THE ACETIC BACTERIA ISOLATION AND SELECTION FOR THE VINEGAR INDUSTRY

Maria Cristiana Garnai (Tarhon), Oancea Ioana
"Dunarea de Jos" University of Galati, 111 Domneasca Street, 800201 Galati
Tel./Fax: +40 236 460165

Abstract

From wines and vinegar there have been isolated and obtained, under the form of pure growing environments, more Acetobacter colonies; these colonies have been tested in view of identification and framing in the classification, having as purpose their selection for vinegar industry.
Keywords: *fermentation acetic acid, vinegar, Acetobacter*

Introduction

Acetic bacteria are widespread in nature, on vegetal products–the place of some alcoholic fermentation-in liquids with low alcohol content (beer, cider, wine), at the surface of which it can form a thin layer–more or less adherent to the walls of the container. These bacteria are aerobian, non-sporulated, mobile or immobile, most of them having sizes of 0.3–3 microns.

The first classification of these bacteria, made on Hansen's morphological criteria, was made in 1894. Afterwards, other classifications have been made, based on the biochemical criteria of these acetic bacteria. Contributions in this sense have been brought by Dupuy (1957), Dupuy and Mangenet (1963), Frateur (1950), Diviers (1973) and others.

In Bergey's Manual (1974) acetic bacteria are represented by two types: *Acetobacter* and *Gluconobacter*, between which there exists both resemblances and differences. Both acetic bacteria oxidize the ethanol at the acetic acid, but only *Acetobacter* oxidizes the acetic acid and the lactic at CO_2. Because acetic bacteria in the genus *Gluconobacter* oxidizes glucose at gluconate is of interest in producing the gluconic acid, while acetic bacteria in the genus *Acetobacter* have an important role as fermentation agent in vinegar technology.

Vinegar industry is, in general, interested in acetic bacteria with superior fermentative potential, with a convenient bioconversion efficiency of ethanol into acetic acid, in different variants of the fermentation environments imposed by the available raw material.

In view of obtaining Acetobacter colonies, which could be later studied, selected and recommended to the vinegar industry, we have isolated more acetic bacteria colonies from alcoholic environments (wine, cider).

The obtained pure colonies have been further submitted to some biochemical tests, besides macro and microscopic observations, in view of their framing into the classification.

Experimental

For the intended purpose we have made colonies from wine and from cider in malt must with 6% refractometric extract, 2% agar and 4% alcohol, using the plate-isolation method.

After thermostating at 25-28°C the most isolated colonies have served as inoculations for resowing both on the above-mentioned agarized environment and on liquid environment.

Then, the over 30 isolated pure cultures have been submitted to the lactate oxidation test (after Bergey's Manual–1974). In this test, there has been used the colony environment made up of yeast extract 0,5%, calcium lactate 2%, gelose 2%. The growth of acetic bacteria on this environment, with calcium precipitation around the colony, separates acetic bacteria in the genus *Acetobacter* from the ones in the genus *Gluconobacter* that do not oxidize the lactate. As a consequence of this test, 14 *Acetobacter* colonies have been kept, which have constituted further study material.

Afterwards, some other tests have been performed, i.e. glycerol oxidation, catalase production, growth on Hoyer-Frateur environment (that is, using the ammonium salts as unique source of nitrogen in the environment), cellulose production, pigment production, alcohol tolerance, the potential of alcohol bioconversion into acetic acid (table 1).

Microscopic and macroscopic observations have been made on the colonies, referring to the form and sizes of the colonies, the aspect, form, colour of the colonies developed on solid environment and the aspect of the membranes grown in alcoholic liquid environment.

In the test regarding glycerol oxidation there has been used the growing environment made up of yeast extract 0,5%, glycerol 2%, gelose 2%. The growth of the colony during 24 hours on this environment highlights the presence of the species *Acetobacter aceti*, capable of metabolizing oxidatively the glycerol.

As far as catalase production is concerned, the test has been performed using a drop of peroxide (H_2O_2, conc. 3%) over a fragment of

bacterian colony (taken with a sterile ear) and put on a glass mount. The instant appearance of gas bubbles is due to catalase action and is interpreted as a positive test.

Table 1: Biochemical tests which can be used in a taxonomical purpose for acetic bacteria

	THE TESTED ACETIC BACTERIA COLONY													
THE ACETIC BACTERIA BIOCHEMICAL TESTS RESULT	Ai	A10	C2	C3	2GI	3GI	S	T10	Bz	3Gi	T5	T6	T8	T14
Lactate oxidation	+++	+	+++	+	+++	+++	+	+++	+	+	+++	+	+	
Glycerol oxidation	+++	+++	+++	++	+++	+++	+	+++	+	+	+++	+++	+++	+
Catalase production	++	++	+	+	+	+	+	++	+	+	+	++	+	+
Growth on a Hoyer-Frateur environment	-	-	+	+	+	+	+	-	+	+	+++	+	++	
Cellulose production	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Pigment production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Max. acetic acid (g/100ml) resulted by alcohol oxidation	6,76	6,89	6,70	6,57	7,02	6,70	6,83	7,08	6,95	6,08	7,09	6,54	6,68	4,70
INTRODUCTION IN CLASSIFICATION	*A.a.-orleansis*	*A.a.-orleansis*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-orleansis*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-aceti*	*A.a.-xylinum*

Table 2: The acetic bacteria biochemical tests result

	SPECIES	SUBSPECIES	THE HIGHLIGHTING OF DIFFERENTIAL CHARACTERS					
			Lactate oxidation	Glycerol oxidation	Catalase production	Growth on a Hoyer-Frateur environment	Cellulose production	Pigment production
Biochemical tests which can be used in a taxonomi-cal purpose for acetic bacteria	*Acetobacter aceti*	*A.a.-aceti*	+	+	+	+	--	--
		A.a.-orleansis	+	+	+	--	--	--
		A.a.-xylinum	+	+	--	--	+	+
		A.a.-liquefaciens	+	+	+	+	--	+
	Acetobacter pasteurianus	*A.p.-pasteurianus*	+	--	--	±	--	--
		A.p.-lovaniensis	+	--	--	+	--	--
		A.p.-estunensis	+	--	--	+	+	--
		A.p.-ascendens	+	--	--	--	--	--
		A.p.-paradoxus	+	--	+	--	--	--
	Acetobacter peroxidans		+	--	+	+	--	--

In the test regarding the acetic bacteria growth on the Hoyer-Frateur environment, there has been used a growing environment that contains: $SO_4(NH_4)_2$–0,1%, PO_4HK_2–0,01%, PO_4H_2K–0,09%, $MgSO_4$–0,025%, FeCl (3%)–0,5 ml, gelose (agar)–2%.

Cellulose production has been highlighted with Lugol solution in the presence of sulphuric acid conc. 60%). A fragment from the tested material

put on a glass mount in the presence of the Lugol solution and of the sulphuric acid, for an hour, gives the colour blue–specific to cellulose presence. In the absence of cellulose, the colour yellow appears.

The capacity to produce acetic acid, as well as the alcohol tolerance of the 14 *Acetobacter* colonies, have been achieved by their growth in 6, 7, 8, 9, 10, 11 and 12% alcohol environments (proceeded from refined alcohol), with 2% acetic acid and 1% corn extract (used as nutrient).

In these environments, 13 of the *Acetobacter* colonies have multiplied (at 25°C) and produced more or less acetic acid depending on the colony, obtaining the maximum acidity written differently in table 2, except for the colony A14 which developed only in the environments with 6 and 7% alcohol.

Results and Discussions

✓ After the lactate test, there have been kept 14 *Acetobacter* colonies. After having interpreted the results from table number 2, and the microscopic and macroscopic observations, we reached the conclusion that the 14 colonies belong to the genus *Acetobacter aceti* (the glycerol and catalase tests being positive).

✓ Three of the *Acetobacter aceti* colonies, which do not grow on the Hoyer-Frateur environment, i.e. Ai, A10, T10 belong to the sub-species *Acetobacter aceti orleansis.*

✓ A14 colony, which produces cellulose (it forms a thick, mucilaginous membrane), has been identified as *Acetobacter aceti xylinum.*

✓ The other 10 *Acetobacter aceti* colonies C2, C3, 2Gl, 3Gl, S, Bz, 3Gi, T5, T6, T8 that grow on the Hoyer-Frateur environment, which do not produce cellulose, and do not produce pigment belong to the sub-species *Acetobacter aceti aceti.*

✓ The isolated acetic bacteria colonies from more wine and cider samples, identified as genus, species and subspecies will constitute the biological material basis for a larger study with useful recommendations for an efficient fermentation in obtaining vinegar.

References

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