# COEI-2-AMIBIO Qualitative method for detection of biogenic amines produced by lactic acid bacteria by thin-layer chromatography (TLC)

## 1. Principle

This method determines the ability to produce biogenic amines (BA) by bacteria in liquid culture media containing the corresponding amino-acid precursor. The method permits the separation and identification of the amines histamine (HIS), tyramine (TYR), putrescine (PUT), cadaverine (CAD) and phenylethylamine (PEA) using thin layer chromatography (TLC).

## 2. Reagents

2.1. Amino acids: L-histidine monohydrochloride, L-tyrosine di-sodium salt, L-ornithine hydrochloride, L-lysine monohydrate and L-phenylalanine;

2.2. Amines: histamine dihydrochloride, tyramine hydrochloride, 1,4-diaminobutane dihydrochloride , 1,5-diaminopentane dihydrochloride, □-phenylethylamine hydrochloride;

2.3. Dansyl chloride

2.4. Acetone

2.5. Chloroform

2.6. Triethylamine

2.7. Isopropanol

2.8. Triethanolamine

2.9. Thin-layer chromatography (TLC) plates (10 x 20 precoated plates with 0.20 mm silica gel 60 $F_{254}$)

## 3. Standard solutions

A stock of standard solutions is prepared by dissolving 0.2 g of each amine (HIS, TYR, PUT, CAD and PEA) in 10mL of 40% ethanol. The working standard solution is prepared by mixing 1 ml of each of these solutions and bringing it to a final volume of 10 mL with water.

Amines are converted to their fluorescent dansyl derivatives as follows: one volume of 250 mM $Na_2HPO_4$, 0.1 volume of 4N NaOH and 2 volumes of dansyl chloride solution (5 mg/mL dansyl chloride in acetone) are added to one volume of the sample. The mixture is homogenized with a Vortex mixer and incubated at 55° C for 1 hour in the

dark.

## 4. Microorganisms and growth conditions

*O. oeni* strains are cultured in pH 4.8 MRS broth (Merck), supplemented with 10% tomato juice. Strains of the genera *Lactobacillus* and *Pediococcus* are cultured in pH 6.3 MRS broth. All the bacteria are incubated at 30° C.

The broths are supplemented with biogenic-amine precursor amino acids such as histidine (5 mg/mL), tyrosine (5 mg/mL), ornithine (5 mg/mL), lysine (5 mg/mL), and phenylalanine (5 mg/mL). Samples are analysed after 9-12 days of growth.

## 5. TLC CONDITIONS

The amines are fractionated on silica gel plates (silica gel 60 F254s). Amine-derivative extracts (10 µl) are applied 2 cm from the base of the plates with capillary pipettes. The dansylated compounds are separated by ascending development for 17 cm in chloroform:triethylamine (4:1). The spots are visualized under UV by using a transilluminator with a system for image acquisition. If a similar instrument is not available, the plate can be sprayed with isopropanol:triethanolamine (8:2) to enhance the fluorescence and visualized under a classical UV source.

The detection limit for the amines TYR, PUT, CAD and PEA is 0.01 mg/ml and the detection limit for HIS is 1 mg/mL. The method showed less sensitivity to HIS, however this detection level in the TLC method described is also adequate to detect HIS production when the bacteria is growing in a culture media supplemented with 5 mg/mL of histidine, as previously described.

## 6. Analysis of biogenic amines from bacterial cultures

Bacterial strains are grown as described in section 4. After incubation, the broth media are centrifuged and the supernatants are analysed for BA content. Analysis of amines produced by bacterial strains is performed directly on bacterial supernatants as described above.

The separation order of the resulting amine spots from the top to the bottom of the plate are: PEA, TYR, HIS, CAD, PUT.

## 7. Bibliography

- Costantini A., Cersosimo M., Del Prete V., Garcia-Moruno E. (2006). Production of biogenic amines by lactic acid bacteria: screening by PCR, TLC and HPLC of

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