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MALATE UTILIZATION BY *SCHIZOSACCHAROMYCES POMBE*

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SUMMARY

Malic acid degradation by *Schizosaccharomyces pombe* was studied in synthetic and natural media. Using different concentrations from 1 to 29 g/l, malic acid was degraded. Initial deacidification rate increased with initial malate concentration. During the stationnary phase, *S. pombe* was able to decompose malate added to the medium before or after sugar exhaustion.

INTRODUCTION

Schizosaccharomyces yeasts present the unusual property of being able to convert malic acid into ethanol with a great efficiency during fermentation.

Malic acid is one of the main organic acids in grapes. Generally, it is necessary to remove it from wines in order to improve their organoleptic qualities and to ensure their biological stability. One means of reducing grape musts acidity might be the use of *Schizosaccharomyces*. Some attempts have been made to deacidify grape musts (GALLANDER, 1977 ; SNOW and GALLANDER, 1979; CHARPENTIER and al. , 1985) or wines (BENDA, 1973) with *S. pombe*. Results were variable because of the broad variety of *S. pombe* strains and because of a lack of data about the yeast kinetic and physiologic behaviour.

In order to develop a suitable biological method for grape musts deacidification using *S. pombe* we had to improve our understanding of growth and malate degradation kinetics as well as malate metabolism.

The present study deals with the linkage between malate metabolism, cellular growth and sugar assimilation.

MATERIALS AND METHODS

Microorganism

The yeast strain used was the *Schizosaccharomyces pombe* strain isolated by Institut Coopératif du Vin of Montpellier (ICV).

Culture medium

Two media were used:

- grape must (Gros Manseng variety) containing 200 g/l sugars (approximatively 100 g/l glucose and 100 g/l fructose) and 5 g/l malate.
- synthetic medium consisted of the following in g/l water: glucose 100; malate 1 to 29; yeast extract 4 ; KH_2PO_4 5 ; $MgSO_4$ 0.4; pH 3.

Culture conditions

Batch experiments were performed in 2 liters fermentors (SETRIC GENIE INDUSTRIEL. Toulouse). Temperature was regulated at 30° C. PH was not regulated. Aeration rate was 0.15 VVM. Agitation rate was 280 r.p.m.

Analytical determinations

Biomass was estimated gravimetrically. Cell viability was determined by methylene blue staining. Glucose, fructose and malic acid were measured using enzymatic procedures of

Boehringer Mannheim. Ethanol was determined by gas chromatography.

RESULTS AND DISCUSSION

Although malate utilization has been studied for some time, the metabolic pathway for malate degradation in *S. pombe* remains unknown. Energetic processes controling malic acid transport and break down are still not elucidated. According to most authors, malate catabolism in *Schizosaccharomyces* is strictly linked with yeast cellular growth (DITTRICH, 1963; BIDAN, 1974) , and with sugar consumption (PEYNAUD and al., 1964; BRUGIRARD and ROQUES, 1972; OSOTHSILP and SUBDEN , 1986).

Malate tolerance

To investige malate uptake in *S. pombe* (saturation kinetics or substrate inhibition) , batch cultures were performed using synthetic media containing 18-29 g/l malic acid ; fermentations with synthetic medium containing 1 to 16 g/l malate have been presented elsewhere (AURIOL and al., 1987).

Concentration of malic acid (1 to 29 g/l) did not affect growth, sugar assimilation or ethanol production ; 100 % of malate was degraded in each experiment. No substrate inhibition of deacidification could be found. Initial deacidification rate was increasing with malate concentration. Malate uptake in *S. pombe* did not follow saturation kinetics that would have indicated a carrier mediated malate transport.

Further experiments were conducted on media containing about 7 g/l malate that is the level occuring in acid grape musts.

Relation between deacidification and cellular growth

As shown in previous work (AURIOL and al. , 1987), when acid level was high, the entire amount of malate could not be metabolized during the growth phase. Therefore, during the stationary phase resting cells continued to break-down malic acid.

Likewise, when malate was added to a culture of *S.pombe* during the stationary phase, it was totally degraded at a high rate (figure 1).

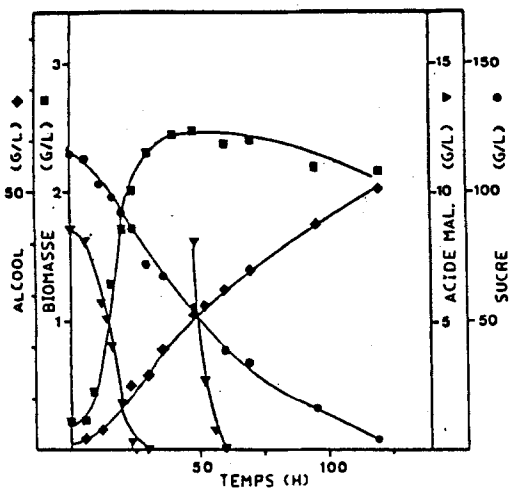

Figure 1: Malic acid degradation during the stationnary phase.

Grape musts deacidification method using S. pombe was tested in a "Jurançon" winery (Cave Coopérative des Producteurs de Jurançon).Grape musts were sulfited before inoculation with S. pombe (1 10^6 cells / ml) in order to delay the growth of natural Saccharomyces cerevisiae flora (figure 2).

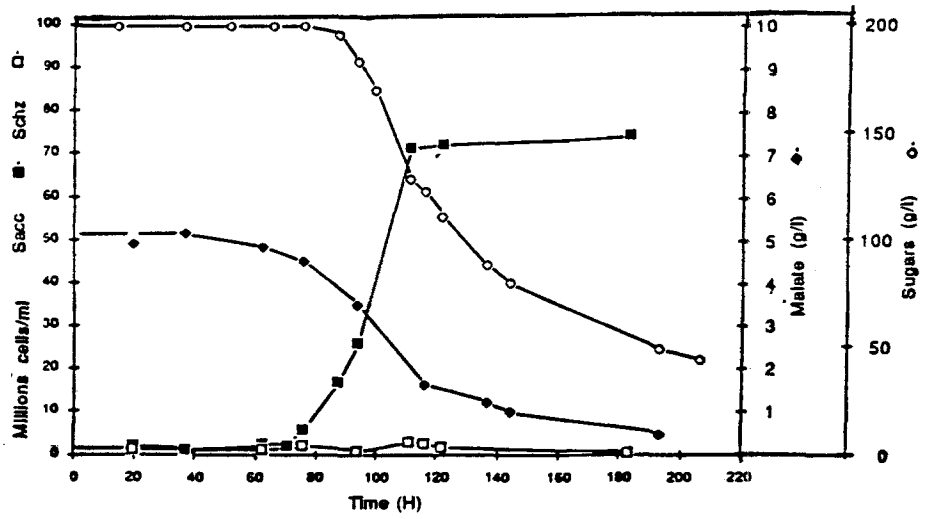

Figure 2: Grape musts deacidification. Sugars consumption. Evolution of *Saccharomyces* and *Schizosaccharomyces* populations.

After about 50 hours , deacidification of musts started before sugar up-take. *Saccharomyces* yeasts started to multiply when the sugar concentration was decreasing. *S. pombe* concentration was never greatly increasing but its viability was always about 90 %. In fact, it seemed that there was little or no *S. pombe* growth.

THIBAULT (1982) noted that malate degradation started before sugar assimilation during fermentation of grape musts with pure *S. pombe* .

These three kinds of experiments clearly demonstrated that with S. pombe deacidification and cellular growth are strictly no-linked .

Relation between deacidification and sugar consumption

Grape musts deacidification attempts made at GAN winery suggested that malate degradation and sugar utilization with *S. pombe* were not so strictly linked as it was previously thought.To verify this, malate was added repeatly to a culture of *S. pombe* after all sugar has been consumed (figure 3).

The decomposition of malate by resting cells was observed before and after sugar exhaustion with a declining deacidification rate. At the same time, the viability was decreasing rapidly from sugar exhaustion, so the decline in deacidification rate may be due to a loss of yeast activity.

In a reference culture where no malate was added after sugar exhaustion, viability remained constant, equal to 95 %. Consequently, it seemed that malic acid was responsible

for the decrease of viability when no sugar was present in the culture medium.

No extracellular deacidification activity was found in the supernatant of a *S. pombe* culture in synthetic medium. Despite their loss of viability, *S. pombe* cells were able to degrade malic acid efficienly without sugar consumption (20 g/l malate consumed during 150 hours after sugar exhaustion).

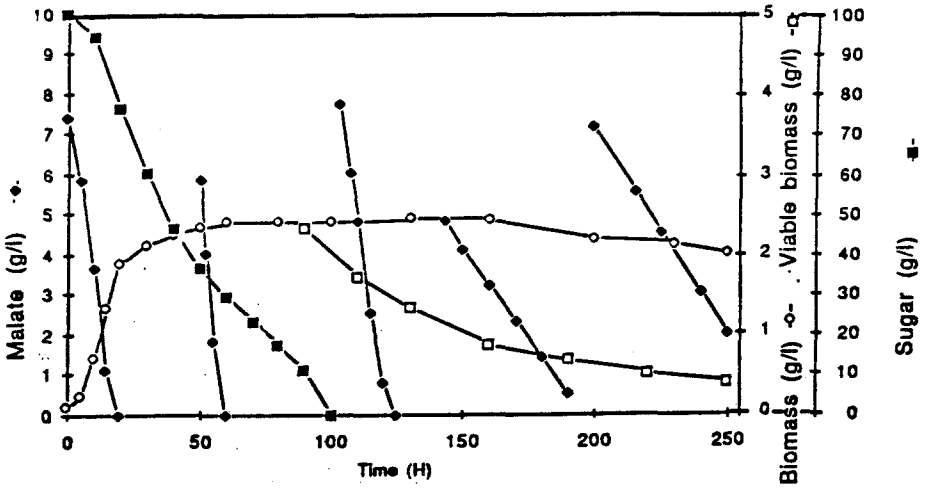

Figure 3: Malic acid degradation during the stationary phase before and after sugar exhaustion

CONCLUSION

The results presented in this communication clearly show that malate degradation by *S. pombe* is neither linked with growth nor with sugar assimilation as it was previously thought. When sugar is no longer present in the culture medium , viability of yeasts decreases whereas the deacidification activity remains. It seems that malic acid is responsible the loss of viability in the absence of sugar. Since malate is not a source of energy and can not be the only source of carbon (KUNKEE, 1967), we do not know why it is metabolized in a medium lacking sugar.

This about malic acid utilization by *S. pombe* can help us to determine the optimal conditions for continuous biological deacidification of grape musts.

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