RESOLUTION OIV/OENO 328/2009

LACTIC ACID BACTERIA- MODIFICATION

The GENERAL ASSEMBLY,

CONSIDERING Article 2, paragraph iii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Upon proposal of the group of experts “Microbiology” and the group of experts "Specifications of oenological products",

DECIDES to replace the existing monograph (Oeno 15/2003) by the following monograph in the International Oenological Codex and to adapt the resolution accordingly (17/2003):

LACTIC ACID BACTERIA

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Lactic acid bacteria are used in oenology to perform malolactic fermentation. The lactic acid bacteria must belong to the Oenococcus, Leuconostoc, Lactobacillus and Pediococcus genus and must be isolated from grapes, musts, wine or have been derived from these bacteria.

The use of genetically modified bacteria will be governed by the currently applicable legislation.

The strains of lactic acid bacteria must be kept under conditions which most favour their genetic stability.

Lactic acid bacteria used in oenology must transform the malic acid in must and wine into lactic acid and carbon dioxide. This should produce biogenic amines in the smallest possible quantities, and must not produce an off taste.

2. LABELLING

The following information must be indicated on the label:

- The genus name and specie(s) in addition to the reference(s) of the strain(s) in the case that there is a registration body.
- Selecting body

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- Operating instructions method and possible reactivation additives recommended by the manufacturer.
- The minimum number of viable cells per gram of preparation that is guaranteed by the manufacturer,
- The manufacturing batch number, in addition to the expiration date and storage conditions with a storage temperature recommended by the manufacturer.
- Where relevant, the indication that lactic acid bacteria were obtained by genetic modifications and their modified character(s).
- The additives.

3. CHARACTERISTICS

Lactic acid bacteria are marketed in liquid, frozen or powder form obtained by lyophilisation or drying, in pure culture or in association with pure cultures.

4. TEST TRIALS

4.1. Humidity for lyophilisated or dried bacteria

Measured by the weight loss of 5 g of the product, dried at 105 °C until constant weight (about 3 hours).

Maximum content should not exceed 8 %.

4.2. Lead

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 2 mg/kg of dry matter.

4.3. Mercury

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

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4.4. Arsenic

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 3 mg/kg of dry matter.

4.5. Cadmium

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

4.6. Viable lactic acid bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex. (method in annex of the present resolution)

The number should be more or equal to 10^8 CFU/ml for frozen or liquid bacteria.

The number should be more or equal to 10^{11} CFU/g for lyophilisated or dried bacteria.

4.7. Mould

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number should be less than 10^3CFU/g.

4.8. Contaminant acetic acid bacteria

Proceed with counting according to the methods in chapter II of the International Oenological Codex.

The number of acetic bacteria should be less than 10^3 CFU/g for frozen or liquid lactic acid bacteria or 10^4 CFU/g for lyophilisated or dried lactic acid bacteria.

The sum of Acetobacter + Gluconobacter should be less than 10^3 CFU/ml for frozen or liquid lactic acid bacteria or 10^4 CFU/g for lyophilisated or dried lactic acid bacteria.

4.9. Yeasts contaminants

Proceed with counting according to the methods in chapter II of the International Oenological Codex

The number of viable cells of total contaminant yeasts must be less than 10^3 CFU/g for lyophilisated or dried lactic acid bacteria or 10^2 CFU/ml for frozen or liquid lactic acid bacteria.

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4.10. Salmonella

Proceed with counting according to the method in chapter II of the International Oenological Codex

Absence should be checked on a 25 g sample.

4.11. Pseudomonas aeruginosa[1]

4.12. Escherichia coli

Proceed with counting according to the method in chapter II of the International Oenological Codex using a selective differential medium for Escherichia coli. MET in the annex. A lactic acid bacteria stock suspension is carried out in a trypone salt solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 2 ml of stock solution is transferred to each dish using 5 different dishes. Absence should be checked on 1 g sample[2] .

4.13. Staphylococci

Proceed with counting according to the method in chapter II of the International Oenological Codex. The presence of staphylococci is evaluated by an enrichment culture in a liquid Giolitti and Cantoni medium followed by a confirmation on a solid Baird Parker medium in the annex.

A lactic acid bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 10 ml of stock suspension is used to inoculate a Giolitti and Cantoni medium to Tween 80 double concentration. Cultures are incubated 48 hours at 37 °C.

In the case that the Giolitti and Cantoni medium gives positive results, the presence of Staphylococci is confirmed by isolation on a solid Barid Parker medium. A positive culture medium loop is used to inoculate solid BP mediums to obtain isolated colonies.

Absence should be checked on 1 g sample[3] .

4.14. Coliforms

Proceed with counting according to the method in chapter II of the International Oenological Codex using a selective differential medium for coliforms, desoxycholate gelose in the annex. A lactic acid bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic acid bacteria for 10 ml of solution (total volume). 2

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ml of stock solution are transferred is each dish using 5 different dishes.

The number of coliforms should be less than 10^2 CFU/g[4].

5. ADDITIVES

They must be in conformity with regulations in force.

6. STORAGE CONDITIONS

Always refer to manufacturer's recommendations.

MICROBIOLOGICAL ANALYSIS METHODS

(amendment of the resolution 17/2003
in chapter II of the International Oenological Codex).

A. point 1

1. Preliminary rehydration of lactic acid bacteria

- weigh 1 g of ADB under aseptic conditions;
- add 100 ml of 5 % saccharose solution in water at 36-40 °C under sterile conditions;
- slowly homogenise using a rod or a magnetic stirrer for 5 min;
- stop stirring and allow to stand for 20 minutes at a temperature of 36-40 °C;
- homogenise again at room temperature for 5 minutes;
- take 10 ml under sterile conditions and then proceed with micro-biological controls on the homogenised reference solution.

B. replace in all the milieu composition Agar by Bacteriological Agar

C. Add the following paragraphs

7.2 For research of Acetobacter

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Acb/s agar environment
Composition

Yeast extract	30 g
Alcohol 95 % per volum after sterilisation	20 ml
Bromocresol green (sol. 2.2 %)	1 ml
Bacteriological Agar	2%
Water	Up to 1000 ml

Sterilisation at 120 °C for 20 min.
Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pimaricine hydroalcoholic solution at 25 % m/v.
Incubate under aerobic conditions at 25 °C for 7 days.

7.3 - Search for Gluconobacter

Gcb/s agar medium
Composition

yeast autolysate	10 g
glucose	3 g
$CACO_3$	3 g
Bacteriological agar	2%
Water	Up to 1000 ml
Sterilisation at 120°C for 20 min	

Add directly to Petri dish 0.1 ml of penicillin solution at 0.25 % in pure alcohol.
Add directly to Petri dish 0.2 ml of pimaricine hydroalcoholic solution at 25 % m/v.
($CACO_3$ facilitates the recognition of Gluconobacter colonies which dissolve and

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produce a lighter circular zone around the colony.)
Aerobic incubation at 25 °C for 7 days.

ANNEX 1 REVIEW OF METHODS OF COLIFORM RESEARCH

Escherichia coli and Staphylococcus

SELECTIVE-DIFFERENTIAL MEDIUM FOR COLIFORMS. DESOXYCOLATE AGAR

Ingredients/l

Peptone	10.0 g
Lactose	10.0 g
Sodium desoxycolate	1.0 g (inhibition of the flora accompanying coliforms)
Sodium chloride	5.0 g
Dipotassium phosphate	2.0 g
Ferric ammonium citrate	1.0 g
Sodium citrate	1.0 g
Agar	15.0 g
Neutral red	0.03 g

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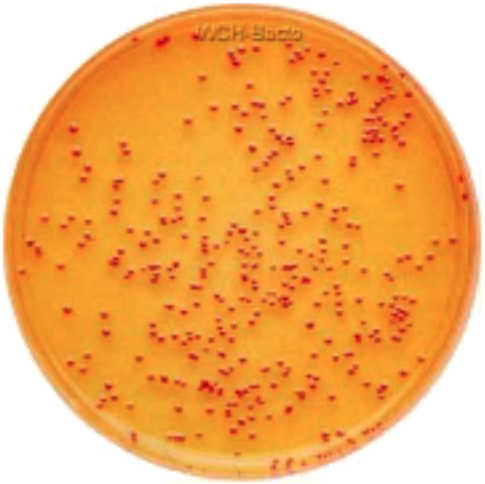
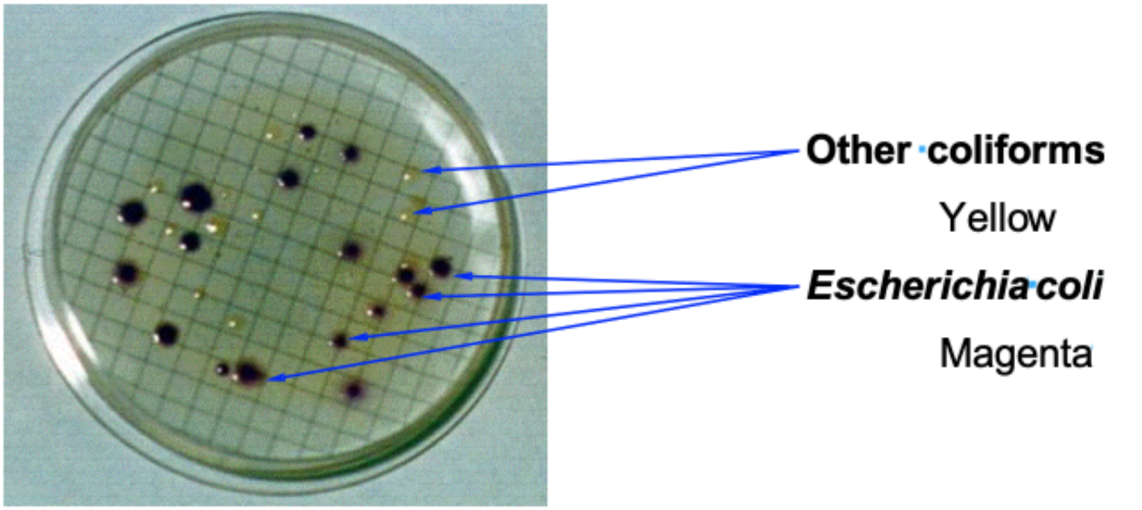


SELECTIVE-DIFFERENTIAL MEDIUM FOR Escherichia coli. MET

Sodium laurisulphate and sodium desoxycolate are used as selective factors, in accordance with their properties to inhibit the development of Gram-positive cocci and sporulated bacteria. The differential nature of the method is provided by the chromogen 5-bromo, 6-chloro-indolyl-□-D-glucuronide.



SELECTIVE-DIFFERENTIAL MEDIAS FOR Staphylococcus

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Giolitti and Cantoni medium

Composition (g) for 1 litre of medium:

Tryptone	10,0.
Meat extract	5,0.
Autolytic yeast extract	5,0.
Glycine	1,2.
Mannitol	20,0.
Sodium piruvate	3,0.
Sodium chloride	5,0.
Lithium chloride	5,0.
Tween 80	1,0.
pH medium	6,9 ± 0,2.

Baird Parker solid medium

Composition (g/l)

Tryptone	10,0.
Meat extract	5,0.
Autolytic yeast extract	1,0.
Sodium pyruvate	10,0.
Glycine	12,0.
Lithium chloride	5,0.

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Bacteriological agar	20
Egg yolk emulsion	47 ml
Potassium tellurite at 3,5%	3 ml
Sulfamehazine	0,05 g/l (if necessary inhibit Proteus)
pH medium	7,2 ± 0,2

[1] Point to be studied at a later date by the expert group “Microbiology”.

[2] Annex 1

[3] Annex 1

[4] Annex 1

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