

# COMPARISON OF BACARA® AGAR, A NEW CHROMOGENIC MEDIUM AND MYP AGAR FOR THE ENUMERATION OF *B. CEREUS* IN FOOD SAMPLE

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## Abstract

### Introduction:

The *Bacillus cereus* Group includes species such as *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycooides*, *B. weihenstephanensis* and *B. anthracis* well known as human food-borne pathogens. *B. cereus* is the second pathogen involved in outbreaks caused by bacterial toxins in Europe.

### Purpose:

The aim of this study was to evaluate the performances of BACARA®, a new chromogenic medium, versus the MYP agar (mannitol egg yolk polymyxin agar - ISO Mandatory Medium) for the direct enumeration of *B. cereus* in Foodstuffs.

### Methods:

Inclusivity tests with 100 *B. cereus* Groups strains and exclusivity tests with 70 non *B. cereus* strains were performed on both BACARA® and MYP.

The assessment of the sensitivity, selectivity and easiness of counting of the BACARA® method compared to the ISO7932 method was carried out on a total of 80 food samples as a second step. A vast majority of those samples were naturally contaminated.

Each sample was diluted in a 1/10 Buffered Pepton Water solution and 0.1 ml of the serial dilutions were inoculated on MYP and BACARA® agar. Both of them, MYP and BACARA® agar were incubated respectively at 30°C and 37°C.

### Results:

Exclusivity data demonstrated a much better selectivity of the BACARA® agar compared to the MYP agar. 27 of non bacillus cereus strains grew on MYP whereas they were all inhibited on BACARA® agar. Although most of these strains didn't have a typical aspect on MYP, they were sources of misinterpretation on high contaminated samples due to an interfering flora on the Petri Dishes.

On the other hand, the combination of a specific nutrient base, a strong selectivity and a chromogenic mixture utilized in BACARA® agar allowed obtaining very large and easy to count colonies.

For both naturally and artificially contaminated samples, BACARA® agar performed well for the enumeration of *B. cereus* compared to the ISO7932 reference method.

### Significance:

BACARA® method is a reliable alternative method for the enumeration of *B. cereus* in foodstuffs. The high level of selectivity and the specific enzymatic detection of typical colonies on this medium can improve interpretation compared to the classical MYP agar. BACARA® will help the end-user save time and avoid any confirmation protocol.

## Introduction

*Bacillus cereus* is responsible for food-borne outbreaks. It produces thermo-resistant spores that make it particularly adapted to foodstuffs submitted to thermal treatment. Some strains of *B. cereus* can grow at refrigeration temperature, which is an emerging risk for ready-to-use products. They represent by themselves 5% of the collective food poisoning in France and are also involved in a lot of opportunistic infections on in-patients.

*Bacillus cereus* belongs to the *Bacillus cereus* group within can be found *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudo-mycoides* and *B. anthracis*. Except the last-mentioned, the phenotypic differentiation of species between *Bacillus cereus* group is impossible with actual culture methods.

BACARA® agar is a selective chromogenic medium that allows the enumeration of *Bacillus cereus* without confirmation. On this medium, typical colonies of *B. cereus* show a pink/orange color due to the metabolism of the substrate and are surrounded with an opaque halo due to the phospholipase activity.

The selectivity of BACARA® agar has been especially optimized to prevent growth of interfering flora and thus to allow an easy interpretation of plates even when matrix highly contaminated with competitive flora are analyzed.

The aim of this study was to compare the performances of selectivity, specificity and accuracy of the BACARA® method with the ISO7932 reference method.

## Material & Methods

### Inclusivity and Exclusivity

100 strains belonging to the *Bacillus cereus* group have been tested on both BACARA® and MYP medium to evaluate the inclusivity and 70 Non *Bacillus cereus* strains have been streaked on these 2 media to study the exclusivity of both methods. All the strains were cultivated overnight in BHI at 37°C for bacteria and in Sabouraud broth at 25°C for yeasts and moulds and the were all streaked on BACARA® and MYP Agar and incubated respectively at 37°C +/- 1°C and 30°C +/- 1°C.

### Method comparison

A comparison between the BACARA® method and the ISO7932 reference method has been carried out on more than 80 samples issued from different food categories: Deep frozen vegetables, mushrooms, pastry, cooked meals, catering food... The majority of these samples were naturally contaminated. The accuracy, the sensitivity, the selectivity and the easiness of counting the typical colonies were compared.

Figure 1: Horizontal Method for the enumeration of presumptive *Bacillus cereus* Standard Method : NF EN ISO 7932 July 2005

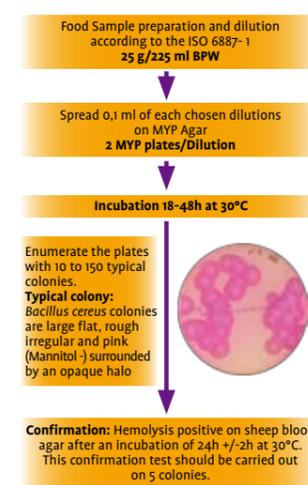
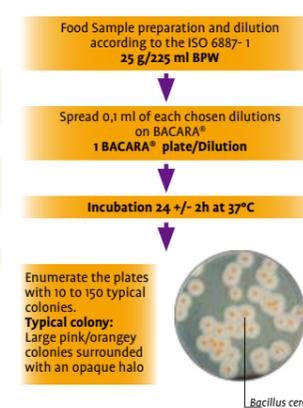


Figure 2: BACARA® method for the enumeration of *Bacillus cereus*



## Results

### Inclusivity and Exclusivity

Fig 3: Comparison of the BACARA® and MYP AGAR specificity on 100 *Bacillus cereus* strains and 70 Non *Bacillus cereus* strains

Strains	Number	MYP 30°C		BACARA® 37°C	
		Growth	Typical	Growth	Typical
<i>Bacillus cereus</i>	53	+++	+	+++	+
<i>Bacillus mycoides</i>	16	+++	+	+++	+
<i>Bacillus pseudomycooides</i>	9	+++	+	+++	+
<i>Bacillus thuringiensis</i>	15	+++	+	+++	+
<i>Bacillus weihenstephanensis</i>	7	+++	+	+++	+

+++ Good growth • ++ Growth • + Growth with small colonies  
+/- Very weak growth with pinpoint colonies • - Complete inhibition

Strains	Number	MYP 30°C		BACARA® 37°C	
		Growth	Typical	Growth	Typical
<i>Aerococcus viridans</i>	1	+/-	-	-	/
<i>Aeromonas hydrophila</i>	1	-	/	-	/
<i>Bacillus licheniformis</i>	3	+++	-	-	/
<i>Bacillus subtilis</i>	6	++	-	-	/
<i>Bacillus megaterium</i>	1	+++	-	-	/
<i>Bacillus circulans</i>	1	+++	-	-	/
<i>Bacillus pumilus</i>	2	+++	-	-	/
<i>Bacillus Firmus</i>	1	-	/	-	/
<i>Bacillus lentus</i>	1	-	/	-	/
<i>Bacillus amyloliquefasciens</i>	2	+++	-	-	/
<i>Bacillus coagulans</i>	2	+++	-	-	/
<i>Bacillus stearothermophilus</i>	1	+++	-	-	/
<i>Candida albicans</i>	1	+++	-	++	-
<i>Citrobacter freundii</i>	1	-	/	-	/
<i>Enterococcus hirae</i>	1	-	/	-	/
<i>Enterococcus faecium</i>	1	+	-	+	-
<i>Enterococcus faecalis</i>	1	+	-	-	/
<i>Enterobacter gergoviae</i>	1	-	/	-	/
<i>Enterobacter sakazakii</i>	1	-	/	-	/
<i>Escherichia coli</i>	3	-	/	-	/
<i>Escherichia fergusonii</i>	1	-	/	-	/
<i>Escherichia vulneris</i>	1	-	/	-	/
<i>Haemophilus influenzae</i>	1	-	/	-	/
<i>Hafnia alvei</i>	1	-	/	-	/
<i>Klebsiella oxytoca</i>	1	-	/	-	/
<i>Klebsiella pneumoniae</i>	1	-	/	-	/
<i>Lactobacillus acidophilus</i>	1	-	/	-	/
<i>Listeria grayi</i>	1	+++	-	-	/
<i>Listeria innocua</i>	1	+++	-	-	/
<i>Listeria monocytogenes</i>	1	+++	-	-	/
<i>Listeria seeligeri</i>	1	+++	-	-	/
<i>Listeria welshimeri</i>	1	+++	-	-	/
<i>Mucor spp</i>	1	++	-	+	-
<i>Penicillium commune</i>	1	-	-	-	/
<i>Proteus mirabilis</i>	1	-	/	-	/
<i>Proteus penneri</i>	1	++	-	-	/
<i>Providencia spp</i>	1	++	-	-	/
<i>Pseudomonas aeruginosa</i>	1	-	/	-	/
<i>Pseudomonas fluorescens</i>	1	-	/	-	/
<i>Saccharomyces cerevisiae</i>	1	+	/	-	/
<i>Salmonella derby</i>	1	-	/	-	/
<i>Salmonella dublin</i>	1	-	/	-	/
<i>Salmonella heidelberg</i>	1	-	/	-	/
<i>Salmonella infantis</i>	1	-	/	-	/
<i>Salmonella typhimurium</i>	3	-	/	-	/
<i>Salmonella virchow</i>	1	-	/	-	/
<i>Salmonella enteritidis</i>	1	++	-	-	/
<i>Serratia marcescens</i>	1	++	-	-	/
<i>Shigella sonnei</i>	1	-	/	-	/
<i>Sphingomonas paucimobilis</i>	1	-	/	-	/
<i>Staphylococcus aureus</i>	1	++	-	-	/
<i>Staphylococcus epidermidis</i>	1	++	-	-	/
<i>Staphylococcus xylosum</i>	1	++	-	+	-
<i>Streptococcus pyogenes</i>	1	++	-	-	-
<i>Vibrio fluvialis</i>	1	-	/	-	/
<i>Yersinia enterocolitica</i>	1	-	/	-	/

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The specificity study clearly showed a better selectivity of the BACARA® Agar compare to the MYP Medium. At least 37 non *Bacillus cereus* strains showed a good growth on MYP (ISO Mandatory Medium) whereas only 4 strains present some colonies on BACARA® (Fig 3). Although these strains were not typical on MYP Medium, they were really sources of misinterpretation on high contaminated samples.

### Methods comparison

Fig 4: Comparison of the accuracy of BACARA® Method and Iso 7932 reference Method on 80 food samples

	ISO 7932 Positive Results	ISO 7932 Negative Results
BACARA® Positive Results	54	4°
BACARA® Positive Results	0	22

\* These strains were confirmed by reference biochemical tests and identified with BIOLOG® System as *Bacillus cereus*

54 samples were confirmed positive with *Bacillus cereus* by both methods. 4 food samples were only found positive on BACARA® and negative on MYP medium. The interfering flora on these 4 samples was very important and the interpretation of the MYP medium was impossible. On the contrary, the high selectivity and specificity of the BACARA® allow easy counting of the typical colonies.

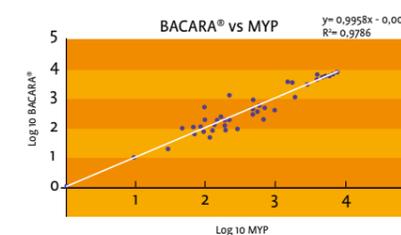
Fig 5: Difference of interpretation on BACARA® (a) and MYP medium (b) with a high level of interfering flora



On BACARA®, the enumeration and differentiation of the typical colonies (orange surrounded by an opaque halo) from the other strains showing a growth was very easy. The majority of the interfering flora was completely

inhibited by the specific mixture of antibiotics (Fig 5 - a). On the other hand, in the presence of such a sample, the interpretation of MYP Medium was not possible. The lack of selectivity permits the growth of many strains which hide the characteristic colonies. In such a case, these standard medium plates were not interpretable and are a source of false-negatives results (Fig 5 - b).

Fig 6: Regression analysis between BACARA® and ISO7932 Methods



The comparison of the enumeration of *Bacillus cereus* by BACARA® method and reference method from the 54 positive samples by a regression analysis gave similar results. The correlation coefficient R2 = 0,97 with an intercept equal to - 0,002 and a slope of 0,99 (Fig 6).

## Conclusion

This study demonstrated the performances of a novel chromogenic medium, the BACARA®, for the enumeration of *Bacillus cereus* in foodstuffs. The combination of its high selectivity and its specific enzymatic detection system improves the sensitivity of the method and the interpretation of the plates compared to the MYP medium.

BACARA® Method is a reliable alternative method for the enumeration of *Bacillus cereus* and will help the end-user save time and avoid any confirmation protocol.