TOS Propionate Agar Base

Medium for the enumeration of bifidobacteria in milk products.

General Information

In combination with the selective supplement Lithium-Mupirocin (MUP) the TOS Propionate Agar allows the direct detection of viable bifidobacteria in starter cultures and various milk products like fermented and unfermented milk, milk powder and baby food. The growth of other lactic acid bacteria is very much inhibited so that a confirmation of presumptive bifidobacteria is in most cases not necessary.

The detection of bifidobacteria is done by using the plate count method with an anaerobic incubation at 37°C.

The composition of the medium is in line with ISO 29981/IDF 220: 2010 Standard.

Mode of Action

Bifidobacteria are gram-positive, non-spore forming anaerobic rods. The combination of peptone from casein and yeast extract gives a rich nutrient base for excellent growth of bifidobacteria. The addition of magnesium sulphate further allows to support growth of preinjured bifidobacteria, Ammonium sulphate serves as nitrogen source, potassium dihydrogen phosphate and di-potassium hydrogen phosphate buffer the medium in the neutral pH zone and L-Cysteine serves as reducing agent to give the necessary anaerobic condition in the media.

Galactooligosaccharide TOS is a specific growth factor for all bifidobacteria whereas other lactic acid bacteria cannot utilize this saccharide. In combination with sodium propionate, which largely inhibits the accompanying flora, TOS Propionate Agar is already very selective and specifically supports the growth of bifidobacteria.

Lithium-Mupirocin inhibits growth of lactobacilli, lactococci, streptococci and leuconostocs, whereas bifidobacteria grow unhindered. The typical accompanying flora of milk products is specifically inhibited. In combination with TOS Propionate Agar Mupirocin is so selctive that in most cases only bifidobacteria grow with visible colonies with no need for confirmation.

Typical Composition (g/L)

Peptone from Casein 10.0; yeast extract 1.0; KH_2PO_4 3.0; K_2HPO_4 4.8; $(NH_4)_2SO_4$ 3.0; $MgSO_4$ $7H_2O$ 0.2; L-cysteine HCl H_2O 0.5; Sodium propionate 15.0; Galactooligosaccharide TOS 10.0; Agar-Agar 15.0.

Preparation

TOS Propionate Agar (Base):

Suspend 62.5 g in 950 ml of demineralized water and heat in a boiling water bath or current of steam, with frequent stirring, until completely dissolved.

The medium is heat sensitive and should be autoclaved in small portions. 95 ml or 190 ml of the dissolved medium are transferred into appropriate bottles and autoclaved at 115°C ± 3°C for 15 minutes. Afterwards the medium is immediately cooled to 48°C ± 1°C in a waterbath.

After autoclaving the medium is slightly opaque, which disappears when the medium is cooled (48°C ± 1°C). The base medium is stable for 4 hours in a waterbath at 48°C.

pH: 6,7 ± 0.2 at 25 °C.

The prepared medium is yellowish in colour and clear.

MUP selective supplement (supplement solution):

Lithium-Mupirocin (MUP) comes as a lyophilisated supplement (Merck Cat. No. 1.00045.0010). Each vial contains 25 mg Lithium-Mupirocin, sufficient for 500 ml of the complete TOS-MUP medium.

To prepare the supplement, suspend the lyophilisate in the original vial by adding 25 ml of sterile purified water. Shake gently until the solution is clear.

TOS-MUP medium (final medium):

Aseptically add 5 ml of MUP Selective Supplement to 95 ml of liquefied base medium at 48°C ± 1°C (190 ml of base medium is supplemented with 10 ml supplement solution). The supplement solution is carefully added to the base medium to avoid air bubbles, which can cause oxydation of the medium.

The complete TOS-MUP medium contains 50 mg/L Lithium-Mupirocin. After adding the Lithium-Mupirocin the medium is used immediately.

Experimental Procedure and Evaluation

Sample preparation:

The samples are prepared according to international standards like ISO 8216/IDF 122 (dried milk products) or ISO 7889/IDF 117 (yogurt products). Each 10 g sample is suspended in 90 ml diluent (1/4-strength Ringer's solution). Additional decimal dilutions in 1/4-strength Ringer's solution with a temperature of 20°C ± 1°C are prepared immediately. Mix carefully without air bubbles, because bifidobacteria are damaged by oxygen from air. In order to keep stress for bifidobacteria as low as possible the total time from the first dilution to the inoculation of the agar should not exceed 15 minutes.

Inoculation of the agar:

TOS-MUP medium is usually inoculated using the pour-plate method. 1 ml of the appropriate dilution is transferred to an empty Petri dish and mixed with 15 ml of the medium cooled to 48°C. Careful pipetting is required to minimize air entry.

Incubation:

Directly after the agar is solidified, the plates are incubated reversed (lid to the bottom) anaerobically (in an anaerobic jar using Anaerocult® A) for 72 h ± 3 h at 37°C. Anaerobiosis should be checked (e.g. Anaerotest®).

Colony count:

All plates showing a colony count of ≤ 300 are counted. Typical bifidobacteria colonies grow white in colour with a smell of acetic acid. The colony morphology can be quite different from lentil to round in the agar and like star to cloverleaf form on the agar surface. The colony diameter is around 1 - 4 mm.

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Colony confirmation:

A confirmation of selected colonies is possible by microscope. Bifidobacteria show irregular formed rods when magnified 100 times using phase-contrast method.

Alternatively a fructose-6-phosphate phosphoketolase (F6PPK) test with the appropriate colonies is used.

Results:

From the colony count of the appropriate dilution the number of bifidobacteria is calculated per gram of sample.

Storage

The dehydrated culture medium is to be stored at +15°C to +25°C in a dry place, tightly closed and can be used until the expiration date on the label.

The prepared base medium is stable for 3 months if stored at +2°C to +8°C, protected from drying and light exposure.

The dissolved supplement is stable for 2 weeks if stored in the refrigerator (+ 2°C to + 8°C) and for 3 months if stored at - 20°C.

Literature

Rada, V., Koc, J. The use of mupirocin for selective enumeration of bifidobacteria in fermented milk products. Milchwissenschaft. 55: 65-67 (2000).

Zitz, U., Kneifel, W., Weiss, H., Wilrich, P.-Th. - Selective Enumeration of Bifidobacteria in Dairy Products: Development of a Standard Method. Bulletin Int. Dairy Fed. 411: 3-20 (2007)

ISO 29981 / IDF 220. Milk products - Enumeration of presumptive bifidobacteria - Colony count technique at 37°C (2010)

Orban, J.I., Patterson, J.A. Modification of the phosphoketolase assay for rapid identification of bifidobacteria. Journal of Microbiological Methods. 40: 221-224 (2000)

Tanaka, R., Takayama, H., Morotomi, M., Kuroshima, T., Ueyama, S., Matsumoto, K., Kuroda, A., Mutai, M. Effects of Administration of TOS and Bifidobacterium breve 4006 on the Human Fecal Flora. Bifidobacteria Microflora. 2: 17-24 (1983)

Ordering Information

Product	Ordering No.	Pack size
TOS Propionate Agar Base	1.00043.0100	100 g
TOS Propionate Agar Base	1.00043.0500	500 g
MUP Selective Supplement	1.00045.0010	1 x 10 vials
Anaerobic Jar	1.16387.0001	1 ea
Anaerocult® A	1.13829.0001	1 x 10 envelopes
Anaerotest®	1.15112.0001	1 x 50 stripes
RINGER's tablets	1.15525.0001	100 tablets

Quality control

Test strains	Inoculum CFU/Petri dish	Recovery	Colour of colony
Bifidobacterium animalis subspecies lactis ATCC 27536	50 - 150	>70 %	white
Bifidobacterium breve ATCC 15700	50 - 150	>70 %	white
Bifidobacterium longum ATCC 15707	50 - 150	>70 %	white
Lactobacillus delbrueckii subspecies bulgaricus ATCC 11842	10^6 - 10^7	no growth	n.a.
Lactobacillus casei ATCC 393	10^6 - 10^7	no growth	n.a.
Streptococcus thermophilus DSM 20259	10^6 - 10^7	no growth	n.a.

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Bifidobacterium breve ATCC 15700

Bifidobacterium longum ATCC 15707