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Short Communication

Effects of soy peptone on the inoculum preparation of *Streptococcus zooepidemicus* for production of hyaluronic acid

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ABSTRACT

Soy peptone (SP) was studied as nutrient source in replacement of the conventional media as Brain–Heart Infusion (BHI) and sheep blood in the first seed culture medium in Petri plates of *Streptococcus zooepidemicus*. This substitution, aimed at meeting the claim of the pharmaceutical and cosmetics industries, for the removal of animal sources of the culture media used in obtaining their products for safety reasons. The animal sources were used as a control. The effects of this substitution were studied in fermentations carried out at 37 °C and 150 rpm in 250 mL Erlenmeyer flasks containing 100 mL culture medium containing glucose and SP only. The replacement of animal nutrient sources by SP to about twice the BHI concentration did not alter the amount of the produced HA, or caused deviations in the metabolism of the microorganism in favor of HA to the detriment of cell growth.

1. Introduction

Hyaluronic acid (HA) is a natural polysaccharide with a wide range of pharmaceutical, medical and cosmetic applications (Weigel, 2004). Due to its biological applications, it is necessary to produce it highly pure and free from viral agents. Nowadays, the microbial production is the preferred alternative, compared to the extraction from animal tissues such as rooster combs, umbilical cord blood or synovial fluid. The microbial route uses *Streptococci* mainly, which produce HA as a protective capsule around its cell wall (Chong et al., 2005; Weigel, 2004).

In general, the first seed culture media for propagation of *Streptococci* contain animal-derived sources, such as sheep blood and/or Brain Heart Infusion (BHI), for the cell growth (Pires et al., 2010a,b; Pires and Santana, 2010).

Although the animal sources are effective for cultivation of the microorganism, there is a risk of species cross contamination in HA produced, even when these components are used in the first steps of inoculum preparation, such as in Petri dishes growth.

Recently, vegetable peptones have been used as nitrogen sources in culture media for cell cultivation (Fakhfakh-Zouari et al., 2010; Heenan et al., 2002; Junguo et al., 2004; Le Marrec et al., 2007; Siaterlis et al., 2008), including the cultivation of *Streptococcus* and *HA production* (Cazzola et al., 1994; Izawa et al., 2010; Kanchankumar et al., 2009; Lee et al., 2009; Zisu and Shah, 2003). On the other hand, the pharmaceutical and cosmetics applications have demanded non-animal nutrients in microbial culture media for the production of their components, for safety reasons. As far as we know, there is no report in the literature about the use of vegetable peptones as a nitrogen source for growth of *Streptococcus zooepidemicus* and for the production of hyaluronic acid.

In this context, the objective of this study was to substitute BHI and sheep blood for soy peptone (SP) in the culture medium for the growth of *S. zooepidemicus* in Petri dishes. Soy protein was chosen because it is commercially available at low cost. The effects of the substitution were studied in fermentations, in which cell growth, HA production, glucose consumption, and secondary metabolites were quantified. Additionally, the average molecular weight and polydispersity of the produced HA was also characterized.

2. Methods

2.1. Microorganism

Streptococcus equi, subsp. *zooepidemicus* ATCC 39920, was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) as a lyophilized culture kept frozen at −80 °C in ampoules containing 10% glycerol as cryoprotectant.

2.2. Solid inocula preparations

In the first culture in Petri dishes, the compositions of the solid medium were: P_c or control (37 g L^{-1} BHI, 5% (v/v) sheep blood); P_I

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