# COEI-1-BALACT 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
- 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.

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[1]

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

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 $10^3$ CFU/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.

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*[2]

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.

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.

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 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.

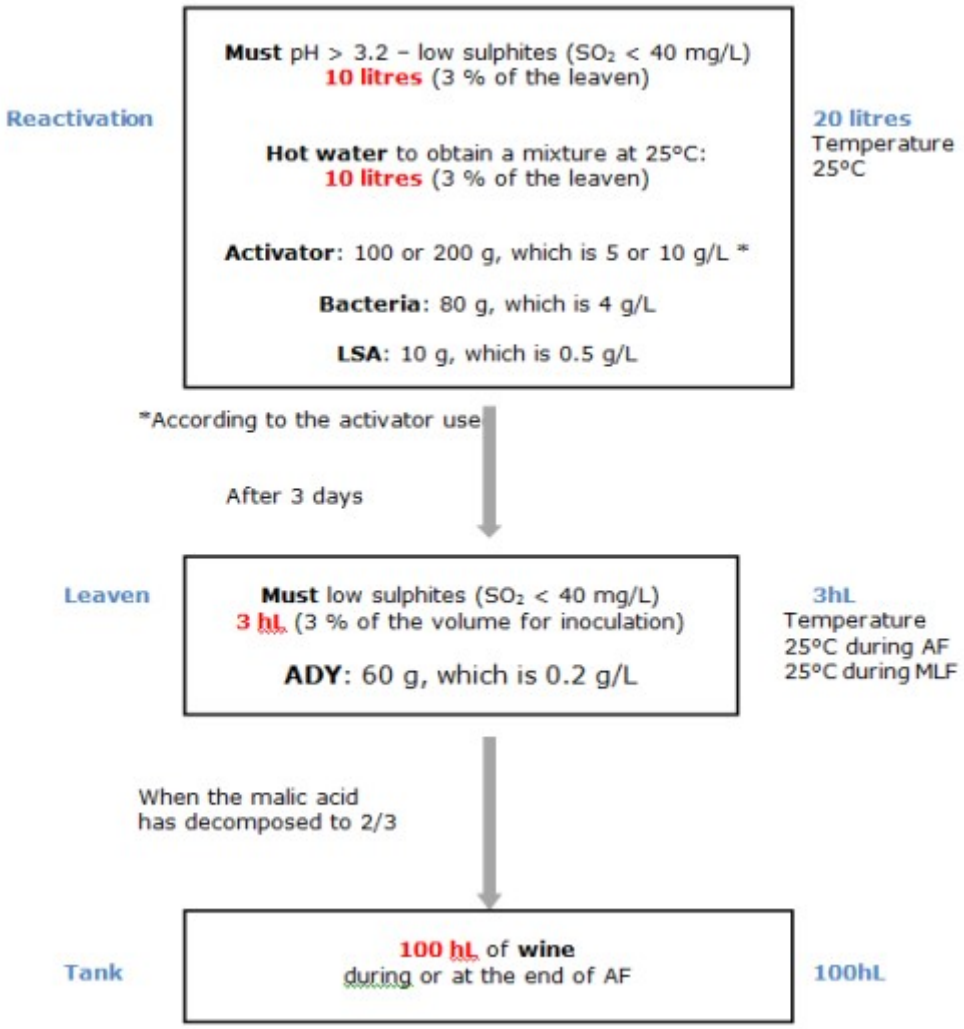
**5. Additives**

They must be in conformity with regulations in force.

**6. Storage conditions**

Always refer to manufacturer's recommendations.

Appendix: Preparation of a leaven "pied de cuve malo" to innoculate 100hL of wine or any volume from the values in brackets in %, the quantities of powder are expressed in g/L




[1] Except for specific bacteria intended for acidic wines (pH up to 2.85) that should be used with a pre-multiplication process (see Annex) in the must or wine, where the population cannot be less than $10^9$ CFU/g.
Reference: Bridier, J., O. Claisse, M. Coton, E. Coton and A. Lonvaud-Funel (2010). "Evidence of distinct populations and specific subpopulations within the species Oenococcus oeni." Appl Environ Microbiol **76**(23): 7754-7764.

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