

Industry guidelines of quality control for Microbial Food Cultures used in Meat Fermentation

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1 Scope

This international industry guideline specifies characteristics of industrial [Microbial Food Cultures \(MFC\)](#) to be used for the manufacture of meat-containing products. The cultures mainly belong to the following genus: *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Leuconostoc*, non-toxic and non-pathogenic staphylococci, *Kocuria*, *Streptomyces*, yeasts and moulds (Bourdichon et al. 2012).

2 Terms and definitions

For the purpose of this international industry guideline the following terms and definitions apply.

2.1. Microbial Food Cultures

Preparation of cultures that contains one or several strains of microorganisms at high counts (in general more than 10^8 colony forming units (CFU) per g or ml) on a carrier being added to provide a desirable metabolic activity (e.g. fermentation of nutrients and carbohydrates resulting in acid production or other metabolic activities directly related to specific product properties).

2.2. Food safety criterion

The conditions that determine the acceptability of a food product placed on the market (Commission Regulation EC No. 2073 2005).

2.3. 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 regulations (Commission Regulation EC No. 2073 2005).

3 Principle

A description is given of the characteristics of MFC regarding cell concentration, contaminants, quality and safety management, and product information. It also provides examples of analytical methods to assess compliance with declared specifications.

4 Essential composition

The following microbiological criteria are recommended for products placed on the market. Products should comply with those criteria for the duration of their shelf-life. Recommended analytical methods are specified in Annex A.

4.1. Viable cell counts

The number of viable cells expressed as CFU per gram, ml or selling unit shall meet the minimum specification claimed by the MFC manufacturer/supplier when using the manufacturers' methods.

In general, population concentration is the main criteria for the functionality of MFC in the manufacture of meat products.

MFC for the manufacture of meat products contain in most cases more than 10^8 CFU/g or ml or the equivalent population concentration measured with the use of alternative methods (e.g. flow cytometry and optical density).

For certain applications, the functionality of the MFC may be measured by controlling the metabolic activity (acidification activity, texturing potential, etc.).

4.2. Contaminants

The manufacturer shall establish control measures to prevent potential contamination according to 5.2.

MFC preparations have to comply with the microbiological specifications shown in Table 1. Microbiological criteria and specifications fall in two categories: process hygiene and food safety. The sensitivity of the available analytical methods (see Annex A) has been considered when setting specifications.

Table 1a — Specifications for mandatory microbiological criteria

| Type of criteria | Contaminants ^{a)} | Units | Liquid and frozen | Dry |
|--------------------------|--|-------------------------------|-------------------|--------------------|
| Process hygiene criteria | <i>Enterobacteriaceae</i> | CFU/g or ml | <10 | <10 ² |
| | Coagulase-positive staphylococci ^{b)} | CFU/g or ml | <50 | <5x10 ² |
| | Other than specified yeasts and moulds | CFU/g or ml | <10 ² | <10 ³ |
| Food safety criteria | Anaerobic sulphite-reducing bacteria | CFU/g or ml | <10 | <10 ² |
| | <i>Salmonella</i> spp. | Absence/presence in 1 g or ml | Absence | Absence |
| | <i>Listeria monocytogenes</i> | Absence/presence in 1 g or ml | Absence | Absence |

a) Contaminants can be tested in process environment and in product samples. The set-up of environmental samples compared to product samples shall be based on HACCP principles (ISO 22000) and justified against the specifications given here.

b) Not relevant for products containing high level of staphylococci due to culture background (risk of false positive, detection limit and/or pressure selection issue).

Table 1b — Specifications for suggested optional microbial criteria (if relevant)

| Type of criteria | Contaminants ^{a)} | Units | Specification |
|--------------------------|--|-------------|------------------|
| Process hygiene criteria | Mesophilic micro-organisms other than specified ones | CFU/g or ml | <10 ³ |
| | <i>Enterococcus</i> | CFU/g or ml | <10 ³ |

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

Additional microbiological criteria than those defined in Table 1b may be relevant depending on the application of the MFC.

The manufacturer shall establish control measures to prevent 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.

5 Quality and food safety management

5.1 Quality management

To control the essential composition of starter cultures the manufacturer shall put in place, implement and maintain a quality management system (e.g. ISO 9001).

5.2 Food safety management

To control the essential composition of MFC, the manufacturer shall put in place, implement and maintain a permanent procedure or procedures based on pre-requisite programs and Hazard Analysis Critical Control Point (HACCP) principles (ISO 22000).

5.3 Product quality

To comply with the levels given in 4.1 and 4.2 product quality shall be secured and documented according to 5.1 and 5.2.

6 Product information

6.1 Labelling, product documentation or accompanying documents

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

The following items are recommended to be on the product label or accompanying documents:

1. Commercial name of product
2. Species name
3. Other ingredients
4. Allergens as required by legislation
5. Type of product (e.g. liquid, frozen or freeze-dried)
6. Net content which may be indicated in one of the following units: g, ml, units, doses (in accordance with any applicable law, if any available)
7. Name and address of the manufacturer, distributor, importer, exporter or vendor
8. Code and lot identification
9. Best before date (month and year)
10. Storage conditions

6.2 Technical data

The following information could be made available to the user:

1. Application areas of use
2. Instructions for use (inoculation rate, etc.)
3. Composition
4. Certificate of Analysis, Certificate of Compliance or similar

7 Methods of analyses

Recommended analytical methods are given in annex A.

In general, analytical methods recommended both for cell counts and contaminants have not been validated for MFC but for food products. Therefore, when appropriate, the validation of methods shall take place by the manufacturer of the relevant product(s). Other methods can be used when validated, and therefore annex A shall be seen as indicative rather than mandatory.

Annex A

Recommended analytical methods

A.1. General

For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

A.2. Preparation of samples

The sample to be tested shall be homogenous and truly representative of the batch of product to be tested. For preparation of the sample, the rules specified in ISO 6887-1 and ISO 6887-2 are recommended.

Diluents listed in ISO 6887-1 and ISO 6887-2 or other equivalent diluents can be used when preparing initial suspensions from samples and further dilutions.

Examples of procedure for diluting

Several methods for dilution can be used (see ISO 6887-1 and ISO 6887-2).

Example 1

Weigh 10 g of well-mixed culture blend under sterile conditions into 90 ml sterile dilution medium **or** add 90 g sterile dilution medium into the Stomacher bag with the culture.

Homogenize until the culture is completely dispersed **either** by shaking the bottle for 7 seconds 20-25 times in an angle of 30° **or** use sterilized Ultra-Turrax bar and homogenize sample 2 x 1 minute at

13500 re/minute **or** treat for 2 minutes in a Stomacher **or** treat on a stirring table for 15 minutes. This is called the 10^{-1} dilution.

If the solution is not transferred right away shake it before transferring.

Transfer 1.0 ml from 10^{-1} dilution into 99 ml of sterile peptone water. Shake the bottle for 7 seconds 20-25 times in an angle of 30° . This is called 10^{-3} dilution. Alternatively transfer 1.0 ml into 9.0 ml of sterile dilution medium and Vortex the tube 3 times for minimum 1 second. This is called the 10^{-2} dilution. Continue to dilute as described above until appropriate dilutions are obtained.

Example 2

For cell count: weigh 1 g of well-mixed culture blend under sterile conditions into 99 ml bottle of sterile dilution medium. Treat on a stirring table for 45 minutes. This is called the 10^{-2} dilution. Transfer 1.0 ml from 10^{-2} dilution into 99 ml of sterile diluent. Shake vigorously the bottle for 30 seconds. This is called 10^{-4} dilution. Continue to dilute as described above until appropriate dilutions are obtained.

Example 3

For contaminants: weight 5 g of well-mixed culture blend under sterile conditions into 45 ml bottle of sterile dilution medium. Homogenize by shaking the bottle for 30 seconds or use stomacher. Rehydrate the culture during 45 minutes. This is called the 10^{-1} dilution. Transfer 1.0 ml from 10^{-1} dilution into 9 ml of sterile diluent and Vortex the tube 3 times for minimum 10 sec. This is called the 10^{-2} dilution. Continue to dilute as described above until appropriate dilutions are obtained.

A.3. Procedure for plating and incubating

See ISO 7218. For double determination: 2 plates for each dilution step.

A.4. Procedure for counting colonies

Counting colonies

For cell counts, plates with 30-300 typical colonies are used. Petri dishes with less than 300 typical colonies are selected for counting, unless described differently in A.6 and A.7. All typical colonies are counted.

For contaminants and if not described differently in chapter A.7, Petri dishes with less than 150 typical colonies are used for counting. All typical colonies are counted.

Enumeration of cell counts

For each sample, perform 1 dilution on 2 plates. After counting, calculate the average and the standard deviation.

After counting, a statistical test needs to be carried out on the plate counts e.g. χ^2 -test (chi square), variation coefficient or an equivalent test. If the statistical test is not accepted, the results must be rejected and the analysis has to be repeated (Niemelä, 1982).

If the statistical test is accepted, the count of colonies is determined by the ‘weighed’ arithmetic average (c). The total amount of counted colonies is calculated as shown below:

$$C = \frac{\Sigma c}{(n1 \times 1 + n2 \times 0.1) \times d}$$

Σc sum of colonies counted on all Petri dishes investigated (lowest dilution and following dilutions).

n1 number of Petri dishes of the lowest (first) dilution.

n2 number of Petri dishes of the following higher (second) dilutions.

d dilution factor corresponding to the lowest (first) dilution put into investigation (n1).

Example:

Enumeration of colonies gave the following results (two Petri dishes per dilution):

- first dilution (10^{-8}): 280 and 299 colonies

- second dilution (10^{-9}): 31 and 36 colonies

$$C = \frac{\Sigma c}{(n1 \times 1 + n2 \times 0.1) \times d} = \frac{280 + 299 + 31 + 36}{(2 + 0.1 \times 2) \times 10^{-8}} = \frac{646}{2.2 \times 10^{-8}}$$

$$C = 294 \times 10^8 \text{ CFU / g} = 2.9 \times 10^{10} \text{ CFU / g}$$

In cases where only one Petri dish per dilution is evaluated, the counted bacteria will be given as c. This fact has to be noted in the report.

Low levels of contaminants

The number of colonies found to be the investigated contaminant is reported in CFU/g or CFU/ml by multiplying the counted colonies with the dilution factor. If no colonies are found, the result is reported as e.g. <1 CFU/g or CFU/ml.

Enrichment procedure

By using enrichment procedure, the determination is expressed as presence or absence in g or ml sample.

A.5. Expression of results

The calculated cell count may be reported as the example above or as rounded number with two significant digits. The results of the count should be expressed as the number of microorganisms per

g or ml of starter culture, i.e. number of colonies x the inverse of the dilution or stated e.g. $<10^2$ CFU/g or CFU/ml if no colonies are detected on -1 dilution surface plated with 0.1 ml.

A.6. Methods for cell counts

An internationally recognized enumeration method which scope is applicable to MFC incorporated in food products only exists today for lactic acid bacteria.

In the absence of a method acknowledged by a legislation, standardized or which has been the subject to a ring test for the enumeration of *Staphylococcus*, yeasts and moulds, microbial food cultures producers may use internal methods validated in house to guarantee the compliance of the MFC product with declared specifications.

Table 2 – Examples of cell count methods

| ISO Standard | Microorganism | Purpose: Methods recommended for enumeration |
|---|-----------------------|---|
| ISO 6887-1 | General | 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 16140 | General | Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods |
| ISO 3534-1 | General | Statistics - Vocabulary and symbols - Part 1: General statistical terms and terms used in probability |
| ISO 15214 | Lactic acid bacteria | Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria -- Colony-count technique at 30 degrees C |
| ISO 6888-1 modified | <i>Staphylococcus</i> | See ISO 6888-1:2000 For distinction between <i>Staphylococcus</i> and <i>Kocuria</i> , the use of agar containing furazolidone is recommended |
| ISO 6611 - IDF 94 ISO 21527-1 ISO 21527-2 | Yeast and moulds | Enumeration of colony-forming units of yeasts and/or moulds - colony-count technique at 25 degrees C Enumeration of colony-forming units of yeasts and/or moulds Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95 - Part 2 Colony count technique in products with water activity less or equal to 0,95 |

A.7. Methods for detection and enumeration of contaminants

The listed methods might be adapted and evaluated on a company level in order to fulfil specific requirements and incorporate experience. Note that when using the following standards, starter cultures may lower the pH to an extent 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.

Table 3 - Recommended methods for contaminants

| Contaminant | Literature |
|---|--|
| <i>Enterobacteriaceae</i> | ISO 21528-1 and ISO 21528-2 NF V08-054 |
| <i>Enterococcus</i> | NMKL 68 Slanetz and Bartley, 1957 APHA 1992. Comp. for microbial examinations of foods in Vanderzant and Splittstoessor, 1992 BVL L 06.00-32 |
| Anaerobic sulphite-reducing bacteria | NMKL 56 ISO 15213 NF V08-061 |
| Coagulase-positive staphylococci | NMKL 66 NF V08-057-1 ISO 6888-1 ISO 6888-2 and ISO 6888-3 |
| Other than specified yeasts and moulds | ISO 6611 - IDF 94 ISO 21527-1 and ISO 21527-2 NF V08-059 NMKL 98 |
| <i>Salmonella</i> spp. | ISO 6579 Amtliche Sammlung (BVL L 00.00-20) NMKL 71 NF V06-052-1 AOAC Official Method 2004.03 |
| <i>Listeria monocytogenes</i> | ISO 11290-1 Amtliche Sammlung (BVL L 00.00-32) NMKL 136 NF V08-028-1 NF V08-055 AOAC Official Method 993.12 AOAC Official Method 2004.06 |
| Mesophilic microorganisms other than specified ones | ISO 4833-1 Enumeration of colony-forming units of yeasts and/or moulds |

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