Effect of Threonine on Growth and Acetaldehyde Production by *Streptococcus thermophilus*

Bennama Rabha, Rechidi-Sidhoum Nadra and Bensoltane Ahmed

**Abstract:** This work evaluates performance of three strains of *Streptococcus thermophilus* on the basis of acetaldehyde production. The studied strains BN1, BN2 and BN3 were isolated from Algerian raw milk. Moreover, the growth of these strains and acetaldehyde determination have been established in skim milk reconstituted at 10% (w / v) supplemented with 5 mmol and 10 mmol of threonine. The results show that the strains have similarity biomass profiles. After 10h of fermentation, biomass reached in average 09.03 ± 0.23 log CFU / ml. For acetaldehyde and after quantification, the studied strains proved producing this compound and in varying amounts. The obtained amounts indicate that a large proportion of this compound flavour comes from threonine. The statistical treatment of data confirmed the significant effect of threonine on the formation of acetaldehyde (P <0.01). The highest amounts were quantified in the presence of 10 mmol of threonine and whose values are 309 ± 0.00 µmol, 400 ± 0.14 µmol and 290 ± 1.60 µmol respectively with BN1, BN2 and BN3 strains.

**Key words:** *Streptococcus thermophilus* • New starter • Acetaldehyde production • Threonine

**INTRODUCTION**

Flavour is usually due to the presence of many volatile and non-volatile compounds with diverse physicochemical properties [1]. The non-volatile compounds contribute mainly to the taste volatile influence the taste and aroma. A vast array of compounds may be responsible for the aroma of food products, such as alcohols, aldehydes, esters, short to medium-chain free fatty acids, lactones, phenolics and sulfur compounds [1, 2].

The aroma compounds are multiple and since the first time, the compounds responsible for flavours were extracted from plant sources. However, after elucidation of their structures, most of these compounds are produced by chemical synthesis. Nevertheless, consumers seek more "natural" or "organic" food products without additives. To overcome these problems an alternative strategy was adopted. It is considered to the natural selection microbial strains producing aromas [1].

In the dairy sector where the flavour is the key property of fermented dairy products, the research of new generally recognized as safe (GRAS) bacteria producing aroma is quite desired. Thus, nowadays a large number of publications explain the function of lactic acid bacteria (LAB) in the production of compounds, which give the taste and flavour to the fermented dairy products [3, 4]. Some strains of LAB are able to produce aromatic compounds such as acetaldehyde. Acetaldehyde is indeed recognized as one of the major aroma compounds of fermented dairy products such as yoghurt [5, 6] and some cheeses [7]; it gives them a fruity and refreshing taste [3, 5, 8]. The production of acetaldehyde by LAB seems to be strains dependent. However, there are many literature contradictions regarding the acetaldehyde amounts formed by both yoghurt starters [5]. For instance, *Lactobacillus delbrueckii* subsp. *bulgaricus* has been reported by some authors to be a greater acetaldehyde producer than *Streptococcus thermophilus*, whereas other authors have reported the contrary [8]. The secreted amounts of acetaldehyde in yoghurt strains
vary from 5 to 21 mg/l [5, 8]. In LAB, acetaldehyde is generally formed directly from the decarboxylation of pyruvate by the action of pyruvate decarboxylase or indirectly from the acetyl- CoA through pyruvate dehydrogenase and aldehyde dehydrogenase [3, 5]. Moreover and especially in *S. thermophilus* bacterium, acetaldehyde can also be produced through Serine Hydroxymethyl Transferase (SHMT), which performs a cleavage on the threonine, forming thus acetaldehyde and glycine [5, 9].

The overall objective of this work concerns the selection of three strains of *S. thermophilus* as dairy starter; on the basis of acetaldehyde production and also to evaluate the effect threonine on the production of this aroma compound.

**MATERIALS AND METHODS**

**Studied Strains And Growth Conditions:** This work has involved three strains of *S. thermophilus* BN1, BN2 and BN3. The strains were reactivated from frozen stock cultures at - 20°C. They were reactivated and usually grown on M17 medium [10]. For the enumeration of strains in milk, LAPT agar [11] was used. Regarding the conditions of incubation, the strains were incubated at 42°C using an anaerobic jar.

**Growth, Acidification Profiles and Acetaldehyde Production in Milk in the Absence and Presence of Threonine**

**Cultures Preparation:** Defined volumes of skim milk reconstituted to 10% (W / V) supplemented with 5 and 10 mmol threonine were inoculated by the studied strains at a final concentration of 1%. Established cultures were incubated at 42°C and after 10h of fermentation, aliquots were taken to determine the following parameters:

**Biomass Evaluation and pH Measurement:** *S. thermophilus* population developed in milk medium after 10h of incubation was performed by conducting serial dilutions in peptone saline solution [peptone (1 g L⁻¹) and NaCl (8.5 g L⁻¹)]. Then, 0.1ml of the chosen dilutions was spread on the surface of LAPT agar. The plates were then incubated at 42°C for 24 to 48h. The number of bacteria is given as colony forming units (cfu/ml). Developed acidification was monitored by measuring pH using a pH meter type "Hanna Instruments, pH 210 microprocessor pH meter.

**Acetaldehyde Determination:** Acetaldehyde was determined after 10h of fermentation. Crops for the assay were established under the same conditions described above. Acetaldehyde was measured by spectrophotometer, using a kit for determining acetaldehyde (R-Biopharm: enzymatic bioanalysis). This assay is based on the reduction of NAD to NADH by acetaldehyde dehydrogenase.

**Data Analysis:** The Student’s t test was used to compare means values of produced biomass (log cfu / ml) and acetaldehyde (µmol) between the strains in the presence of different concentrations of threonine.

**RESULTS AND DISCUSSION**

**Growth, Milk Acidification and Acetaldehyde Production:** After 10h of fermentation in reconstituted skim milk in the absence and presence of 5 and 10 mmol of threonine, the strains BN 1, BN2 and BN3 grew in milk and showed similar appearance in growth, as no significant difference (P>0.05) was observed on the biomass profiles (in log cfu / ml) (Figure 1). In addition, the pH decrease recorded in this growth reflects the acidification potential of the strains in milk. The values reached after 10h of fermentation were in average 4.78, 4.67 and 4.58 respectively in the absence and the presence of 5 mmol and 10mmol of threonine (Figure 1). These results describe one of the metabolic aspects of *S. thermophilus* species in milk; which consist to the rapid conversion of lactose into lactic acid causing a decrease in pH [12, 13].

Since the hypothesis of this study was to demonstrate the performance of the strains towards the production of acetaldehyde and to check the influence of threonine concentration on its produced amounts. As shown in Figure 2, the strains BN1, BN2 and BN3 are able to produce acetaldehyde. The production appears to be variable and proportional to the concentration of threonine used in the medium. These results indicate also that the addition of threonine had a significant effect on the amounts of acetaldehyde quantified after 10h of fermentation. Several reports [3, 5, 6, 9] have been stated that acetaldehyde produced by thermophilic lactic acid bacteria may have different metabolic pathways. Ott *et al.* [8] showed that glucose appears to be the main precursor of acetaldehyde in milk fermented by *S. thermophilus*. The same authors reported that large proportions of acetaldehyde were derived from glycolytic pathway.
In fact, taking into account our results and assuming that lactose is the main sugar of milk and when metabolized by *S. thermophilus*, it is converted into glucose and galactose. This one is generally excreted in the external environment [13, 14]. Of all these considerations, it appears that acetaldehyde formed during the growth of the studied strains is essentially and primarily related to the threonine metabolism. In addition, the obtained amounts with threonine-free milk are very low (Figure 2). For example, BN2 strain produces in milk alone about 15.4µMo of acetaldehyde, whereas these amounts increased significantly (P <0.01) in the presence of 5 mmol and 10mmol of threonine. They reached respectively 280 ± 0.00 and 400± 0.14µmol. Similar observations have been recorded on the amounts formed by BN1 and BN3 strains. Chaves *et al.* [5] found that a five to ten-fold increase in acetaldehyde production was resulting from addition of threonine (10mmol) to the growth medium.

The same authors were observed a nearly linear relationship between the amounts of acetaldehyde formed during milk fermentation and SHMT activity measured in different *S. thermophilus* strains. Attention regarding the influence of selected factors on acetaldehyde production was also reported by other authors [15]. These authors have been demonstrated that amino acids supplementation, lactose hydrolysis and heat-shock treatments had a significant effect on acetaldehyde formation in yoghurt starter cultures. However, based on the amounts of acetaldehyde obtained under different culture conditions including the concentrations of threonine, it is clearly that acetaldehyde formation of by the BN1, BN2 and BN3 strains was non-growth associated, but rather, it was quite variable depending on the strain. The production of acetaldehyde by LAB appears to be strains dependent [5, 8]. The variability of acetaldehyde production among a number of
S. thermophilus strains was in fact widely documented [16-19]. Otherwise, as it was reported above, threonine has no effect on the obtained biomass, since no significant differences were observed on the cell concentration in the absence and presence of threonine. The present results confirmed that threonine is required for acetaldehyde synthesis.

CONCLUSION

The results thus obtained are promising to use the studied strains as dairy starters. They showed that the three strains produced significant and varying amounts of acetaldehyde in the presence of threonine.

REFERENCES