Development of in vitro screening of EPS-producing LAB for cereal based products

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The bacteria are capable of producing exopolysaccharides (EPSs), a long chain branches made of repeated units of sugars or their derivatives excreted outside the cell wall. The EPSs improve the rheology and texture of many fermented foods like yogurt and baked goods and seem have a positive effect on health. Today, consumer demand is increasingly directed towards products with low levels of additives and use of EPSs could be a viable alternative. The selection of EPS-producing strains is a fundamental step to allow their more scope in cereal-based food processes.
Qualitative test on solid media: several experiments were carried out to decide the optimal medium composition to promote the EPS production using 12 *Leuconostoc*, 8 *Lactobacillus* and one *Streptococcus* strains isolated from sourdough for sweet dough. LAB strains were inoculated on the following different media: modified Agar Chalmers (Pepe *et al.,* 2001); modified Agar Chalmers with sucrose (5% w/v), with and without $CaCO_3$. Since $CaCO_3$ resulted in inhibition of EPS production, Agar Chalmers without $CaCO_3$ was further tested with the addition (5%) of one of the following sugars: maltose, glucose, galactose, fructose, lactose. Subsequently, the EPS-producer strains were also tested on Agar Chalmers containing a mixture of sucrose (1.6 % w/v), fructose (1.6% w/v) and maltose (1.6% w/v). EPS production was detected by the presence of slime colonies and ropy behaviour of the positive strain (Vescovo *et al*., 1989).
Quantitative analysis: the best EPS-producing LAB strains were assayed in the quantitative analysis following an ad hoc method: 1 ml of the overnight culture was surface inoculated on the modified Chalmers agar with sucrose, fructose and maltose. The yeast extract influence was also evaluated during this experiment. After 48 h of incubation, the EPS produced were directly dissolved with 20 ml of distilled water and collected in sterile falcons. The cells were removed by centrifugation at 6500 rpm for 10 min at room temperature. EPS were precipitated by adding volumes of chilled 100% ethanol and after 1 h, the pellet was collected, freeze-dried and weighed using analytical balance. The EPS production was expressed in milligrams of EPS ml^{-1}. The most strains used in this study produced slimy colonies (EPS) only when propagated in modified Chalmers Agar with sucrose (5% w/v) as the only carbon source, for its capability to promote the EPS biosynthesis, and without $CaCO_3$ that, at the concentration used in this study (2%), inhibited EPS production. We tested also modified agar Chalmers with three different sugars with the aim to the selection of EPS-producers in a medium with a similar sugars composition of dough for sweet baked products. EPS quantification is often tested using broth complex media medium. The most common procedures provide TCA precipitation and protein removal by centrifugation, followed by ethanol or/and acetone precipitation and several steps of purification (Cerning *et al*., 1994). Therefore, the method applied in this study revealed simpler. We used a solid medium in order to avoid the interference of some proteins and/or other high-molecular mass molecules. This method of screening will be applied to putative EPS-producing LAB strains isolated from cereal based products.

Keywords: exopolysaccharides (EPS), LAB, screening.

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