



COMPASS *Enterobacter sakazakii* Agar

INTENDED USE

COMPASS *Enterobacter sakazakii* Agar is a selective medium used for the detection of *Enterobacter sakazakii* in milk powder, dehydrated products and raw materials often used in infant formulations.

HISTORY

Enterobacter sakazakii is a motile, non-sporeforming, facultatively anaerobic Gram negative bacillus that forms yellow pigmented colonies after 24 - 48 hours of incubation on a non-selective medium. Prior to its definitive nomenclature in 1980 based on DNA sequencing, biochemical reactions, pigmentation and sensitivity to antibiotics, *Enterobacter sakazakii* was known as yellow-pigmented *Enterobacter cloacae* (Lehner & Stephan, 2004). As an opportunistic pathogen, *Enterobacter sakazakii* has been identified as the causative agent in meningitis and colitis, particularly in newborns and infants (Simmons *et al.*, 1989 ; Gurtler *et al.*, 2005), with a relatively low frequency of 1 per 100000, but with a high mortality rate between 20 and 50%. Although strains of *Enterobacter sakazakii* have been isolated from different food products, only those destined for use in infant or baby foods have been implicated in infectious episodes.

Studies have demonstrated that 100% of *Enterobacter sakazakii* are positive for α -glucosidase while 100% of other *Enterobacter* species are negative for this enzyme (Muytjens *et al.*, 1984). On the basis of these observations, the chromogenic substrate 5-bromo-4-chloro-3-indolyl- α -D-glucopyranoside (X- α -glucoside) has been proposed to differentiate *Enterobacter sakazakii* from other members of the *Enterobacteriaceae* family (Iversen *et al.*, 2004 ; Guillaume-Gentil *et al.*, 2005). The enzyme α -glucosidase hydrolyzes the X- α -glucoside and liberates the aglycon 5-bromo-4-chloro-indolol. In the presence of oxygen, this aglycon is dimerized and forms the bromo-chloro-indigo pigment.

PRINCIPLES

- Tryptone stimulates growth of the genera *Enterobacter*.
- Yeast extract is a source of complex B vitamins.
- Sodium chloride maintains the osmotic balance.
- The choice of an incubation temperature of 44°C, associated with sodium desoxycholate and crystal violet contributes to the inhibition of background flora.
- X- α -glucoside insures the chromogenic revelation of the α -glucosidase activity of *Enterobacter sakazakii*. On solid media, these bacteria present green to bluish-green colonies after hydrolyzing the substrate.

PREPARATION

- Suspend 30.2 g of dehydrated media (BK188) in one liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.

NOTE :

A partial dissolution of the agar will invariably lead to a significant alteration of the solidity and gel strength of the solidified media, after sterilization and cooling.

INSTRUCTIONS FOR USE

- Cool and maintain the medium at 44-47°C.
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.
- Dry the plates in an incubated, covers ajar.
- On the surface of plates prepared as above, or on pre-poured media (BM120) previously brought to room temperature, inoculate by streaking from an enrichment media recommended for the detection of *Enterobacter sakazakii*, such as Modified Laurylsulfate-Tryptose broth + Vancomycin (mLST + Vancomycin).
- Incubate at (44 ± 1)°C for (24 ± 2) hours.

RESULTS / INTERPRETATION

Colony aspect is as follows :

Microorganisms	Colony characteristics
<i>Enterobacter sakazakii</i>	Blue-green colonies
<i>Escherichia coli</i>	Grey to violet colonies
<i>Enterobacter</i> spp., <i>Klebsiella</i> spp.	Grey to violet colonies
Gram (+) bacteria	Inhibited

- It is necessary to confirm typical colonies with an appropriate method such as : production of yellow pigment, biochemical identification tests, etc.

NOTE :

Strains of *Enterobacter sakazakii* generally present a characteristic blue-green pigmentation. However, some strains may be only weakly α -glucosidase-positive, and be confused with non-targeted Gram negative microorganisms, which by incorporation of crystal violet, present a similar aspect.

TYPICAL COMPOSITION

(can be adjusted to obtain optimal performance)

For 1 liter of medium :

- Tryptone	7.00 g
- Yeast extract.....	3.00 g
- Sodium chloride.....	5.00 g
- Sodium desoxycholate	0.60 g
- Crystal violet.....	2.0 mg
- 5-bromo-4-chloro-3-indolyl, α -D-glucopyranoside	150.0 mg
- Agar	14.40 g

pH of the ready-to-use media at 25°C : 7.0 \pm 0.2.

QUALITY CONTROL

- Dehydrated media : beige powder, free-flowing and homogeneous.
- Prepared media : violet agar.
- Typical cultural response after 24 hours incubation at 44°C :

Microorganisms	Growth	Colony characteristics
<i>Enterobacter sakazakii</i> DSMZ 4485	good, score 2	blue-green
<i>Enterobacter sakazakii</i> CIP 104951	good, score 2	blue-green
<i>Escherichia coli</i> ATCC® 25922	good, score 1-2	violet
<i>Enterobacter cloacae</i> ATCC 13047	good, score 2	violet
<i>Staphylococcus aureus</i> ATCC 25923	inhibited, score 0	

STORAGE / SHELF LIFE

Dehydrated medium : 2-30°C

- The expiry date is indicated on the label.

Prepared media (benchmark value*) :

- Media in Petri dishes : 8 days at 2-8°C, shielded from light.

Pre-poured media in Petri dishes :

- Store between 2 - 8°C, shielded from light.
- The expiry date is indicated on the label.

PACKAGING

Dehydrated medium :

- 500 g bottle

Code

BK188HA

Pre-poured media in Petri dishes (Ø 90 mm) :

- 20 plates

BM12008

BIBLIOGRAPHY

MUYTJENS, H.L., van der ROS-van de REPE, J., and van DRUTEN, H.A.. 1984. Enzymatic profiles of *Enterobacter sakazakii* and related species with special reference to the alpha-glucosidase reaction and reproducibility of the test system. *Journal of Clinical Microbiology*, **20** : 684-686.

SIMMONS, B.P., GELFAND, M.S., HAAS, M., METTS, L., and FERGUSON, J.. 1989. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infection Control and Hospital Epidemiology*, **10** : 398-401.

IVERSEN, C., DRUGGAN, P., and FORSYTHE, S.. 2004. A selective differential medium for *Enterobacter sakazakii*, a preliminary study. *International Journal of Food Microbiology*, **96** : 133-139.

LEHNER, A., and STEPHAN, R.. 2004. Microbiological, epidemiological and food safety aspects of *Enterobacter sakazakii*. *Journal of Food Protection*, **67**(12) : 2850-2857.

XP CEN ISO/TS 11133-2 (V 08-104-2). Janvier 2004. Microbiologie des aliments. Guide pour la préparation et la production des milieux de culture. Partie 2 : Guide général pour les essais de performance des milieux de culture.

GUILLAUME-GENTIL, O., SONNARD, V., KANDHAI, M.C., MARUGG, J.D., and JOOSTEN, H.. 2005. A simple and rapid cultural method for detection of *Enterobacter sakazakii* in environmental samples. *Journal of Food Protection*, **68**(1) : 64-69.

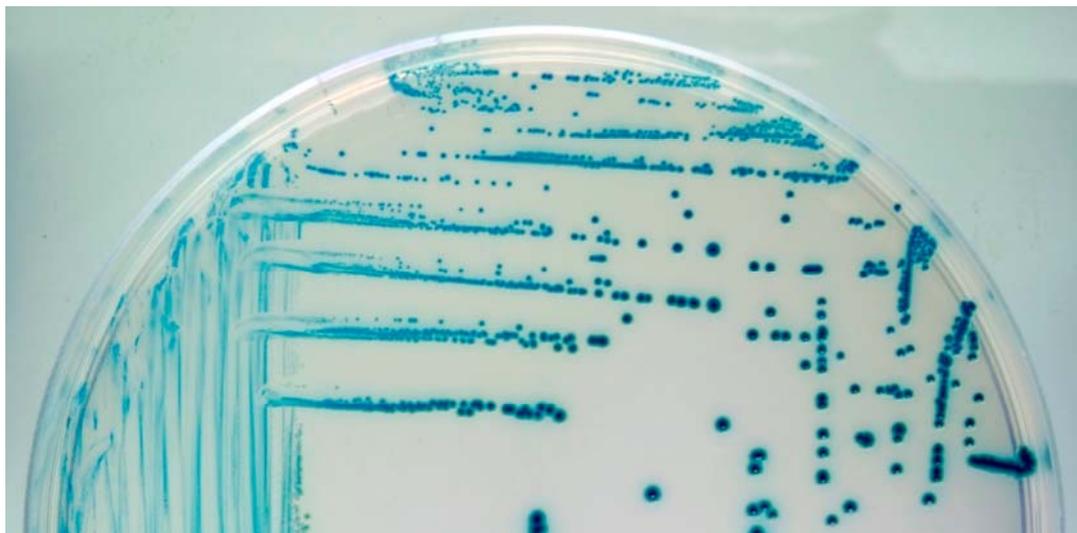
GURTLER, J.B., KORNACKI, J.L., and BEUCHAT, L.R.. 2005. *Enterobacter sakazakii*: a coliform of increased concern to infant health. *International Journal of Food Microbiology*, **104** : 1-34.

ISO/TS 22964 – IDF/RM 210. Février 2006. Lait et produits laitiers. Détection de l'*Enterobacter sakazakii*.

SUPPORT PHOTO :

Reference : BK188HA, BM12008

Media used for : Detection of *Enterobacter sakazakii* primarily in milk powder.



Enterobacter sakazakii

COMPASS *Enterobacter sakazakii* Agar

Ref : BM12008

Incubation : 24 hours / 44°C (surface inoculation pictured)

Characteristics : blue-green colonies revealing α -glucosidase activity.

*Benchmark value refers to the expected value under standard laboratory conditions following manufacturer's instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.

The information provided on the package labels take precedence over the formulations or instructions described in this document.

The information and specifications contained in this technical data sheet date from 2009-02-17.

They are susceptible to modification at any time, without warning.

Code document : BK188/A/2006-06 : 3