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Fermented milk products — Bacterial starter cultures — Standard of identity

Produits laitiers fermentés — Levains de cultures bactériennes — Norme de composition

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Foreword

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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 27205 | IDF 149 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO 27205 | IDF 149 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Project Group *Dairy starter cultures of lactic acid bacteria* of the Standing Committee on *Analytical Methods for Dairy Microorganisms* under the aegis of its project leaders, Mrs. S. Casani (DK) and Mrs. D. Ellekaer (DK).

This edition of ISO 27205 | IDF 149 cancels and replaces IDF 149A:1997, which has been technically revised.

Fermented milk products — Bacterial starter cultures — Standard of identity

1 Scope

This International Standard specifies characteristics of industrial bacterial starter cultures, which are principally lactic acid bacteria (LAB), but which also include bifidobacteria and propionibacteria used for the manufacture of fermented milk products such as yoghurt, sour cream, cultured butter and cheese.

This International Standard does not apply to bacterial cultures which are added as an ingredient to foods only because of their probiotic properties.

2 Terms and definitions

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

2.1
bacterial starter culture
prepared culture that contains one or several strains of microorganisms at high counts (in general more than 10^8 CFU/g or 10^8 CFU/ml of viable bacteria) being added to bring about a desirable enzymatic reaction (e.g. fermentation of lactose resulting in acid production, degradation of lactic acid to propionic acid or other metabolic activities directly related to specific product properties)

EXAMPLE The most important bacterial starter cultures consist of lactic acid bacteria (2.2), propionibacteria (2.3) and bifidobacteria (2.4) as described in this International Standard.

2.2
lactic acid bacterium
LAB
Gram-positive, non-motile, non-sporeforming, catalase-negative, nitrate-reductase-negative and cytochrome oxidase-negative bacterium that does not liquify gelatine or produce indole

NOTE LAB has a fermentative metabolism which is mainly saccharolytic. Lactic acid is the major end product from carbohydrate utilization.

EXAMPLE LAB of importance for the dairy industry are:

Streptococcus thermophilus	*Lactococcus lactis*	*Pediococcus*
Enterococcus	*Leuconostoc*	*Lactobacillus*

2.3
propionibacterium
Gram-positive, non-motile, non-sporeforming, generally catalase-positive, anaerobic to aerotolerant pleomorphic rod, that is often diphteroid or club shaped and may also be coccoid, bifid or branched

NOTE Propionibacterium is a chemoorganotroph and its fermentation products include large amounts of propionic and acetic acids and carbon dioxide. Its optimum growth temperature is between 30 °C and 37 °C.

2.4
bifidobacterium
Gram-positive, non-motile, non-sporeforming, catalase-negative bacterium, that is often branched rod shaped and which has obligate anaerobic properties

NOTE Bifidobacterium is a chemoorganotroph and ferments sugars producing acetic and lactic acid. Its optimum growth temperature is between 37 °C and 41 °C. Its rods are arranged singly, in pairs, in V-arrangements, in chains, in palisades of parallel cells or in rosettes, occasionally exhibiting swollen coccoid forms.

2.5
food safety criterion
condition determining the acceptability of a product or a batch of a foodstuff applicable to products placed on the market

NOTE See Reference [18].

2.6
process hygiene criterion
condition determining the acceptable functioning of the production process, but which is not applicable to products placed on the market, setting an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law

NOTE See Reference [18].

3 Principle

A description is given of the characteristics of bacterial starter cultures regarding bacterial composition, cell concentration, contaminants, quality and safety management, and product information. It also provides a list of methods of analysis to assess compliance.

4 Description of bacterial starter cultures

4.1 Grouping depending on type and number of strains

4.1.1 Single-strain starter culture

A single-strain starter culture is a starter culture that contains only one strain of a defined species.

4.1.2 Single-species, multiple-strains starter culture

A single-species, multiple-strains starter culture is a starter culture that contains more than one strain belonging to the same species. It may be an undefined mixed strain starter culture.

4.1.3 Multiple-species starter culture

A multiple-species starter culture is a starter culture that contains more than one species. It may contain one or more strains of each species. It may be an undefined multiple-species starter culture.

4.2 Grouping depending on application temperature

4.2.1 Mesophilic bacteria used as starter cultures

Mesophilic bacteria used as starter cultures are applied at temperatures ranging from about 18 °C to 37 °C. Mesophilic strains are widely used in cheese manufacturing and in other fermented milk products, such as buttermilk and sour cream.

The following bacteria are examples of mesophilic bacteria and can be used alone or in combination in the starter cultures specified in 4.1.1 to 4.1.3.

Lactoc. lactis	*Lactob. rhamnosus*
Leucon. mesenteroides	*Propionibacterium freudenreichii*
Pedioc. pentosaceus	*Bifidobacterium animalis*
Lactob. casei	*Bifidob. longum* subsp. *longum*
Lactob. paracasei	*Brevibacterium linens*

Examples of mesophilic single- and multiple-species starter cultures can be found in Table 1. Mesophilic LAB can be further differentiated depending on their metabolism. O-cultures are homofermentative and produce exclusively lactic acid. Citrate-positive organisms are contained in L-, D- and DL-cultures, which produce characteristically lactic acid plus volatile compounds with a characteristic odour, e.g. ethanol, acetaldehyde, diacetyl and acetate, and/or carbon dioxide during fermentation. Acidifying bacteria and *Leuconostoc* species are present in L-cultures, while D-cultures consist of acidifying bacteria and biovar. *diacetylactis*. DL-Cultures consist of L- and D-cultures.

Table 1 — Examples of mesophilic single- and multiple-species LAB starter cultures

Type		Examples
O	Single species	*Lactoc. lactis* subsp. *lactis* and/or *Lactoc. lactis* subsp. *cremoris*
L	Multiple species	*Lactoc. lactis* subsp. *lactis* and/or *Lactoc. lactis* subsp. *cremoris* and, in addition, strain(s) of *Leuconostoc* e.g. *Leucon. mesenteroides* subsp. *cremoris, Leucon. lactis, Leucon. mesenteroides* subsp. *dextranicum* and *Leucon. mesenteroides* subsp. *mesenteroides*
D	Single species	*Lactoc. lactis* subsp. *lactis* and/or *Lactoc. lactis* subsp. *cremoris* and, in addition, strain(s) of *Lactoc. lactis* subsp. *lactis* biovar. *diacetylactis*
DL	Multiple species	*Lactoc. lactis* subsp. *lactis* and/or *Lactoc. lactis* subsp. *cremoris*, and, in addition, strain(s) of *Lactoc. lactis* subsp. *lactis* biovar. *diacetylactis* and of *Leuconostoc* (e.g. *Leucon. mesenteroides* subsp. *cremoris*, *Leucon. lactis, Leucon. mesenteroides* subsp. *dextranicum*, and *Leucon. mesenteroides* subsp. *mesenteroides*)

4.2.2 Thermophilic bacteria used as starter cultures

Thermophilic bacteria used as starter cultures are applied at temperatures ranging from 30 °C to 45 °C. The culture is used in the production of fermented milks, e.g. yoghurt, and certain cheeses, e.g. Emmental and Grana.

The following bacteria are examples of thermophilic acidifying bacteria that can be used as starter cultures.

Strep. thermophilus	*Lactob. acidophilus*	*Bifidob. adolescentis*
E. faecium	*Lactob. fermentum*	*Bifidob. longum* subsp. *infantis*
Lactob. helveticus	*Lactob. gasseri*	*Bifidob. bifidum*
Lactob. delbrueckii subsp. *bulgaricus*	*Lactob. reuteri*	*Brevib. breve*
Lactob. delbrueckii subsp. *lactis*	*Lactob. rhamnosus*	

The aforementioned thermophilic bacteria may be used alone or in combination in the starter cultures specified in 4.1.1 to 4.1.3.

Examples of thermophilic single-species starter cultures are *Lactob. acidophilus* and *Lactob. helveticus*. An example of a thermophilic multiple-species starter culture (4.1.3) is yoghurt containing *Strep. thermophilus* and *Lactob. delbrueckii* subsp. *bulgaricus*.

4.3 Grouping depending on physical form

Starter cultures may have one of the following physical forms:

a) liquid;

b) frozen;

c) dried.

5 Essential composition

5.1 General

The microbiological criteria specified in 5.2 and 5.3 are recommended for products placed on the market during their shelf-life.

A list of recommended methods used for analysis of microbiological criteria is specified in Annex A.

5.2 Viable bacteria

The number of viable cells expressed as colony forming units per gram shall meet the minimum specifications claimed by the starter culture manufacturer or supplier.

In general, bacterial starter cultures (2.1) contain more than 10^8 CFU/g or 10^8 CFU/ml of viable bacteria. For certain applications, testing for acidification activity, texture, optical density, flow cytometry or other alternative new technologies instead of viable cells may be more appropriate.

5.3 Contaminants

The manufacturer shall establish control measures for preventing potential contamination according to 6.2.

Starter cultures shall comply with the specifications shown in Table 2. The microbiological criteria and specifications for process hygiene and food safety criteria have been set in order to define the acceptability of the processes and the product.

The sensitivity of the available analytical methods (see Annex A) has also been considered when setting specifications.

Table 2 — Specifications

Type of criterion	Contaminant[a]	Unit	Liquid and frozen	Dry
Process hygiene	Non-lactic acid bacteria[b]	CFU/g	< 500	< 500
	Yeasts and moulds	CFU/g	< 1	< 10
	Enterobacteriaceae	CFU/g	< 1	< 10
	Coagulase-positive staphylococci	CFU/g	< 1	< 10
Food safety	*Salmonella* spp.	Absence/presence in 1 g	Absence	Absence
	Listeria monocytogenes	Absence/presence in 1 g	Absence	Absence

[a] Contaminants can be tested in process environment and in process or product samples. The set-up of environmental samples compared to process or product samples shall be based on HACCP principles (in accordance with 6.2) and justified against the specifications given here.

[b] This criterion is only relevant as a contaminant in cultures containing only lactic acid bacteria.

Other microbiological test criteria or other concentrations than defined in Table 2 may be relevant depending on the application of the starter culture.

The manufacturer shall establish control measures for preventing potential cross-contamination from other products that might affect the quality of the product.

It shall also be evaluated whether cross-contamination testing needs to be implemented on product, process samples or in the process environment.

6 Quality and food safety management

6.1 Quality management

To control the essential composition of starter cultures, the manufacturer shall put in place, implement and maintain a quality management system.

6.2 Food safety management

To control the essential composition of starter cultures, the manufacturer shall put in place, implement and maintain a permanent procedure or procedures based on pre-requisite programmes and hazard analysis critical control point (HACCP) principles (see ISO 22000[16]).

6.3 Product quality

To comply with the levels given in 5.2 and 5.3, product quality shall be secured and documented according to 6.1 and 6.2.

7 Product information

7.1 Labelling

Labelling shall be in accordance with national legislation, where applicable.

The following items should appear on the product label:

a) name of product;

b) type of product (e.g. mesophilic culture) or bacterial composition in accordance with international scientific nomenclature (see, for example, Reference [19]) and with those mentioned in Clause 4 as appropriate (optional);

c) type of product (e.g. freeze-dried, concentrated);

d) net contents which may be indicated in one of the following units: grams, millilitres, units, doses (in accordance with any applicable law);

e) name and address of the manufacturer, packer, distributor, importer, exporter or vendor;

f) country of manufacture (optional);

g) code and lot identification;

h) expiry date (month and year);

i) storage conditions.

7.2 Technical data

The following information shall be made available to the user:

a) application areas of use;

b) instructions for use (inoculation rate, incubation temperature, etc.);

c) composition (types of bacteria, type of culture, etc., according to the descriptions in 4.1 to 4.3);

d) certificate of analysis, certificate of compliance or similar.

8 Methods of analysis

Recommended methods of analysis are given in Annex A.

In general, methods of analysis recommended for contaminants have not been validated for starter cultures, but for food products. Therefore, when appropriate, methods shall be validated by the manufacturer of the relevant product(s). Other methods can be used when validated, and therefore Annex A is informative.

Annex A
(informative)

Recommended methods of analysis

A.1 Preparation of samples

The rules specified in ISO 6887-5[3] are recommended.

A.2 Methods for enumeration of lactic acid bacteria in starter cultures

A.2.1 Enumeration of *Lactobacillus delbrueckii* subsp*. bulgaricus*

The MRS agar method specified in ISO 7889 | IDF 117[7] is recommended.

A.2.2 Enumeration of *Lactobacillus acidophilus*

The MRS agar method with clindamycin and ciprofloxacin specified in ISO 20128 | IDF 192[15] is recommended.

A.2.3 Enumeration of *Enterococcus faecium,* pediococci and lactobacilli

The MRS agar method specified in ISO 7889 | IDF 117[7] is recommended.

The pH of the medium should be between 6,0 and 6,4. The incubation should be performed under anaerobic conditions at 37 °C ± 1 °C for 72 h.

A.2.4 Enumeration of lactococci and *Streptococcus thermophilus*

The M-17 agar method specified in ISO 7889 | IDF 117[7] is recommended.

A.2.4.1 M-17 agar pH

The pH for M-17 agar (after sterilization) should be 7,2 ($M\text{-}17_{7,2}$) for lactococci and pH 6,8 ($M\text{-}17_{6,8}$) for *Strep. thermophilus*.

A.2.4.2 Plating procedure

Mix appropriate dilutions with the molten medium cooled to 44 °C to 47 °C. After solidification, invert the Petri dishes and incubate for lactococci aerobically at 30 °C ± 1 °C for 72 h and for *Strep. thermophilus* aerobically at 37 °C ± 1 °C for 48 h.

In multiple species starter cultures composed of *Strep. thermophilus* and lactococci, aerobic incubation at 45 °C ± 1 °C for 48 h in $M\text{-}17_{6,8}$ is used for *Strep. thermophilus* and aerobic incubation at 20 °C ± 1 °C for 5 days in $M\text{-}17_{7,2}$ for lactococci.

A.2.4.3 Reading the Petri dishes

After incubation, all colonies should be counted. If the starter culture is of the multiple species type, use differential examination (e.g. growth at different temperatures) to ascertain whether the colonies are lactococci or *Strep. thermophilus*.

A.2.5 Enumeration of citrate-fermenting lactic acid bacteria

The Nickels and Leesment method specified in ISO 17792 | IDF 180[10] is recommended.

A.2.6 Enumeration of *Leuconostoc* spp.

The Nickels and Leesment plus vancomycin method specified in ISO 17792 | IDF 180[10] is recommended.

A.3 Methods for enumeration of *Propionibacterium* spp. in starter cultures

The modified yeast extract-lactate medium described in Reference [20] is recommended.

A.4 Methods for enumeration of *Bifidobacterium* spp. in starter cultures

The method using TOS agar medium containing mupirocin specified in ISO 29981 | IDF 220[17] is recommended.

A.5 Methods for detection and enumeration of contaminants

Note that when using the International Standards specified in this clause, starter cultures may lower the pH to a degree that may inhibit the contaminants (target organisms) and therefore may need neutralization. This is seen as part of the validation of methods for relevant products.

A.5.1 Enumeration of non-lactic acid bacteria

The method specified in ISO 13559 | IDF 153[9] is recommended.

A.5.2 Enumeration of yeasts and moulds

The methods specified in ISO 6611 | IDF 94[1], ISO 21527-1[11], and ISO 21527-2[12] are recommended.

NOTE It is possible that ISO 21527-1[11] and ISO 21527-2[12] will replace ISO 6611 | IDF 94[1].

A.5.3 Enumeration of Enterobacteriaceae

The methods specified in ISO 21528-1[13] and ISO 21528-2[14] are recommended.

A.5.4 Enumeration of Coagulase-positive staphylococci

The methods specified in ISO 6888-1[4], ISO 6888-2[5], and ISO 6888-3[6] are recommended.

A.5.5 Detection of *Salmonella spp.*

The method specified in ISO 6785 | IDF 93[2] is recommended.

For the detection of *Salmonella* spp. in matrices with lactic acid bacteria and bifidobacteria, it is often necessary to modify the composition of the pre-enrichment broth to ensure that the organic acids produced by the starter bacteria and the concomitant pH decrease do not kill *Salmonella* spp. The required modification depends on the type of lactic acid bacteria present and also its quantity.

Based on work carried out with several starter cultures, the following guidelines are provided. Depending on the strain and quantity these guidelines may need to be adapted.

a) For matrices containing up to 10^8 CFU/g lactic acid bacteria or bifidobacteria: use buffered peptone water (BPW) supplemented with vancomycin (10 mg/l). Filter sterilization is applied, but that is not suitable for vancomycin-resistant lactic acid bacteria and bifidobacteria.

b) For matrices containing up to 10^{11} CFU/g lactic acid bacteria or bifidobacteria: use double strength BPW supplemented with vancomycin (10 mg/l), malachite green (40 mg/l) and milk (10 g/l). Instead of using double strength BPW, BPW in which only the phosphate buffer concentration has been doubled, compared with standard BPW, can also be used.

c) For matrices containing more than 10^{11} CFU/g: use double strength BPW supplemented with vancomycin (10 mg/l), malachite green (40 mg/l) and milk (10 g/l) (see Note). Apply a higher dilution factor to ensure that the maximum level of lactic acid bacteria and bifidobacteria in the pre-enrichment broth, immediately after adding the sample to the pre-enrichment broth, does not exceed 10^{10} CFU/ml.

NOTE The addition of milk is only necessary for matrices not containing milk. The addition is necessary to reduce the toxic properties of malachite green versus *Salmonella*.

A.5.6 Detection of *Listeria monocytogenes*

The method specified in ISO 11290-1[8] is recommended.

For pathogen tests, dilute 1 g of test sample with 25 g of suitable sterile diluent. Blend the mixture carefully and add 225 g of convenient strength of broth medium in order to reach a total weight of 250 g. Ensure that the broth contains the correct concentration of selective agents.

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[2] ISO 6785|IDF 93, *Milk and milk products — Detection of* Salmonella *spp.*

[3] ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

[4] ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci* (Staphylococcus aureus *and other species*) *— Part 1: Technique using Baird-Parker agar medium*

[5] ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci* (Staphylococcus aureus *and other species*) *— Part 2: Technique using rabbit plasma fibrinogen agar medium*

[6] ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci* (Staphylococcus aureus *and other species*) *— Part 3: Detection and MPN technique for low numbers*

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[8] ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of* Listeria monocytogenes *— Part 1: Detection method*

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[10] ISO 17792|IDF 180, *Milk*, *milk products and mesophilic starter cultures — Enumeration of citrate-fermenting lactic acid bacteria — Colony count technique at 25 °C*

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[15] ISO 20128|IDF 192, *Milk products — Enumeration of presumptive* Lactobacillus acidophilus *on a selective medium — Colony count technique at 37 °C*

[16] ISO 22000, *Food safety management systems — Requirements for any organization in the food chain*

[17] ISO 29981 | IDF 220, *Milk products — Enumeration of presumptive bifidobacteria — Colony count technique at 37 °C*

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