*. These strains correspond to gender and species commonly found in food products (for example: *B. subtilis, B. licheniformis, B. megaterium, B. amyloliquefaciens, B. simplex, Paenibacillus spp. Sporosarcina spp. Viridibacillus spp*., ...) but also to the B. mycoides species in order to establish whether the medium is specific for the species *B. cereus* group or to cereus group.

II. 2 Analysis of potentially naturally contaminated samples

The technical approach proposed is a comparison of enumerations obtained on CHROMagar medium for presumptive B. cereus with those obtained on the Mossel medium (AES) on 20 samples likely to have a natural contamination of presumptive *B. cereus* because of the nature of the selected food matrices. Counts were performed according to standard NF EN ISO 7932-July 2005 with reading at 24 and 48 hours on both media. Confirmations were made by hemolysis test with sheep blood agar on one single typical colony (note: the standard 7932 application ask for 5 colonies for confirmation) after overnight incubation at 30°C.

II. 3 Analysis of artificially contaminated samples

The technical approach proposed is a comparison of enumerations obtained on CHROMagar medium for presumptive *B. cereus* with those obtained on the Mossel medium (AES) on 3 food matrices (dairy products, surimi and mashed vegetables) artificially contaminated with one mesophilic strain of *B. cereus* (vegetative form) at different rates (10, 100, 1000 CFU/g) with 3 replications. Analyses were performed after 1h at 4°C (time to adapt to the matrix). The analyses were performed according to standard NF EN ISO 7932-July 2005 with reading at 24 and 48 h on both media.

Note: In all cases, products from CHROMagar method tested were given free of charge by CHROMagar Company.

III. RESULTS

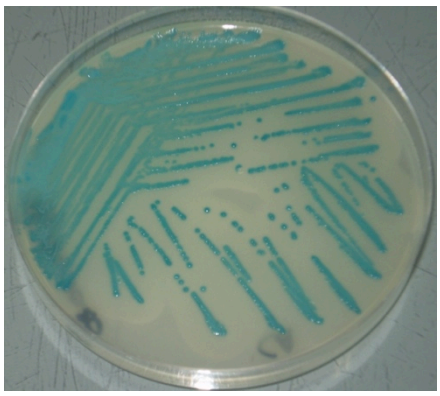
III. 1 Study of the selectivity

The detailed results are provided in Appendix 1

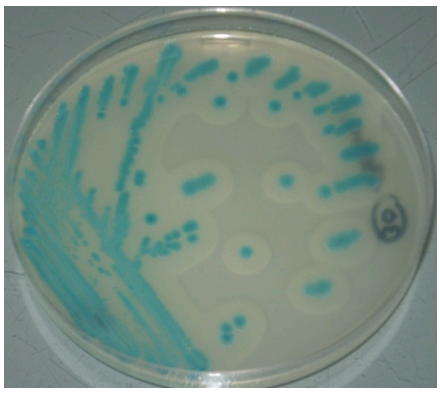
III.1.1 Inclusivity study

Thirty pure strains of presumptive *Bacillus cereus* were selected with respect to their origin or genetic groups: 15 strains from dairy products, surimi, 4, 5 egg products, 2 and 4 surface samples are strains of collection. These strains are divided into several genetic groups (group II to group VII). 30 isolates (except one confirmed non *B. cereus*) have the expected characteristics of the medium tested namely a turquoise color more or less intense, with a presence of a halo around the colonies. However, we can distinguish three groups of colonies relative to the observed macroscopic appearance:

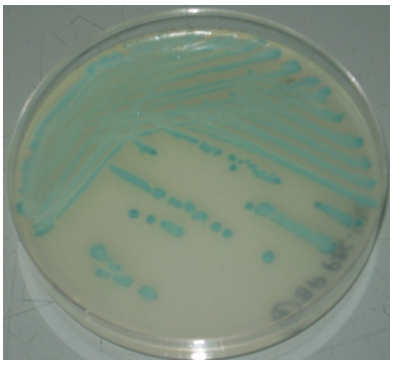
- Group 1: represents the largest group with presence after 24 h of incubation at 30 ° C in small colonies (1-2 mm diameter), regular and intense turquoise color



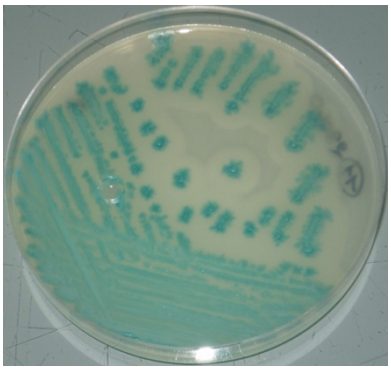
- Group 2: colonies of 3 to 5 mm in diameter after 24 h of incubation at 30 ° C



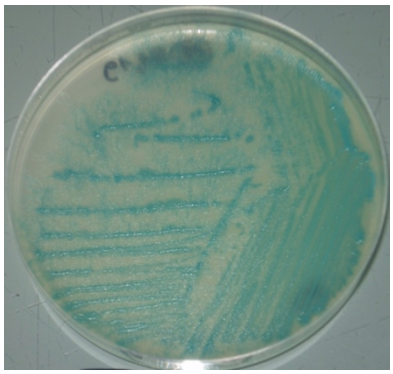
- Group 3: different types of colonies are represented in this group: less intense color (blue-green) with the presence of a halo less marked, irregular colonies and colonies characteristic of Bacillus mycoides



Very intense color, reduced halo



regular colonies



Bacillus mycoides

After 48 h of incubation, the same features are found with some drying visible colonies or a slight increase in the diameter of others.

In all cases, the CHROMagar B. cereus has allowed the identification of typical colonies of B. cereus and B. mycoides. From macroscopic observations we can conclude that the medium is discriminating after 24 hours of incubation at 30 ° C.

III.1.2 Exclusivity Study

Twenty pure strains of genus and species close to *B. cereus* were isolated on CHROMagar B. cereus medium. Observation of the potential growth and the macroscopic appearance was performed for all selected strains. The results of macroscopic observations showed that growth was inhibited for most strains tested. When growth was observed (after 24h or 48h), it was not characteristic of *B. cereus* (turquoise blue colonies without halo or white or pinkish).

Conclusions about the selectivity of the medium

The study of the selectivity of the medium CHROMagar B. cereus showed the usefulness of the medium to identify strains belonging to B. cereus group (at least *B. cereus* and *B. mycoides*). Of the 50 pure strains tested, no false-positive or false-negative was observed under the conditions used. In addition, results were obtained after 24 h of incubation. Finally, no concern for practicability of use of the medium was observed.

Appendix 1

Step 1 *Bacillus cereus* studied strains

	CODE	GROUP	ORIGIN	Appearance at 24H		Appearance at 48H	
				color	growth	color	growth
1	**8P P26-44**	II	milk powder	pale	+++, localised halo	same	same
2	**A7 ovo10**	II	Egg product	Nothing to say	+++, 2mm	same	dry colonies with white outline
3	**AS 11 ovo14**	II	Egg product	Nothing to say	+++, 2mm	same	dry colonies with white outline
4	**44009**	II	Plumber	Nothing to say	+++, 2mm	same	dry colonies with white outline
5	**29014**	II	floating islands	Nothing to say	+++, 2mm	same	dry colonies with white outline
6	**28056**	II	Rum/grapes	Nothing to say	+++, 2mm	same	dry colonies with white outline
7	**27009Vg**	II	Egg cream	Nothing to say	+++, 2mm	same	dry colonies with white outline
8	**12P P26-52**	III	milk powder	Nothing to say	+++, 2mm	same	same
9	**15001 22**	III	surimi	Nothing to say	+++, 2mm	same	same
10	**33007**	III	Milk product	Nothing to say	+++, 2mm	same	same
11	**28040**	III	coconut	Less intense	+++, 4mm irregular	same	same
12	**INRA 91**	III	collection	Nothing to say	+++, 4 mm	same	same
13	**13049-22**	III	surimi	No growth : confirmed strain non *B.cereus*			
14	**B1 ovo 20**	IV	Egg product	Nothing to say	+++, 2mm	same	same
15	**07015-2**	IV	surimi	Nothing to say	+++, 2mm	same	same

Appendix 1 (following table, step 1)

	CODE	GROUP	ORIGIN	Appearance at 24H		Appearance at 48H	
				color	growth	color	growth
16	**49006 RVg**	IV	Panacotta	Nothing to say	+++, 2mm	same	Dry colonies with white outline
17	**46001 Msp**	IV	Egg waste before heating	Nothing to say	+++, 2mm	same	4mm colonies
18	**51007 Rsp**	IV	Egg waste after heating	Nothing to say	+++, 2mm	same	same
19	**5012**	V	Floating islands	Nothing to say	+++, 2mm	same	same
20	**AS 44-1**	V	product testing witness	Nothing to say	+++, 2mm	same	same
21	**AS 27-1**	V	Milk product	Nothing to say	+++, 2mm	same	same
22	**AS 36**	V	Milk product	Nothing to say	+++, 2mm	same	same
23	**42007 RVg**	V	Panacotta	Nothing to say	+++, 2mm	same	same
24	**KBAB4**	VI	collection	Nothing to say	+++, 2mm	same	same
25	**13P' P26-54''**	VI	Milk powder	Nothing to say	+++, 2mm	same	4mm colonies
26	**A11 I Mossel**	VI	Surface sample	Nothing to say	+++, 2mm	same	same
27	**D2PPC**	VI	Milk product CAR coffee	Nothing to say	+++, 2mm	same	4mm colonies
28	**51,19**	VI	collection		*B.mycoides*		
29	**D19**	VII	collection	Nothing to say	+++,4mm	same	same
30	**13051-1**	?	surimi	Nothing to say	+++,4mm	same	same

non-*Bacillus cereus* studied strains

	CODE	GROUP	ORIGIN	Appearance at 24H		Appearance at 48H	
				color	growth	color	growth
A	**LMG 19409**	*B. licheniformis*	?	/	No growth	/	same
B	**Ad 978**	*B. licheniformis*	collection	Blue without halo uncharacteristic	+	same	same
C	**1003006 2 FAM**	*B. licheniformis*	rice flour	Blue without halo uncharacteristic	+	same	same
D	**16001-22**	*B. subtilis*	surimi	/	No growth	+/- blue uncharacteristic	+
E	**932001**	*B. subtilis*	Milk dessert	Blue without halo uncharacteristic	+	same	same
F	**1040008**	*B. circulans*	Chocolate-orange dose	Blue without halo uncharacteristic	+	same	same
G	**1001003 aé spo**	*B. subtilis*	rice flour	Blue without halo uncharacteristic	+	same	same
H	**1042001**	*B. subtilis*	dessert caramel cream	Blue without halo uncharacteristic	+	same	same
I	**1033012**	*B. megaterium*	wipe VP	Blue without halo uncharacteristic	+	same	same
J	**114 10**	*B. amyloliquefaciens*	rice pudding	Blue without halo uncharacteristic	+	same	same
K	**B Nov 42037**	*B. amyloliquefaciens*		Blue without halo uncharacteristic	+	same	same
L	**18022-3**	*B. simplex*	shredded surimi	/	No growth	white	+
M	**1010003**	*P. lautus*	Egg product	/	No growth	/	same
N	**1009001 majo**	*P. spp*	chocolate	Blue without halo uncharacteristic	+	same	same
O	**1023015 FAM**	*P. spp*	Mashed potatoes	Blue without halo uncharacteristic	+	same	same
P	**1007001 PC**	*Sp. aquimarina*	Chocolat cream	White uncharacteristic	+++	Pinkish uncharacteristic	same
Q	**17033-4**	*Sp. aquimarina*	shredded surimi	White uncharacteristic	+++	pinkish	same
R	**0915004 PCA**	*B. pumilus*	shredded 3 chocolates	/	No growth	White uncharacteristic	+
S	**1001002 aé spo**	*B. pumilus*	rice	/	No growth	White uncharacteristic	+
T	**3040053**	*B. circulans*	Milky pudding	Blue without halo uncharacteristic	+	same	same