Food Chemistry 151 (2014) 93–100

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Primary recovery of a bacteriocin-like inhibitory substance derived from *Pediococcus acidilactici* Kp10 by an aqueous two-phase system

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ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 12 September 2013
Accepted 4 November 2013
Available online 14 November 2013

Keywords:
Pediococcus acidilactici
Bacteriocin-like inhibitory substance
Aqueous two-phase system
Recovery

ABSTRACT

A polymer–salt aqueous two-phase system (ATPS) consisting of polyethylene–glycol (PEG) with sodium citrate was developed for direct recovery of a bacteriocin-like inhibitory substance (BLIS) from a culture of *Pediococcus acidilactici* Kp10. The influences of phase composition, tie-line length (TLL), volume ratio (V_R), crude sample loading, pH and sodium chloride (NaCl) on the partition behaviour of BLIS was investigated. Under optimum conditions of ATPS, the purification of BLIS was achieved at 26.5% PEG (8000)/11% sodium citrate with a TLL of 46.38% (w/w), V_R of 1.8, and 1.8% crude load at pH 7 without the presence of NaCl. BLIS from *P. acidilactici* Kp10 was successfully purified by the ATPS up to 8.43-fold with a yield of 81.18%. Given that the operation of ATPS is simple, environmentally friendly and cost-effective, as it requires only salts and PEG, it may have potential for industrial applications in the recovery of BLIS from fermentation broth.

1. Introduction

Increasing consumer awareness of the risks not only from food-borne pathogens but also from chemical preservatives has led to renewed interest in "green technologies". These include novel minimal processing methods and exploitation of bacteriocins for food biopreservation. Bacteriocins are small ribosomally synthesised antimicrobial peptides, which are mostly cationic, amphiphilic and membrane-permeabilising. Bacteriocins are unstructured in aqueous solution, but have the propensity to form an α-helical structure when exposed to structure-promoting solvents or membrane-mimicking media (Moll, Konings, & Driessen, 1999).

Numerous purification strategies have been reported for bacteriocins all with varying degrees of success, which may be attributable to the extremely heterogeneous nature of bacteriocin (Klaenhammer, 1993). The purification methods commonly employed include ammonium sulphate precipitation, ion-exchange chromatography, hydrophobic chromatography coupled with Mono S cation-exchange column chromatography, reverse-phase high-performance liquid chromatography (HPLC), Amberlite XAD-2, Sephadex G-25 gel filtration, ultrafiltration and gel permeation chromatography and ethanol precipitation. Each purification method has its own drawbacks, which may include issues with low yield and purity, cost, and the requirement for a skilled operator (Abriouel, Valdivia, Martinez-Bueno, Maqueda, & Galvez, 2003).

The aqueous two-phase system (ATPS) has been proposed as an ideal purification technique for the separation, extraction and concentration of biomolecules because of its high productivity, simplicity, short processing time, cost effectiveness, scalability and versatility. ATPS, consisting of a polyethylene glycol (PEG)/salt system has been widely used for the bioseparation of proteins due to its low cost and the wide range of hydrophobic differences between the two-phase systems that allow enhancement of the partition selectivity of the target protein (Antov & Omorjan, 2009). ATPS has been applied in the extraction and purification of various compounds, such as enzymes, biopharmaceutics and natural products. The system has also been used in selected biotechnological fields possessing, medium industrial maturity and resolution

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http://dx.doi.org/10.1016/j.foodchem.2013.11.019