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Effect of fermentation conditions (culture media and incubation temperature) on exopolysaccharide production by *Streptococcus thermophilus* BN1

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Abstract. This work investigates the influence of incubation temperature and culture media on exopolysaccharides (EPS) production. The *S. thermophilus* BN1 an EPS-producer strain isolated from cow milk was examined in this study. Skimmed milk, whole milk and cheese whey, were selected as culture media and two different temperatures were also tested. The EPS produced in the different studied conditions was purified and quantified. The strain BN1 was able to produce EPS in all established conditions. However, significant differences were observed in the amounts of produced EPS. The production of EPS reached the maximum values at 37°C in skimmed milk with amount of 548 PDM mg l^{-1}, followed by the amounts of 375 PDM mg l^{-1} and 325 PDM mg l^{-1} respectively in whey and whole milk. In addition, a slight significant difference was noted on the biomass, pH and lactic acid values obtained from the three fermented substrates ($p < 0.05$). The present results demonstrate that incubation temperature (37°C) had a significant ($P<0.05$) effect on the EPS production by *S. termophilus* BN1.

Keywords: *S. thermophilus*, expolysaccharide production, temperature, substrate.

1. Introduction

In the food industry, many polysaccharides are commonly used as feed additives for their gelling, stabilizing or thickening properties. These additives are produced by plants (cellulose, pectin and starch), seaweeds (alginate and carrageenan) and bacteria (alginate, dextran, gellan, pullulan, and xanthan) [1]. Lactic acid bacteria (LAB) play a key role during the fermentation process since they contribute to the texture, flavour, quality and conservation of the fermented products. Several strains of LAB, are also able to produce exopolysaccharides (EPS), these compounds have attracted great interest since they can act as natural thickeners that improve the texture properties, decrease syneresis and reduce the fat levels in fermented dairy foods. EPS-producing LAB belong to different genera such as *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus* [2].

In this work we focus on the production of EPS by *S. thermophilus* BN1. *S. thermophilus* is one of the main starters used in the dairy industry for the manufacture of cheese and yogurt [3]. Its EPS production has been related to the growth phase and the culture conditions. Whereas in fermented milks, EPS concentrations were less than 600 mg l^{-1}, M17 media yielded up to 1500 mg l^{-1} depending on the nature of the carbohydrate source used and the carbon/nitrogen ratio [4, 5]. These observations show the importance of growth

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conditions on EPS production. In this paper, the influence of temperature and the type of fermentation media on EPS production by *S. termophilus* BN1 will be analyzed.

2. Material and Methods

2.1. Strain, media and growth conditions

S. thermophilus BN1, a strain isolated from raw milk [6], was grown in M17 supplemented with lactose (2% w/v) (LM17) at 42ºC under anaerobic conditions. EPS production by this strain was evaluated using as substrates: skimmed milk, whole milk, (Candia, Algeria) and cheese whey collected from the cheese factory "Sidi Saâda" (Relizane, Algeria).

2.2. Growth on different fermentation substrates and EPS determination

EPS production was determined in cultures performed in skimmed milk, whole milk and cheese whey. 250 ml of each medium was inoculated at 0.5% (v/v) with *S. thermophilus* BN1 previously grown in LM17 as has been described before. These cultures were incubated at 37°C and 42°C for 17 h and 12 h respectively. Then, aliquots were taken from each medium to determine different parameters mentioned below.

2.3. Biomass evaluation

From the established cultures, serial dilutions were made in peptone saline. Appropriate dilutions were spread on LM17 agar and incubated for 48 h at 42°C. Growth was estimated as log CFU/ml.

2.4. Determination of total titratable acidity (TTA) and pH measurement

The concentration of lactic acid in the media was determined by titration following the protocol described by Accolas et al. [7]. The pH was also measured using a pH meter type "Hanna instruments microprocessor pH meter".

2.5. Isolation and purification of EPS

Isolation of EPS was performed using the protocol described by Salazar et al. [8]. Briefly, this protocol is based on proteins elimination by precipitation with TCA (12 %) and subsequently precipitation of EPS using two volumes of cold ethanol. The precipitated EPS samples were dialyzed against water using a dialysis membrane (Sigma) with a molecular cut-off weight of 12-14 kDa.

2.6. Quantification of the purified EPS

EPS quantification was done by gravimetric analysis [1], the polymer dry mass (PDM) of the purified EPS; was determined after 48h of drying at 42°C. The measured values are subtracted from that obtained with a PDM of the control media.

2.7. Data analysis

All experiments were repeated two times. The results are means of two replicates.

3. Results and discussion

3.1. Bacterial growth and acidification activity

The biomass developed after 12h of incubation at 42°C and 17h at 37°C is shown in Fig 1. *S. thermophilus* BN1 was able to grow in the three tested substrates, with an average increase of 2.5 log CFU/ml compared to the initial rate of inoculation. The biomass at 37ºC in skimmed milk is quite similar to that obtained in whole milk in the conditions analyzed. By contrary, the biomass at 42ºC in whey was slightly higher to that posted at 37ºC. It is also interesting to note that in whole milk the difference between the growth at 42ºC is bigger than in whey or skimmed milk ($P<0.05$). The increase in biomass caused a significant acidification in the three studied media. This was confirmed by the pH and TTA values measured after 12h and 17h of fermentation (Fig.2, 3). The acidification in the cultures of skimmed and whole milks provoked the clotting of the milk. However, in the whey culture a thick aspect was observed. The ability of *S. thermophilus* BN1 to grow on the tested media is, in fact, due to the capacity of this species to metabolize lactose present naturally in these media. Its conversion into lactic acid decreases the final pH of the medium [9].

3.2. Effect of incubation temperature on growth and EPS production

Many authors emphasize that the growth temperature is a parameter having a significant impact on EPS production in *S. thermophilus* [10]. In this context, the effect of temperature on EPS production was analyzed. EPS production was observed at both temperatures. The higher EPS production was reached at 37ºC compared to 42ºC independently of the media culture. At 37ºC amounts of 548 mg PDM l^{-1}, 375 mg PDM l^{-1} and 325 mg PDM l^{-1} were produced in skimmed milk, whey and whole milk respectively (Fig. 4). It is well known that 37°C is a sub-optimal growth temperature of *S. thermophilus*. Although, several studies showed that suboptimal temperatures influence positively EPS production by mesophilic and thermophilic LAB [2, 11]. Nevertheless, de Vuyst et al. [1] obtained maximal EPS production, when *S. thermophilus* grew at its optimum temperature (42°C). These data show that EPS production could vary among the different strains of *S. thermophilus* under similar growth conditions.

This characteristic is not specific for *S. thermophilus,* a large diversity in EPS production was also observed in other LAB for instance in *Lactobacillus delbrueckii ssp. bulgaricus* from 57 to 424 mg l^{-1} [12],

30-85 mg l^{-1}, 100-600 mg l^{-1} and 105-150 mg l^{-1} in *L. lactis subsp. Lacti* or*, L. lactis subsp. cremoris and Lb. casei subsp. casei*, respectively [11] and 105-168 mg l^{-1} with bifidobacteria [13]. It is also interesting to note, that at 37°C the fermented skimmed milk had a firm and compact texture difficult to break. This textural aspect was reported by many authors in other EPS-producing LAB strains incubated at suboptimal temperatures. The EPS overproduction at sub-optimal temperature has been proposed as answer mechanism to the physiological stress at these temperatures, especially on that species such as *S. thermophilus* with a deficient proteolytic system. In addition, Vaningelgem et al. [14] reported that the use of high-level EPS-producing strain displaying a stronger milk-clotting ability.

3.3. Effect of fermentation media on growth and EPS production

Several authors have stated that medium composition either carbon source, nitrogen source, or ion source are important parameters in EPS biosynthesis [2, 14]. The results have showed that BN1strain is able to grow and produce EPS in all substrates (Fig 4). The maximal EPS production occurred at 37ºC in skimmed milk with 548 PDM mg l^{-1} compared to whey (375 PDM mg l^{-1}) and whole milk (325 PDM mg l^{-1}). The obtained results are according to the findings of de Vuyst et al. [1] indicate that milk is the best media for EPS production in *S.* thermophilus comparing to other media (MRS, M17 or SDM). Other authors [14] have observed that EPS production in some strains of *S. thermophilus* increases only when the skim-based media are enriched with peptone or yeast extract. Moreover, at 42ºC the EPS production by *S. thermophilus* BN1 in the used substrates was approximately three times lower to that observed at 37°C (Fig. 4).

In this study, the interesting finding was the ability of *S. thermophilus* BN1 to produce EPS in the whey. Most studies [15] on EPS production by *S. thermophilus* were realized using whey, but only as based medium and not as a single substrate. Our results demonstrate the capacity of BN1 strain to produce EPS using whey as a substrate without exogenous nutrients. Whey can be considered as a rich medium; besides lactose it provides amino acids. The presence of amino acids is essential to EPS production by *S. thermophilus* BN1, the enrichment of milk with amino acids yielded a significant increase on EPS production (data not shown). According to the literature, the amino acids can generate the metabolic energy, based on the production of ATP through the phosphorylation and decarboxylation of the substrates and the transport of generator, respectively [16]. This energy is essential for cell growth and growth associated with EPS production. The production of EPS in whey has a great economic interest because; it allows to upgrade this product that is usually rejected by most of dairy industries in Algeria.

On the other hand, in the three fermented media a shooting or slime texture was observed at 42°C, while at 37°C, a firm texture has characterized the samples of fermented skimmed milk. These textural characteristics could be related to the production of two types of EPS, previously observed in this strain [6]. Lemoine et al. [17] suggested that the textural properties of several EPS-producing *S. thermpohilus* are due to the synthesis of polysaccharides of similar chemical composition, but different structure. In dairy industry, the firm and shooting textures are very important. At this scale, an appropriate processing allows the interaction of the EPS with milk proteins, somehow breaking the protein matrix. Once broken, it would modulate the organoleptic properties of the end-product.

4. Conclusion

This study has demonstrated that the EPS production by *S. thermophilus* BN1 is depending on the growth conditions; i.e. the incubation temperature (37°C), which stimulated strongly the EPS production. Similarly, the nature of the fermentation substrate has also influence on EPS production. These factors associated may have positive effects on the organoleptic quality of the fermented product. EPS production by *S. thermophilus* BN1 strain in whey substrate shows promising possibilities to valorize this waste milk product.

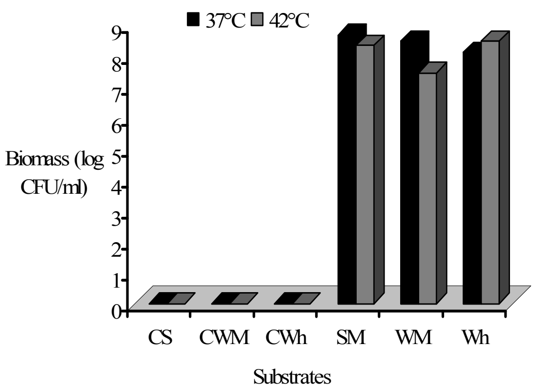

Fig. 1: Biomass developed (log CFU/ml) by BN1 strain after 12h and 17h of incubation in the fermented substrates.
SM: Skimmed milk; WM: Whole milk; Wh: whey; CS: Control Skimmed milk; CWM: Control whole milk; CWh: Control whey.

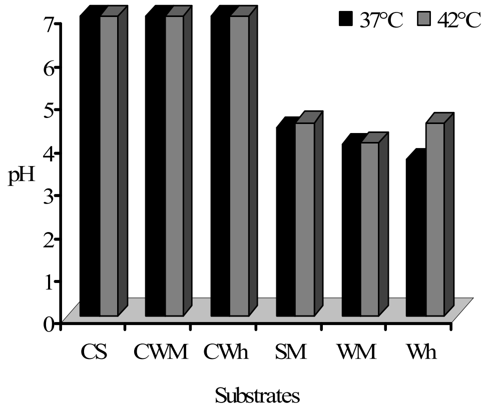

Fig. 2: The pH values measured after 12h and 17h of incubation in the substrates fermented by BN1 strain.
SM: Skimmed milk; WM: Whole milk; Wh: whey; CS: Control Skimmed milk; CWM: Control whole milk; CWh: Control whey.

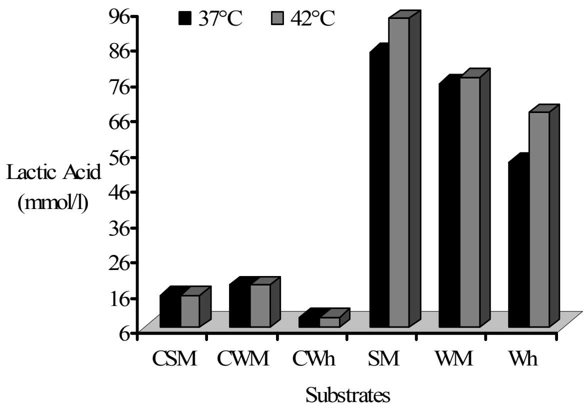

Fig. 3: Lactic acid (mmol/l) produced by BN1 strain after 12h and 17h of incubation in the fermented substrates.
SM: Skimmed milk; WM: Whole milk; Wh: whey; CS: Control Skimmed milk; CWM: Control whole milk; CWh: Control whey.

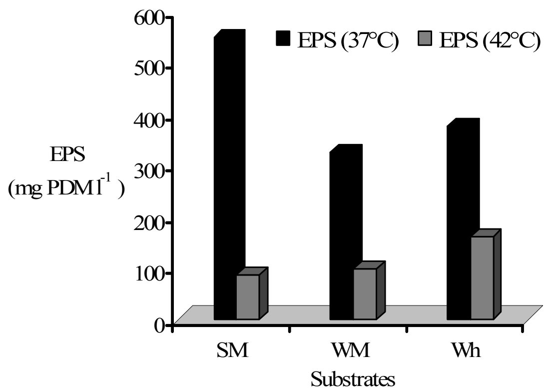

Fig. 4: The real values of EPS (in mg PDM/l) produced by BN1 strain after 12 h and 17h of incubation in the fermented substrates.
SM: Skimmed milk; WM: Whole milk; Wh: whey

5. References

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