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Yogurt — Identification of characteristic microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*)

Yaourt — Identification des micro-organismes caractéristiques (Lactobacillus delbrueckii *subsp.* bulgaricus *et* Streptococcus thermophilus)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 9232 | IDF 146 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Lactic acid bacteria and starters,* of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Prof. B. Bianchi Salvadori (IT).

Yogurt — Identification of characteristic microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*)

1 Scope

This International Standard specifies tests for the identification of the characteristic microorganisms in yogurt on the basis of their morphological, cultural and physiological properties.

It is applicable to strains isolated from yogurts in which both characteristic microorganisms are present and viable.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 7889|IDF 117:2002, *Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C*

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
characteristic microorganisms in yogurt
Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus*

4 Principle

4.1 The morphological, cultural and biochemical characteristics of *L. delbrueckii* subsp. *bulgaricus* are determined.

4.2 The morphological, cultural and biochemical characteristics of *S. thermophilus* are determined.

5 Culture media, diluents and reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and glass-distilled or demineralized water or water of equivalent purity. The water used for the preparation of the enzyme solutions should be at least double glass-distilled. See also ISO 6887-1 and ISO 8261|IDF 122. For other materials, see ISO 7889|IDF 117.

5.1 Culture media

Use only freshly prepared culture media which shall not be exposed to direct sunlight. If the prepared culture media are not used immediately, they shall, unless otherwise specified, be cooled and stored at between 2 °C and 4 °C for no longer than 1 week and under conditions which do not produce any change in their composition. As for reagents, see storage conditions in ISO 7218.

5.1.1 Skimmed milk

5.1.1.1 Composition

Low-heat-treated, spray-dried skimmed milk, free from growth inhibitors	100 g
Water up to	1 000 ml

5.1.1.2 Preparation

Dissolve the dried milk in the water. Distribute 10 ml portions of the obtained solution in test tubes of 16 mm × 160 mm (6.5). Sterilize in an autoclave at 110 °C ± 1 °C for 30 min or at 115 °C ± 1 °C for 20 min. After sterilization and before use, check the sterility by incubating the test tubes in the incubator (6.1) set at 37 °C for 3 days.

5.1.2 MRS broth

5.1.2.1 Composition

Peptone 1(tryptic digest of casein)	10,00 g
Meat extract	10,00 g
Yeast extract (dried)	5,00 g
Glucose ($C_6H_{12}O_6$)	20,00 g
Tween 80 (sorbitan mono-oleate)	1,00 ml
Dipotassium hydrogen phosphate (K_2HPO_4)	2,00 g
Sodium acetate trihydrate ($CH_3CO_2Na{\cdot}3H_2O$)	5,00 g
Diammonium citrate [$C_6H_6O_7(NH_4)_2$]	2,00 g
Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$)	0,20 g
Manganese sulfate tetrahydrate ($MnSO_4{\cdot}4H_2O$)	0,05 g
Water up to	1 000 ml

5.1.2.2 Preparation

Separately dissolve each component in the already boiling water. Cool in a water bath (6.8) to 50 °C. Adjust the pH so that after sterilization it is 6,5 ± 0,2 at 25 °C ± 1 °C, by using a reagent (5.2) and checking with the pH-meter (6.4).

Distribute 20 ml portions of the obtained medium in test tubes of 20 mm × 200 mm (6.5). Sterilize in an autoclave at 121 °C ± 1 °C for 15 min.

NOTE When using commercially available MRS media, the obtained results may differ significantly from one supplier to the other. Therefore, always check commercially MRS medium against the medium prepared as described above.

5.1.3 Basic medium for fermentation tests

5.1.3.1 Composition

Use the composition as described in 5.1.2.1 for the MRS broth, but omitting the meat extract and the glucose component.

5.1.3.2 Preparation

Prepare the basic medium as described in 5.1.2.2 for the MRS broth, using the components described in 5.1.3.1 and adjusting the pH so that after sterilization it is 6,95 ± 0,05 instead of pH 6,5 at 25 °C ± 1 °C.

5.1.4 Culture medium for production of CO_2

5.1.4.1 Composition

Use the composition as described in 5.1.2.1 for the MRS broth, but omitting the meat extract component and replacing the 20 g of glucose with 50 g of glucose.

5.1.4.2 Preparation

Prepare the culture medium as described in 5.1.2.2 for the MRS broth using the components described in 5.1.4.1 and adjusting the pH so that after sterilization it is 6,95 ± 0,05 instead of pH 6,5 at 25 °C ± 1 °C.

Distribute 10 ml portions instead of 20 ml (as described in 5.1.2.2) of the obtained medium in the test tubes of 16 mm × 160 mm (6.5). Sterilize in an autoclave at 121 °C ± 1 °C for 15 min.

5.1.5 Overlay agar

5.1.5.1 Composition

Bacteriological agar	20 g
Water up to	1 000 ml

5.1.5.2 Preparation

Dissolve the agar in the water. Distribute 10 ml portions in tubes of 16 mm × 160 mm (6.5). Sterilize in an autoclave at 121 °C ± 1 °C for 15 min.

5.1.6 Litmus milk

5.1.6.1 Composition

Litmus powder	0,70 g
Skimmed milk (5.1.1) up to	1 000 ml

5.1.6.2 Preparation

Prepare the litmus milk as described in 5.1.1.2 for the skimmed milk, using the components as described in 5.1.6.1.

NOTE Litmus powder or skimmed milk with litmus is commercially available.

5.1.7 M17 broth

5.1.7.1 Basic medium

5.1.7.1.1 Composition

Peptone 1 (tryptic digest of casein)	2,50 g
Peptone 2 (peptic digest of meat)	2,50 g
Peptone 3 (papain digest of soya)	5,00 g
Yeast extract (dried)	2,50 g
Meat extract	5,00 g
β-Glycerophosphate (disodium salt) ($C_3H_7O_6PNa_2$)	19,00 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,25 g
Ascorbic acid ($C_6H_8O_6$)	0,50 g
Water up to	950 ml

5.1.7.1.2 Preparation

Separately dissolve the components in the boiling water. Cool on a water bath (6.8) to 50 °C. Adjust the pH so that after sterilization it is 6,8 ± 0,2 at 25 °C ± 1 °C by using a reagents (5.2) and checking with the pH-meter (6.4).

Distribute 19 ml portions of the obtained medium in test tubes of 20 mm × 200 mm (6.5). Sterilize for 15 min in an autoclave at 121 °C ± 1 °C.

5.1.7.2 Lactose solution

5.1.7.2.1 Composition

Lactose ($C_{12}H_{22}O_{11}$)	10 g
Water up to	100 ml

5.1.7.2.2 Preparation

Dissolve the lactose in the water. Sterilize for 15 min in an autoclave at 121 °C ± 1 °C.

5.1.7.3 Complete medium

5.1.7.3.1 Composition

Lactose solution (5.1.7.2)	1 ml
Basic medium (5.1.7.1)	19 ml

5.1.7.3.2 Preparation

Immediately before use, add the lactose solution to the test tubes with the basic medium (5.1.7.1). Mix by swirling.

NOTE When using commercially available M17 media, the obtained results may differ significantly from one supplier to the other. Therefore, always check commercially M17 medium against the medium prepared as described above.

5.1.8 Culture medium for growth in presence of 6,5 % NaCl

5.1.8.1 Composition

Use the composition as described in 5.1.7.1.1 for the M17 broth, but replacing the 19 g of β-glycerophosphate component with 65 g of sodium chloride (NaCl).

5.1.8.2 Preparation

Prepare the culture medium as described in 5.1.7.1.2 for the M17 broth but distributing 10 ml instead of 19 ml (as described in 5.1.7.1.2) of the obtained medium in the test tubes of 16 mm × 160 mm (6.5). Sterilize for 15 min in an autoclave at 121 °C ± 1 °C.

5.2 Reagents for adjustment of pH

5.2.1 Sodium hydroxide solution, c(NaOH) = 0,1 mol/l approximately.

5.2.2 Hydrochloric acid solution, c(HCl) = 0,1 mol/l approximately.

5.3 Reagent for staining, ethanolic solution of methylene blue, 6 g/l.

5.4 Reagent for catalase reaction, hydrogen peroxide (H_2O_2), 1,5 % (volume fraction).

6 Apparatus and glassware

Sterilization of equipment that will come into contact with the test sample or the culture medium shall be carried out in accordance with the requirements of ISO 8261 | IDF 122. The glassware shall be resistant to repeated sterilization.

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Incubators, capable of operating at 10 °C ± 1 °C, at 15 °C ± 1 °C, at 37 °C ± 1 °C and at 45 °C ± 1 °C.

6.2 Test tube agitator, for example a vortex mixer.

6.3 Magnifying lens, magnification × 8 to × 10.

6.4 pH-meter, with temperature compensation, accurate to ± 0,1 pH unit at 25 °C ± 1 °C (see also ISO 7218).

6.5 Test tubes, with rubber stoppers or caps, of diameter and length 16 mm × 160 mm and 20 mm × 200 mm, to hold the culture medium.

6.6 Graduated pipettes, for bacteriological use, sterilized and calibrated to the tip, capable of delivering 1 ml ± 0,02 ml and 10 ml ± 0,2 ml (see ISO 6887-1).

Presterilized pipettes made of synthetic materials may be used instead of glass pipettes.

6.7 Glass rod.

6.8 Water baths, capable of operating at 10 °C ± 1 °C, at between 44 °C and 47 °C, at 45 °C ± 1 °C, at 50 °C ± 1 °C, and capable of boiling.