RESOLUTION OIV/OENO 329/2009

CODEX - ACTIVE DRY YEASTS - Modification

The GENERAL ASSEMBLY,

CONSIDERING Article 5, paragraph 2 iii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

Upon the proposal of the group of expert “Microbiology” and the "Method of Analysis" Sub-commission,

DECIDES to replace the existing monograph (Oeno 16/2003) by the following monograph in the International Oenological Codex and to adapt the resolution (17/2003) accordingly:

ACTIVE DRY YEASTS (A.D.Y.) Saccharomyces spp.

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Yeasts are used for the inoculation of musts and wine. They are proposed under dehydrated form

The rate of inoculation is at the user’s discretion.

Yeasts used must be isolated from grapes, musts or wine or result from hybridisation, or have been derived from these same yeasts. The use of genetically modified oenological yeasts will be submitted to prior authorisation of competent authorities.

Oenological yeasts must be kept under conditions which most favour its genetic stability.

2. LABELLING

The following information must be indicated on the label:

- The genus name and specie(s) name in addition to the reference(s) of the strain(s) in the case that there is a registration body.
- Selecting body
- Operating instructions or method and reactivation media recommended by the manufacturer.

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- The minimum number of viable cells per gram of powder (CFU as determined in the annex) guaranteed by the manufacturer, with a storage temperature lower than 15 °C.
- The manufacturing batch number, the expiration date and storage conditions.
- Where relevant, the indication that the yeasts were obtained through genetic modifications and their modified character(s).
- Additives, including substances used during drying operations

3. CHARACTERISTICS

Active dry yeast is in typically in the form of round or vermiculated pellets obtained by drying a concentrated yeast culture.

4. TEST TRIAL METHODS AND LIMITS

4.1. Humidity

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

Maximum level should be less than 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.

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.

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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 yeasts

Proceed with counting according to the method in chapter II of the International Oenological Codex. Content should be above or equal to 10^{10}CFU/g.

NB: Counting is not applied when marketed yeasts are not Saccharomyces spp. or if they are mixtures of Saccharomyces spp and non Saccharomyces.

4.7. Yeasts of species different from the species indicated on the label

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

Content should less than 10^5CFU/g.

4.8. Moulds

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

Content should be less than 10^3CFU/g of powder.

4.9. Lactic acid bacteria

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

Content should be less than 10^5 CFU/g.

4.10. Acetic acid bacteria

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

Content should be less than 10^4 CFU/g.

4.11. 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.

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4.12. Escherichia coli

Proceed with counting according to the method in chapter II of the International Oenological Codex using the selective differential medium for Escherichia coli MET in annex. A lactic bacteria stock solution is carried out in a Tryptone salt solution with 1g of lactic bacteria for 10 ml of solution (total volume). 2 ml of stock solution are transferred to each dish using 5 different dishes.

Absence should be checked on a 1 g sample.

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 bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic 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 a 1 g sample.

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 bacteria stock suspension is carried out in a salt tryptone solution using 1 g of lactic bacteria for 10 ml of solution (total volume). 2 ml of stock solution are transferred is each dish using 5 different dishes.

Number should be less than 10^2 CFU/g .

5. ADDITIVES

They must be in conformity with regulations in force.

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6. STORAGE CONDITIONS

Storage should be below 15 °C in unopened packs.
Always refer to manufacturer's recommendations.

METHODS OF MICROBIOLOGICAL ANALYSIS

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

A. point 1

1. Preliminary rehydration of active dry yeasts (ADY)

- weigh 1 g of ADY 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 between 30-40 °C referring to the manufacturer's recommendations;
- 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 "Environment" paragraphs replace Bacteriological agar agarby Bacteriological agar

C. Add the following paragraphs

7.2 – For research of Acetobacter
Acb/s agar environment
Composition

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Yeast extract	30 g
Bromocresol green (sol. 2.2 %)	1 ml
Bacteriological Agar	2%
Water	Up to 1000 ml

Sterilisation at 120 °C for 20 min.
Add 20 ml of alcohol 95 % per volume
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 pymaricine hydroalcoholic solution at 25 % m/v.
($CaCO_3$ facilitates the recognition of Gluconobacter colonies which dissolve and produce a lighter circular zone around the colony.)
Aerobic incubation at 25 °C for 7 days.

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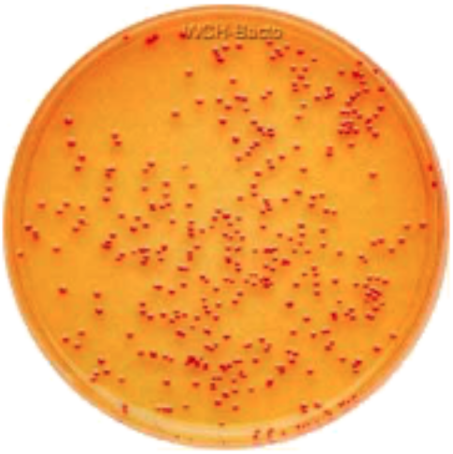
ANNEX 1 REVIEW OF METHODS OF COLIFORM RESEARCH

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

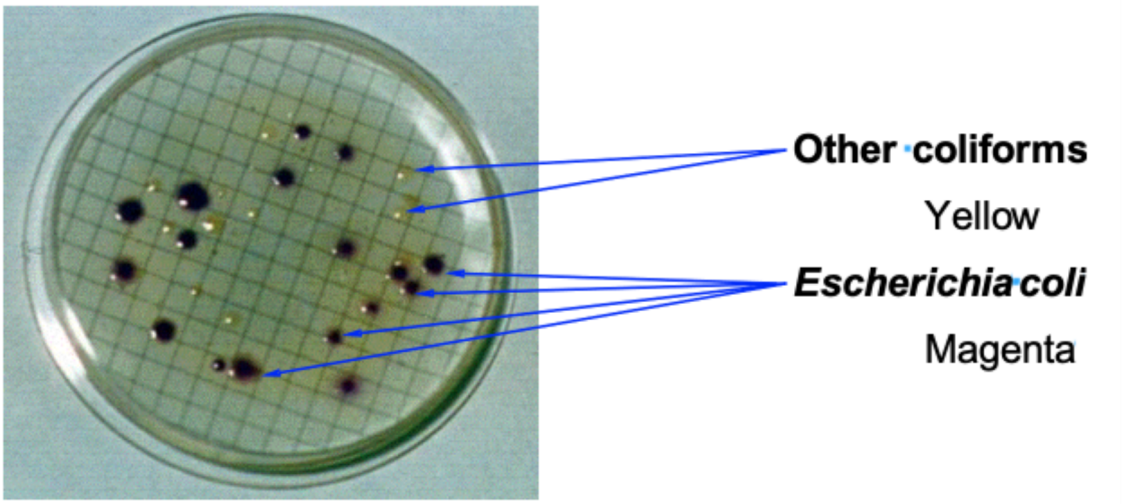


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SELECTIVE-DIFFERENTIAL MEDIUM FOR Escherichia coli. MET

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-β-D-glucuronide.



SELECTIVE-DIFFERENTIAL MEDIA FOR Staphylococcus

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.

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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.
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

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