Influence of Lactose and Sucrose on Growth and Acetaldehyde Production by Three Strains of *Streptococcus thermophilus*

Rabha Bennama [1], Victor Ladero [2], Miguel A Alvarez[2], María Fernández [2], Ahmed Bensoltane [3]

[1] Department of Biotechnology, University of Mostaganem, Mostaganem, Algeria

[2] Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa, Spain

[3] Department of Biology, University of Oran, Algeria

Abstract

This investigation describes three strains of *Streptococcus thermophilus* on the basis of production of acetaldehyde as aroma compounds. The strains under study (BN1, BN2 and BN3) were isolated from Algerian raw milk and were identified according to microbiological, biochemical, and genetic criteria. The growth of the strains and the determination of acetaldehyde were performed on M17 medium added to 0.5 and 3% (w/v) of lactose and sucrose. It was observed that the produced biomass (log cfu/ml), reached high values in the presence of 3% (w/v) of lactose and sucrose compared to that posted with 0.5% (w/v). The strains appeared to produce acetaldehyde. This production was more powerful in the case of concentration 3% (w/v) of lactose and sucrose. Strain BN1 produced approximately 205±75µmol and 218±90µmol of acetaldehyde respectively in the presence of 3% (w/v) of lactose and sucrose. This ratio was significantly higher ($P<0.01$) compared to that quantified with strains BN2 and BN3.

Keywords: *Streptococcus thermophilus*, growth, lactose, sucrose, acetaldehyde production.

1. Introduction

Fermented dairy products have become commonly consumed food in many countries around the world. These products were industrially developed using lactic acid bacteria, which were at the origin of an individual transformation process that affected the texture, flavour, quality, and the conservation of fermented dairy products [1]. *Streptococcus thermophilus* is one of the species that plays a great, interesting role for its contribution to the rapid transformation of lactose milk in lactate, the secretion of exopolysaccharides, synthesis of vitamins like folic acid, and production of some flavour compounds such as acetaldehyde [2]. Acetaldehyde is the major component responsible for the typical flavour of yogurt and a number of cheeses [3-4]**.** It is produced by the two yoghurt bacteria: *S. thermophilus* and *Lactobacillus bulgaricus*, but *S. thermophilus* species is deemed to be a good acetaldehyde producer [3]. The exact mechanism for the production of acetaldehyde from *S. thermophilus* is not well understood. In general, it is formed directly from the pyruvate decarboxylation through the action of the pyruvate decarboxylase or indirectly from the acetyl – CoA, through the pyruvate dehydrogenase and aldehyde dehydrogenase [5-6]**.** Moreover, acetaldehyde can be produced through the serine hydroxyl-methyl transferase

(SHMT), which catabolizes threonine into acetaldehyde and glycine [3]. SHMT is not the only shunt involved in the formation of acetaldehyde in yogurt, but also in the formation of glycine, serine, and significant amounts of folic acid [7].

Concerning this compound, the focus was on improving among other things of biosynthesis conditions, the composition of the culture media. In this context, this work is interested to the screening of three strains of *S. thermophilus* by studying the effect of carbon source (lactose and sucrose) on the growth and production of this aromatic compound.

2. Materiel and Methods

2.1. Strains and Culture Conditions

Three strains of *S. thermophilus* BN1, BN2, and BN3 isolated from Algerian raw milk were used in this study. Strains were identified by phenotypic and biochemical criteria and confirmed by molecular methods as described by Bennama et al. [8]. They were routinely grown on M17 medium [9] containing 0.5% (w/v) of lactose (LM17) and incubated anaerobically at 42°C.

2.2. Fermentation and Growth Parameters

Experimental cultures for growth were established using M17 medium containing as sole carbon sources 0.5 and 3% (w/v) of lactose or sucrose (LM17 or SM17) (Biochemika); adjusted to pH 7.0. Fermentation was initiated by inoculating the media with 1% ($1x10^8$ cfu/ml) overnight cultures of the studied strains. After 8h of fermentation at 42°C, viable bacterial counts were performed by serial dilution in peptone-saline water [[(1 $g.l^{-1}$) and NaCl (8.5 $g.l^{-1}$)]. Selected dilutions were then plated onto LM17 agar. Plates were incubated at 42°C for 48h; growth is expressed as log cfu/ml and analyzed by comparison to the initial rate of inoculation. Acidification that developed in the cultures was measured with a pH meter (Hanna Instruments, pH210 microprocesor pH meter). All experiments were triplicated.

2.3. Acetaldehyde Estimation

Estimation of acetaldehyde was carried out under the same conditions outlined above with LM17 or SM17. In order to avoid evaporation, it should be noted that cultures destined for these experiments were prepared in centrifuge tubes hermetically sealed. However, acetaldehyde was determined after 8h of fermentation at 42°C. It was measured by spectrophotometer (Jenway J7305) using an assay kit (R-Biopharm: enzymatic bioanalysis, Germany), based on the reduction of the NAD to NADH in the presence of aldehyde dehydrogenase. All assays were repeated three times.

2.4. Data Analysis

In all experiments mentioned above, the values of results are the mean ±standard error. Statistical analysis was done with student's test.

3. Results and Discussion

3.1. Growth of Strains in the Presence of Lactose and Sucrose

Bacteria obtain energy by multiple ways. Most of this energy is used in the biosynthesis of many metabolites or anabolism. Performance of different metabolic reactions depends essentially on the nature of the carbon source and its concentration**.** Thus in this work, the importance of the carbon source was checked with strains BN1, BN2, and BN3 of *S. thermophilus* by studying their growth in the presence of lactose and sucrose. These have been incorporated to M17 medium at final concentration of 0.5 and 3% (w/v). It is well known that *S. thermophilus* exhibits a highly affinity to grow on lactose**;** sucrose can also be used nevertheless with lower efficiency than lactose [10-11]. This affinity towards both carbon sources is related to the presence of a large variety of important genes and enzymatic equipment of sugar metabolism and central carbon pathways [11-12].

The results related to the increase in biomass (log cfu/ml) and pH variations after 8h of growth on LM17 and SM17 media are illustrated in figures 1 and 2. From these results, it appears that strains displayed a high level of growth with lactose and sucrose used at 3% (w/v). Comparatively to the initial rate of inoculation, the strains showed an average increase in the biomass of 3.20±0.09 and 3.10±0.14 log cfu/ml respectively in the presence of 3% (w/v) of lactose and sucrose. A significant difference ($P<0.05$) in the biomass was only observed between the sucrose used at 0.5 and 3% (w/v).

During the growth, a significant decrease in the pH of the cultures was observed. The pH values decreased, in average, from 7.0 to 4.5 in the presence of 3% (w/v) of both carbon sources (figure 2). The pH decreasing over time raises the existence of a specific metabolic activity in the different fermentative pathways used by *S. thermophilus*, which acidifies the medium by the production of lactate or other acids [11]. Furthermore, it is important to point that the level of growth of the strains was not influenced by the decrease in pH, even after 24h of incubation (data not shown).

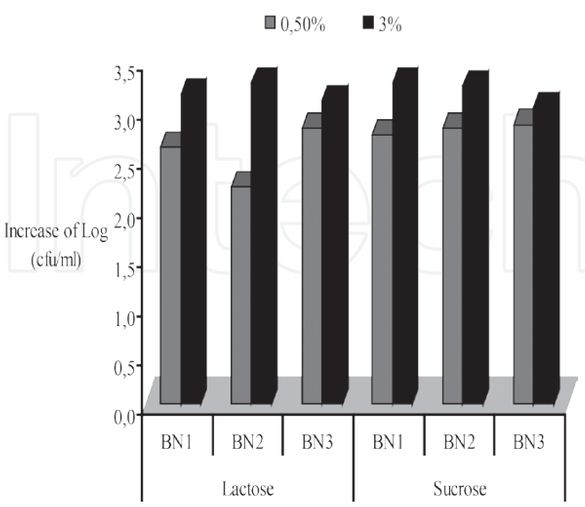

Fig 1. Mean values of increase in biomass (log cfu/ml) of the strains BN1, BN2 and BN3 after 8h of fermentation at 42°C in LM17 and SM17 media.

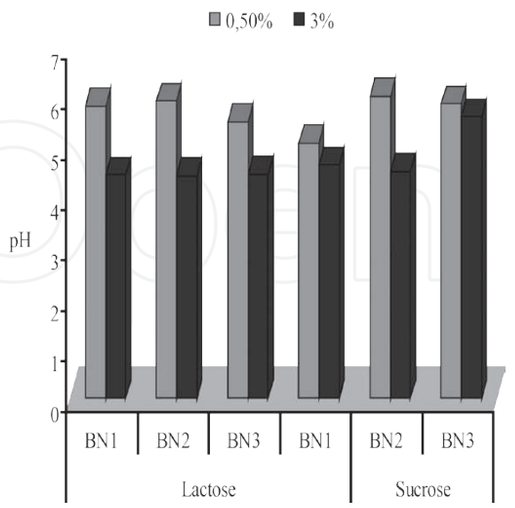

Fig 2. Mean values of pH measured after 8h of fermentation at 42°C in LM17 and SM17 media inoculated with strains BN1, BN2 and BN3.

3.2. Acetaldehyde Production

The ability to produce acetaldehyde from a carbon source was determined in strains BN1, BN2, and BN3 in the presence of 0.5 and 3% (w/v) lactose and sucrose. The acetaldehyde amounts were quantified after 8h of incubation at 42°C (figures 3, 4). The results indicated clearly that the three strains produced acetaldehyde, but the production appeared closely related to the concentration of carbon source and strain-dependent. The amounts of acetaldehyde formed by the strains were proportional to the concentration of carbon source. In the presence of 3% (w/v) of either lactose or sucrose, the strains formed a high amount of acetaldehyde compared to the 0.5% (figures 3, 4**),** with the exception of the strain BN2, which formed about 83.50±4.90 and 85.20±4.00 µ mol respectively with 0.5% and 3% (w/v) of sucrose (P> 0.05). However, the most potent acetaldehyde producer was BN1 strain. It was capable of producing up to 205±75µmol and 218±90µmol of acetaldehyde with 3% (w/v) of lactose and sucrose respectively. This ratio was significantly higher (P <0.01) than the one produced by the BN2 and BN3 strains, which produced 70.50±3.70, 85.20±4.00, 54±12 and 80±7µmol, respectively. These results indicate that there is variability in the amounts of acetaldehyde formed by the strains of *S. thermophilus* under study. This variability has been widely reported by many authors [3-13]**.** Moreover, Ayhan et al. [13] noticed a large variability in the amounts of acetaldehyde produced by 30 strains of *S. thermophilus*. Chaves et al. [3] signaled that the production of acetaldehyde appeared to be strain specific and variable. The same authors reported that the high levels of acetaldehyde were obtained when L-threonine was added to the culture medium. This observation was also reported by Bennama et al**.** [4].

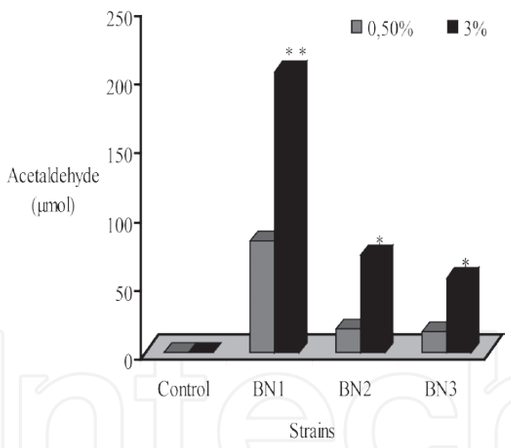

Fig 3. Acetaldehyde amounts (µmol) formed by the strains BN1, BN2 and BN3 after 8h of fermentation at 42°C in LM17 medium.

**: Highly significant difference (P<0.01) compared to mean values of acetaldehyde obtained with BN2 and BN3 strains.

*: No significant difference.

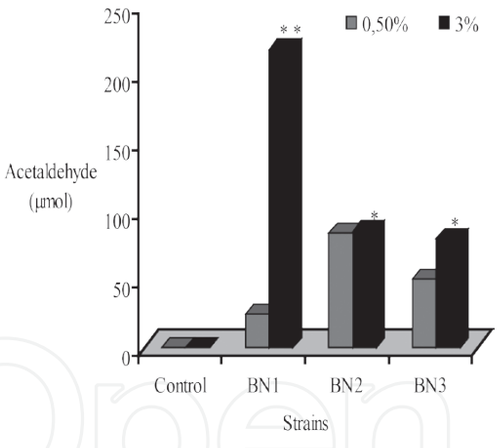

Fig 4. Acetaldehyde amounts (µmol) formed by the strains BN1, BN2 and BN3 after 8h of fermentation at 42°C in SM17 medium.

**: Highly significant difference (P<0.01) compared to mean values of acetaldehyde obtained with BN2 and BN3 strains.

*: No significant difference.

According to Ott et al. [5]**,** the production of acetaldehyde in milk by lactic acid bacteria seems to be strain-dependent too. These authors also showed that glucose appeared as the main precursor

of acetaldehyde in milk fermented with *S. thermophilus.* They confirmed that the major production of acetaldehyde was related to the glycolitic pathway. These observations explain the origin of acetaldehyde amounts formed by strains under study especially by BN1 strain. Furthermore, Oïzer and Atasoy [14] reported that lactose hydrolysis induced by the β-galactosidase caused a significant increase in the level of acetaldehyde in yoghurt samples prepared using viscous starter cultures. The results of this study show that production of acetaldehyde by BN1 strain seemed efficient with lactose and sucrose, whereas for BN3 strain, the production was more important with 3% (w/v) of sucrose.

4. Conclusion

In this study, it was found that strains synthesized significant amounts of acetaldehyde in the presence of 3% (w/v) lactose and sucrose. However, with 0.5% (w/v) small quantities were formed. The results reveal that acetaldehyde production was strain-specific and influenced by the concentration of carbon source added to the medium. Following these promising findings, it appears that depending on the strains of *S. thermophilus*, a determined concentration of carbon source constitutes one of the optimal conditions for the synthesis of acetaldehyde in this thermophilic species. This property, mainly for sucrose, could be useful in dairy technology to enhance natural production of this aroma compound by *S. thermophilus*.

5. References

[1] Tamime, A. Y., 2002. Fermented milks: a historical food with modern applications-a review. European Journal of Clinical Nutrition, 56 (4): p. S2-S15.

[2] Delorme, C., 2008. Safety assessment of dairy microorganisms: *Streptococcus thermophilus*, International Journal of Food Microbiology, 126: p. 274- 277.

[3] Chaves, A.C.S.D., M. Fernandez, A.L.S. Lerayer, I. Mierau, M. Kleerebezem, and J. Hugenholtz, 2002. Metabolic Engineering of Acetaldehyde production by *Streptococcus thermophilus.* Applied and Environmental Microbiology, 68 (11): p. 5656-5662.

[4] Bennama, R., Rechidi-Sidhoum, N. and A. Bensoltane, 2011. Effect of threonine on growth and acetaldehyde production by *Streptococcus thermophilus*. World Applied Sciences Journal, 15 (2): p. 160-163.

[5] Ott, A., J. E. Germond, and A. Chaintreau, 2000. Origin of acetaldehyde during milk fermentation using ^{13}C-labeled precursors. Journal of Agriculture and Food Chemistry, 48: p. 1512-1517.

[6] Bongers, S. R., M.H.N. Hoefnagel, and M. Kleerebezem, 2005. High-Level acetaldehyde Production in *Lactococcus lactis* by metabolic engineering. Applied and Environmental Microbiology, 71 (2): p. 1109-1113.

[7] Ott, A., L.B. Fay, and A. Chaintreau, 1997. Determination and origin of the aroma impact compounds of yogurt flavour. Journal of Agricultural and Food Chemistry, 45: p. 850-858.

[8] Bennama, R., M. Fernández, V. Ladero, M. A. Alvarez, N. Rechidi-Sidhoum, and A. Bensoltane, 2012. Isolation of an exopolysaccharide-producing *Streptococcus thermophilus* from Algerian raw cow milk. European Food Research Technology, 234: p. 119-125.

[9] Terzaghi, B. E., and W. E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophages. Applied and Environmental Microbiology, 29: p. 807-813.

[10] Van Den Bogaard, P.T.C., P. Hols, O.P. Kuipers, M. Kleerebezem, and M.W. De Vos, 2002: Sugar utilization and conservation of gal-lac gene cluster in *Streptococcus thermophilus*. Systematic and Applied Microbiology, 27: p. 10-17.

[11] Hols, P., F. Hancy, L. Fontaine, B. Grossiord, D. Prozzi, N. Leblond-Bourget, B. Decaris, A. Bolotin, C. Delorme, S.D. Ehrlich, E. Guédon, V. Monnet, P. Renault, and M. Kleerebezem, 2005. New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative genomics. FEMS Microbiology Reviews, 29: p. 435-463.

[12] Iyer, R., S.K. Tomar, T. Uma Maheswari, and R. Singh, 2010. *Streptococcus thermophilus* strains: Multifunctional lactic acid bacteria. International Dairy Journal, 20: p.133-141.

[13] Ayhan, K., F.Durlu-Ozkaya, and N. Tunail, 2005. Commercial important characteristics of Turkish origin domestic strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp.bulgaricus.* International Journal of Dairy Technology, 58 (3): p. 150-156.

[14] Ozer, B., and F. Atasoy, 2002. Effect of addition of amino acids treatment with ß-galactosidase and use heat shocked on the acetaldehyde level in yoghurt. International Journal of Dairy Technology, 55: p. 1-6.