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THE RESISTANCE OF ACETIC BACTERIA AT THE ACETIC ACID

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SYNOPSIS

The acetic acid is remarked for its cite-toxicity and for the degradation of the growing process of the microorganisms when the concentration is about 5g/liter (s.carlsen, 1990).these toxic effects refer to the weak lipofilic nature of the undiscounted acid, giving the possibility to the molecule to penetrate the citoplasmatic diaphragm.

In the industrial process, acetobacter aceti can develop in a concentration of the acetic acid of 60g/liter (y.kawamura 1991) and even in an excess of the concentration of 140g/liter in the semi-continuous processes.(h.follman,1983). Lack of oxygen in the case of acetobacter leads in a few seconds to the blocking of the energetic exchanges.

The purpose of these researches was to isolate and identify roots of the acetic bacteria from the spontaneous flora which are able to produce wine vinegar and cider. For these experiments there were used solid cultures, in petri boxes. The bacteria were submitted to identification tests , then there were made experiences regarding the resistance to alcohol and the fermentation potential .

The oxidation of the ethanol by the bacteria which belong to the two types is verified through the cultures which contain 5g/liter extract of levura and 2-3% ethanol.

INTRODUCTION

The acetic acid is remarked for its cite-toxicity and for the degradation of the growing process of the microorganisms when the concentration is about 5g/liter (Bech Jensen E.,Carlsen S., 1990 Diez – Gonzalez F., J.B. Russell. 1997).These toxic effects refer to the weak lipofilic nature of the undiscounted acid, giving the possibility to the molecule to penetrate the citoplasmatic diaphragm.This spreading leads to the dissipation of ionic gradient, the internal concentration increases in a cetates and

disturbs the membraneous processes to the destruction of the cell (Axe D.D, J.E Bailey. 1995, Diez-Gonzalez F., J.B. Russell. 1997, Durre P, H. Bahl, G. Gottschalk. 1988, Russel J.B. 1992).

While the majority of the microorganisms are sesitive to the high concentration of the acetic acid, there are some relatively resisting at this acid. An important example of the resisting microorganisms at the acetic acid is the one which refus to the acetic bacteria, especially the Acetobacter and Gluconobacter types, types used for centuries for producing diluted acetic acid or steel (Ebner H, H. Follmann. 1983).

In the industrial process, Acetobacter aceti can develop in a concentration of the acetic acid of 60g/liter (Park Y.S.,K.Toda,M.Fukaya, H.Okumura,Y.Kawamura. 1991) and even in an excess of the concentration of 140g/liter in the semi-continuous processes.(Ebner H.,H.Follmann,1983). Lack of oxygen in the case of Acetobacter leads in a few seconds to the blocking of the energetic exchanges (Hitschmann A., H.Stockinger.1985).

As a consequence of their genetic variability, acetic bacteria lose rapidly their resistance at the acetic acid when they are no longer in contact with it. (Ebner H, H. Follmann. 1983). That is why, acetic bacteria used in industry are kept in very concentred acetic acid, the process of preparing vinegar continues for years without stopping due to the adaption of the microorganisms to high concentrations. This adaption seems to be an essential condition for tolerance to high concentration, aspect wich cannot be found at the wild types from spontaneous flora. (Lasko D.R., C. Schwerdel, J.E. Bailey, U. Sauer. 1997).

In most of the scientific works are pointed out resistances to the acetates of termofilic stem by A. Aceti, on solid medium, which posseses a set of resisting genes, aarABC, (Fukaya M., H. Takemura, H. Okumura, Y. Kawamura, S. Horinouchi, T. Beppu. 1993),and pointed out that this proteins offers resistance to the asimilation of the acetate with a local reduction of the concentration, but only on solid medium. In liquid medium, the asimilation of the acetate is not a pertinent mechanism of resistance, particularly when the acetate is produced continuously by the microorganism.

THE AIM OF THE PAPER

The purpose of these researches was to isolate and identify roots of the acetic bacteria from the spontaneous flora which are able to produce wine vinegar and cider. For these experiments there were used solid cultures, in Petri boxes. The bacteria were submitted to identification tests , then there were made experiences regarding the resistance to alcohol and the fermentation potential .

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WORKING METHODS AND MATERIALS.
BIOLOGICAL MATERIAL AND MEDIUM.

The biological material neccessary for the experiment was isolated from the following resources:

- viticultural soil, Viticultural Centre Banu Maracine
- washing water of the grapes, Viticultural Centre Banu Maracine
- red wine from the Viticultural Centre Banu Maracine
- turned sour red wine
- white wine from the Viticultural Centre Banu Maracine
- turned sour white wine
- turned sour white wine
- apple cider, S.D. Banu Maracine
- turned sour apple cider

For the research of Acetobacter type (which is intensivly from producing vinegar point of view) it was prepared a medium with the following composition:

Leaven extract – 30g
Alchool 95% vol – 20 ml
Agar 2%
Distilled water – 1000 ml

After dissolving components and after homogenization, sterilization of the enviroment was realized at 120°C for 20 minutes, after the distribution of the medium in the Petri`s boxes, in everyone was put 0.1 ml alcholic solution of penicilin.

The incubation was realized in the thermostat at 28°C for 7 days.

For the microscopic study of the bacterium and their indentification, was used the differential method of colouring Gram. For this purpose we followed some steps:

- Making dry and fixed smears
- Colouring in violet (this was applied for one minute, then rinsing with water)
- Treatment with Lugol (I2/1K) (applied for one minute, then rinsing with water)
- Washing with a mixture of ethylic alcohol and acetone 3:1 for removing the colouring and the iodine from the cell (duration of the contact 15 sec, then rinsing with water)
- Application of the colouring of contrast – fuchsine (duration one minute, then rinsing with water and drying it in warm air)
- Studying the microscop with 100X objective, sinked in cedaroil

As a result, in the microscopic positive gram bacteria appear coloured in violet while the negative gram bacteria appear in contrast colour (red) of the secondary colouring fuchsine.

Doing the analysis of the images and at the same time, using a specialized program (Cell explorer) it was possible to make a digital processing of the images.

THE RESULTES OBTAINED AND THEIR INTERPRETATION. THE MORFOLOGIC STUDY OF THE COLONIES.

After the developing of the colonies in the Petri`s boxes (6-14days), these were morphologically analized, following especially the colour, form and the size of the colonies, the appearance and the way they are organised on the medium surface.

The results of the observations were compared depending on the sources of which biological tests were reaping.

This way, the acetate bacteria colonies isolated in the viticultural soil (fig.2) have colours which differ from dark red to brown; have very small diametre and are spread along the route previously marked with the loop of introduction.

Acetic bacteria isolated from the washing water of the grapes, from lighter coloured colonies (fig.1), are spherical of small dimensions and very dense on the surface of the medium. Around the colonies and fixed on these is formed a mucilagious, shinny veil.

The acetic bacteria colonies isolated from the red wine (fig.3,4) are spherical and very clear outlined, dark red and present a small density of population, compared to the one isolated on grapes.

From the turned sour wine were developed colonies (fig.5,6), identical, in form and colour with the ones isolated from red wine.

Isolated colonies from white wine (fig.7, 8) are grey, in dark shades, round. They are less dense (as far as the population is concerned) compared to the ones isolated from red wine.

CONCLUSIONS

Acetobacter aceti is specific for wine vinigar having a fermentative potential of 7,2%.

Acetobacter ascendens makes larger quantities of acid acetic compared to the other two species, but disturbs the liquid on the surface where it makes a film with delicate structure, having an extraordinary capacity to climb on the walls of the vessel where it is.

Acetobacter xylinum has a low fermentation potential and makes zooglee, for this reason it is not desired in the vinigar industry.

Fig.1 Acetic bacteria – from the washing water of the grapes (morphology of colonies)

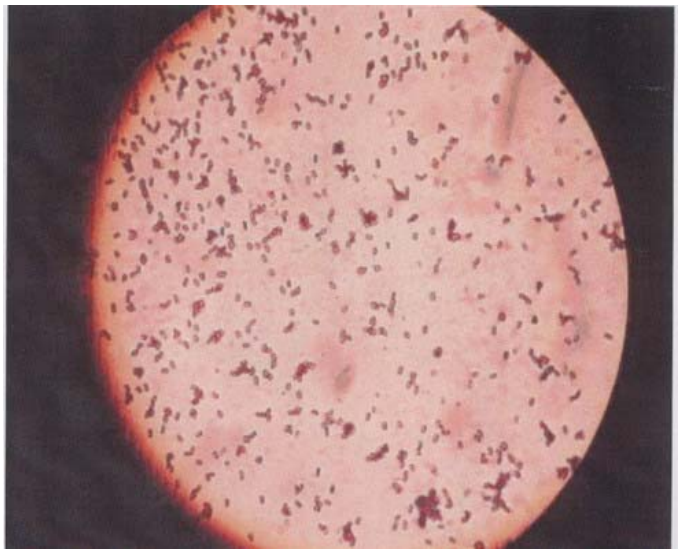

Fig.2 Acetic bacteria – sol (morphology of cells)

Fig.3 Acetic bacteria – from red wine (morphology of colonies)

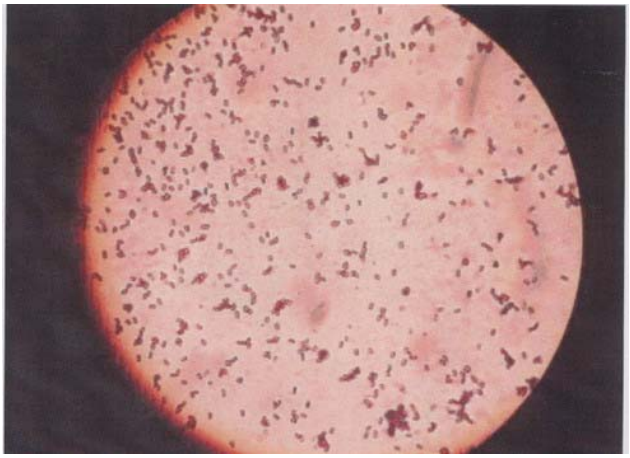

Fig.4 Acetic bacteria – from red wine (morphology of cells)

Fig.5 Acetic bacteria – from sour red wine (morphology of colonies)

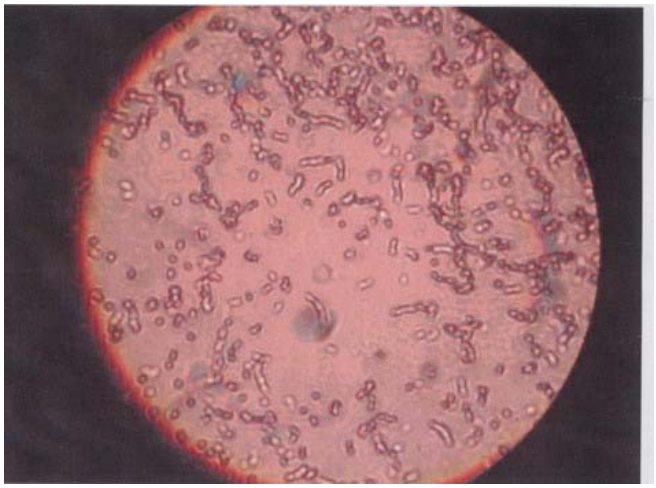

Fig.6 Acetic bacteria – from sour red wine (morphology of cells)

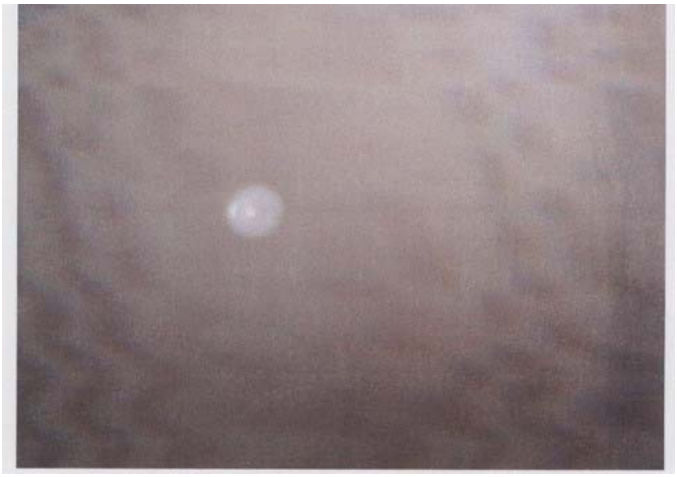

Fig.7 Acetic bacteria – from white wine (morphology of colonies)

Fig.8 Acetic bacteria – from white wine (morphology of cells)

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