



TOS MUP Medium

TOS Propionate Agar & Lithium Mupirocin

TOS MUP Medium



Merck's new medium for the enumeration for
Bifidobacteria in

- Probiotics yoghurts,
- Yoghurt like products
- Dried milk products such as Bifidobacteria containing infant milk formulae

TOS Propionate Agar
&
MUP Selective Supplement

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Probiotics

“Probiotics are **live** microorganisms thought to be healthy for the host organism.

According to the currently adopted definition by FAO/WHO, probiotics are:

- **Live** microorganisms which when administered in adequate amounts confer a health benefit on the host
- **Lactic acid bacteria** (LAB) and **Bifidobacteria** are the most common types of microbes used as probiotics; but certain yeasts and bacilli may also be helpful.
- Probiotics are commonly consumed as part of fermented foods with specially added **active live cultures**; such as in yogurt, soy yogurt, or as dietary supplements”.

see <http://en.wikipedia.org/wiki/Probiotic>

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Bifidobacteria

- “***Bifidobacterium*** is a genus of Gram-positive, non-motile, often branched anaerobic bacteria inhabiting the Gastrointestinal tract and Vagina
- Bifidobacteria are one of the major genera of bacteria that make up the gut flora, the bacteria that reside in the colon.
- Bifidobacteria aid in digestion, are associated with a lower incidence of allergies and also prevent some forms of tumor growth .
- Some bifidobacteria are used as probiotics.
- Before the 1960s, *Bifidobacterium* species were collectively referred to as "*Lactobacillus bifidus*".

see:<http://en.wikipedia.org/wiki/Bifidobacterium>

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Bifidobacteria

- *Bifidobacterium adolescentis*
- *Bifidobacterium animalis* subspecies *animalis*
- *Bifidobacterium bifidum*
- *Bifidobacterium breve*
- *Bifidobacterium infantis*
- *Bifidobacterium longum*

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Lactobacillus

- “***Lactobacillus*** is a genus of Gram-positive facultative anaerobic or microaerophilic bacteria.
- They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid.
- They are common and usually harmless. In humans they are present in the vagina and the gastrointestinal tract, where they are symbiotic and make up a small portion of the gut flora.”

They are the organisms for fermenting milk and they are the accompanying flora in probiotic milk products

see:<http://en.wikipedia.org/wiki/Lactobacillus>

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Lactobacillus

- *Lactobacillus delbrückii* **subspecies bulgaricus**
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Lactobacillus rhamnosus*

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Regulation

- ISO/DIS 29981/IDF 220

Milk products-Enumeration of presumptive Bifidobacteria-Colony count technique at 37°C

Applicable to milk products such as fermented and non fermented milks , milk powders, infant formulae, and starter cultures where these microorganisms are present and **viable**, and in combination with other lactic acid bacteria

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Composition TOS Propionate Agar (Base)

Merck

- Peptone from Casein 10,0
- Yeast extract 1,0
- KH_2PO_4 3,0
- K_2HPO_4 4,8
- $(\text{NH}_4)_2\text{SO}_4$ 3,0
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0,2
- L-cysteine HCl H_2O 0,5
- Sodium propionate 15,0
- Galactooligosaccharide TOS 10,0
- Agar-Agar 15,0

ISO DIS 29981/IDF 220

- Trypticase peptone 10,0
- Yeast extract 1,0
- KH_2PO_4 3,0
- K_2HPO_4 4,8
- $(\text{NH}_4)_2\text{SO}_4$ 3,0
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0,2
- L-cysteine HCl H_2O 0,5
- Sodium propionate 15,0
- Galactooligosaccharide TOS 10,0
- Agar-Agar 12,0-18,0

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Customer

- Dairy industry
- Yoghurt producer
- Dried infant milk producer like e.g. Nestlé
- Producer of starter cultures for Yoghurts

If their products includes bifidobacteria as probiotics

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Advantage

- Competition

- Yakult,

- Offers only the base medium

Merck offers Base Medium and Supplement (Lithium-Mupirocin).

Customer can work immediately according the ISO/DIS Standard.

- Availability

- March 2010

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Benefits

- **Unique**

Best growth performance of Bifidobacteria due to included Galactooligosaccharide (TOS-S)

- **Selective**

Superior suppression of lactic acid bacteria found in milk products due to Lithium-Mupirocin. Growth of Bifidobacteria is not influenced.

- **Reliable**

Reliable enumeration of Bifidobacteria in milk and milk products even in presence of high titer of accompanying flora.

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TOS MUP Medium preparation

- Aseptically add 5 ml of MUP-Selective Supplement to 95 ml of liquefied base (TOS Propionate Agar) medium at $48^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- 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 oxidation of the medium.
- The complete TOS-MUP medium contains 50 mg/L Lithium-Mupirocin. After adding the Lithium-Mupirocin the medium is used immediately.

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Sample preparation

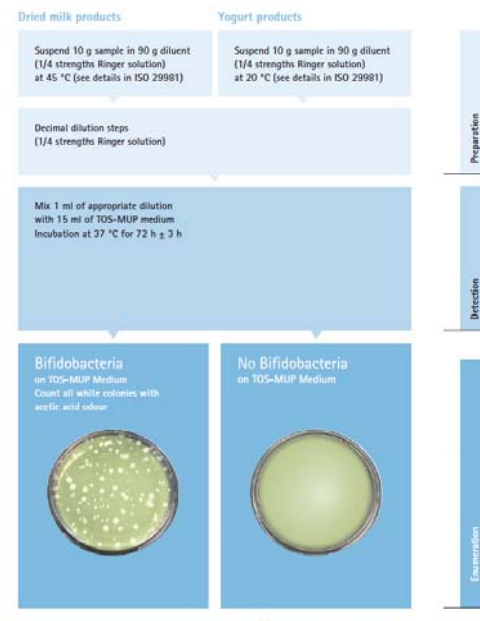
- The samples are prepared according to international standards like ISO 8216/IDF 122 (dried milk products) or ISO 7889/IDF 117 (yogurt products).
- Each 10 g sample is suspended in 90 ml diluent (1/4-strength Ringer's solution). Additional decimal dilutions in 1/4-strength Ringer's solution with a temperature of $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ 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.

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Protocol according ISO/DIS 29981/IDF 220

Enumeration of Bifidobacteria in milk products ISO 29981/ IDF 220 Protocol



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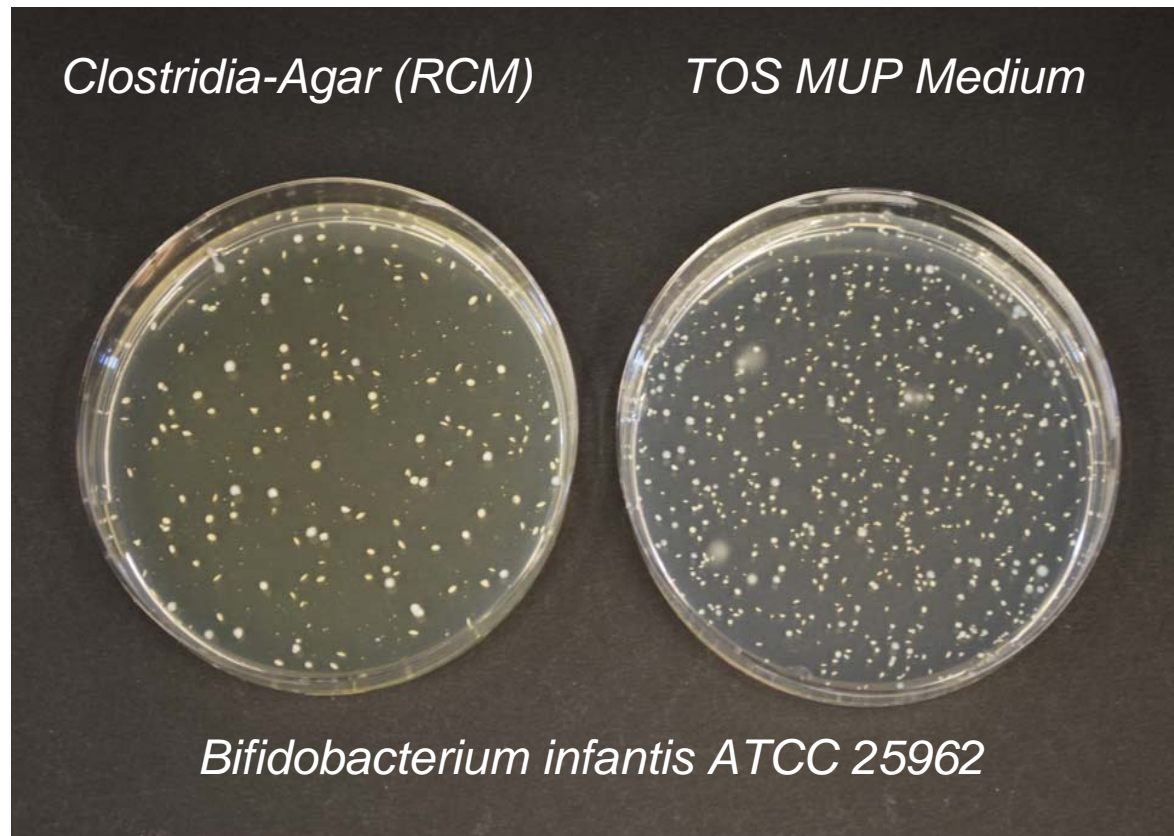
Confirmation

- 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. (not available from Merck)

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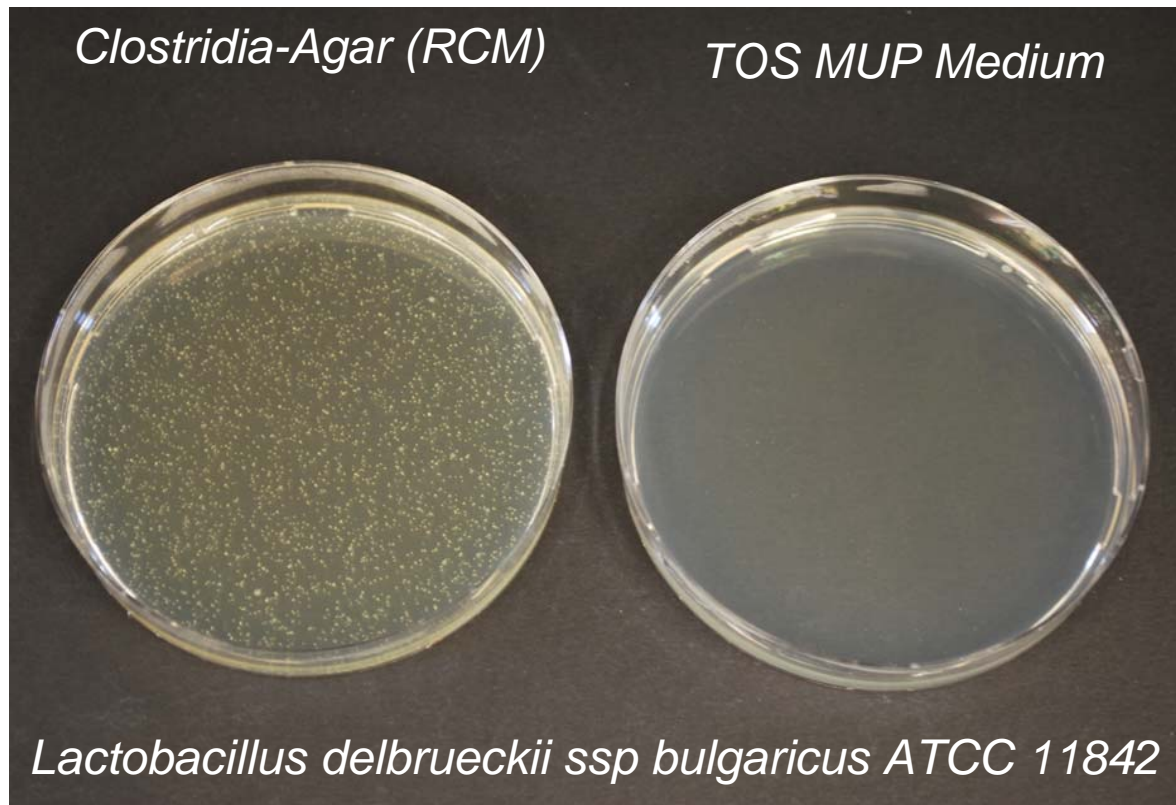
TOS MUP Medium compare with Clostridia-Agar (RCM)



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TOS MUP Medium compare with Clostridia-Agar (RCM)



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Additional information

Brochure



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Additional information

Literature

- Effects of Administration of TOS and Bifidobacterium breve 4006 on the Human Fecal Flora

Ryuichiro Takai, Hiroo TAKAVAMA, Masami MOROTOME,
Toshikata KUROSWAMA, Sadao TAJIMA, Keisuke MATSUMOTO,
Akio KUROTA, and Masahiko Murata

Yakult Central Institute for Microbiological Research, 2976 Y&W, Shinjuku-ku, Tokyo 186

(Received 27 December, 1982)

- Modification of the phosphoketolase assay for rapid identification of bifidobacteria

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Received 13 October 1999; received in revised form 4 February 2000; accepted 6 February 2000

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Additional information Literature

The use of mupirocin for selective enumeration of bifidobacteria in fermented milk products

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