Food Chemistry 232 (2017) 245–252

Contents lists available at ScienceDirect

Food Chemistry

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

Recovery of a bacteriocin-like inhibitory substance from *Pediococcus acidilactici* Kp10 using surfactant precipitation

Fadzlie Wong Faizal Wong [a,b,c], Arbakariya B. Ariff [b,c], Sahar Abbasiliasi [d], David C. Stuckey [a,*]

[a] Department of Chemical Engineering, Imperial College London, London SW7 2AZ, United Kingdom
[b] Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Bioprocessing and Biomanufacturing Research Centre, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[d] Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

A R T I C L E I N F O

Article history:
Received 24 March 2016
Received in revised form 19 November 2016
Accepted 18 March 2017
Available online 23 March 2017

Keywords:
Surfactant precipitation
AOT
Bacteriocin-like inhibitory substance
Pediococcus acidilactici Kp10
Downstream processing

A B S T R A C T

Bacteriocin is an important peptide which can be used as an anti-microbial agent in food. However, simpler and more cost-effective purification methods need to be developed compared to chromatography to enhance its commercial viability. Surfactant precipitation was employed for the first time to purify bacteriocin-like inhibitory substance (BLIS) from a fermentation broth of *Pediococcus acidilactici* Kp10, and the amount precipitated was investigated as a function of anionic surfactant (AOT) concentration, and pH. Protein recovery from the precipitate was accomplished using solvent extraction, and solvent type, NaCl concentration, and ionic strength of the final solution were optimised. Optimal conditions were; 1.05 mM of AOT at pH 4 for precipitation, and acetone extraction (with 1 mM NaCl), which resulted in an 86.3% yield, and 53.8 purification factor. This study highlighted the fact that surfactant precipitation can be used as a primary recovery method for BLIS from a complex fermentation broth.

© 2017 Published by Elsevier Ltd.

1. Introduction

Bacteriocin is an extracellular secondary metabolite produced by lactic acid bacteria, and is a ribosomally synthesized, small (<10 kDa) cationic peptide (Pingitore, Salvucci, Sesma, & Nader-Macias, 2007). It is used as a biopreservative in food products as it is active against pathogenic bacteria such as *Listeria monocytogenes*, *Bacillus cereus* and *Salmonella* spp. (Bali, Panesar, & Bera, 2016; Masuda et al., 2011; Pingitore et al., 2007). It has also been granted a GRAS (generally recognized as safe) status (Ahern, Verschueren, & van Sinderen, 2003; Sharma, Kapoor, Gautam, & Kumari, 2011), and interest in it is driven by the commercial need to have a natural antimicrobial as an alternative to chemical additives that sometimes cause adverse effects (Martin-Visscher et al., 2008). Previously, Abbasiliasi et al. (2012) had isolated a bacteriocin-producing lactic acid bacterium, *Pediococcus acidilactici* Kp10, from dried Iranian milk curd. The bacteriocin-like inhibitory substances (BLIS) were shown to be active against the pathogen *Listeria monocytogenes* (between pH 2 and 9), and its activity was stable in the presence of pepsins such as α-amylase and catalase, but not proteinase K or trypsin.

In the downstream processing of bacteriocin, conventional multi-step methods such as precipitation by ammonium sulphate or ethanol, ion-exchange chromatography, molecular filtration chromatography and hydrophobic interaction chromatography were mainly employed (Pingitore et al., 2007; Saint-Hubert, Durieux, Bodo, & Simon, 2009). However, better separation methods compared to conventional techniques are always needed due to issues such as complexity and cost (Carolissen-Mackay, Arendse, & Hastings, 1997; Kelly, Reuben, Rhoades, & Roller, 2000). Nevertheless, the actual concentration of bacteriocin in the broth has never been quantified due to the high protein background. However, it was reported to be less than 5% of the total protein, and hence measuring the amount of bacteriocin separated has been based on activity recovered from the initial broth (Abbasiliasi et al., 2014; Saint-Hubert et al., 2009; Sharma et al., 2011).

In order to develop more effective separation methods, researchers have used reverse micelles for liquid-liquid extraction of proteins (Aires-Barros & Cabral, 1991; Pires, Aires-Barros, & Cabral, 1996; Shin, Weber, & Vera, 2003). In this technique, the target proteins are solubilised inside the polar core of the micelles (entrapment), and stabilised by the surfactant shell that protects them from denaturation by the organic phase (Aires-Barros & Cabral, 1991; Pires, Aires-Barros, & Cabral, 1996). Even though reverse micelles have been shown to be effective, proteins can be

* Corresponding author.
E-mail address: d.stuckey@imperial.ac.uk (D.C. Stuckey).

http://dx.doi.org/10.1016/j.foodchem.2017.03.102
0308-8146/© 2017 Published by Elsevier Ltd.

lost at the bi-phasic interface by precipitation caused by surfactant interactions (Jauregi & Varley, 1998; Lye, Asenjo, & Pyle, 1995; Shin, Rodil, & Vera, 2003).

Because of this precipitation, Shin, Rodil et al. (2003) proposed a new method of direct surfactant precipitation of proteins. This method was much simpler, with shorter processing times, comparable performance to reverse micellar extraction, and more economic since the surfactant required was orders of magnitude less than for reverse micellar extraction. In addition, during protein recovery its denaturation is less of a problem than in reverse micellar extraction (Cheng & Stuckey, 2011; Shin, Rodil et al., 2003; Shin, Weber et al., 2003). Fundamental studies on surfactant precipitation, i.e. a single protein in buffer, have been carried out, basic insights gained, and potential benefits highlighted (Cheng & Stuckey, 2012; Shin, Wahnon, Weber, & Vera, 2004). Nevertheless, to our knowledge, there are only two reports in the literature on the application of this precipitation technique to a "real world" complex sample: (1) Lysozyme from chicken egg white (Shin, Weber et al., 2003), and; (2) Xylanase from an industrial cellulase product of *Trichoderma* (Shin et al., 2004). Therefore, in order to prove the viability of this technique in downstream protein processing, it is very important to widen its application to separate proteins from complex fermentation broths.

In this context, a first attempt was made in this work to use surfactant precipitation with Bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT) to separate BLIS from a real complex sample of *Pediococcus* sp. in M17 fermentation broth. The influence of pH and AOT concentration on the precipitation and overall recovery of BLIS was investigated, and the BLIS was recovered from the surfactant-protein insoluble complex by solvent extraction.

2. Materials and methods

2.1. Materials

M17 broth used for the fermentation of *Pediococcus* was purchased from Merck (Darmstadt, Germany). Brain Heart Infusion broth (BHB) used for growing indicator strain, *Listeria monocytogenes*, was also purchased from Merck (Darmstadt, Germany).

2.2. Instruments

All chemicals were weighed using an analytical balance (ALPS-AL204, Mettler Toledo, UK). A pH meter (S47-K, Mettler Toledo, UK) was used for all pH measurements. An ultracentrifuge (Sorvall Evolution RC) and Sorvall SLA-1500 rotor, (Thermo Electron Corporation, USA) was used to separate the biomass, while a UV–Vis Spectrophotometer (Lambda 25, PerkinElmer, UK) and 1 cm quartz cuvettes were used to measure the protein concentration. Finally, an end-over-end mixer, Reax 2 (Heidolph. Germany) was used to mix the surfactant and protein mixture.

2.3. Microorganism and BLIS production

The BLIS producing strain, *Pediococcus acidilactici* Kp10, was isolated from dried curd and the details have been described in a previous study (Abbasiliasi et al., 2012). The primary culture was prepared by taking a single bacterial colony from an agar plate and growing it in a 50 mL tube containing 10 mL of M17 broth, followed by incubation at 37 °C for 24 h without shaking. The inoculum was prepared by inoculating 1% (v/v) of the primary culture into a 50 mL tube containing 10 mL of M17 medium, and incubating it at 37 °C on a shaker agitated at 100 rpm for 24 h.

2.4. Preparation of BLIS crude extract

The cell free culture supernatant was prepared by ultracentrifugation (Sorvall Evolution RC) with a Sorvall SLA-1500 Rotor, (Thermo Electron Corporation, USA) for 30 min, 30,000g, 4 °C.

2.5. Preparation of AOT phase

The AOT stock solutions were prepared by dissolving it in deionised water (DI) water with a concentration range of 2.89–43.23 mM. However, after AOT addition to the sample the final AOT concentration in solution ranged from 0.26 to 3.93 mM. Therefore, AOT was present in a monomeric form at these concentrations as the critical micelle concentration (CMC) of AOT in water is 4.1 mM at 25 °C (Linfield, 1976).

2.6. Precipitation method

100 μL of AOT solution was added to 1 mL of a BLIS-containing fermentation broth, and the mixtures were mixed for 5 min with an end-to-end mixer. The samples were then centrifuged at 14,000*g* for 5 min, and the pellet collected and treated to recover the protein. Meanwhile, the supernatant (remaining fraction) was analysed for BLIS activity and protein content.

2.7. Recovery of BLIS from the AOT-BLIS complex

The BLIS was recovered from the AOT-BLIS precipitate by first washing it with 1 mL of distilled water to remove any remaining impurities. Following a centrifugation step (14,000*g*, 5 min), the complex was separated and 1 mL of acetone was added. 10 μL of 0.1 M NaCl solution was later added to the solvent phase followed by a second centrifugation step. The protein precipitate was collected and washed with acetone to remove the remaining AOT. After centrifugation, the precipitate was dissolved in fresh 20 mM, pH 7 PBS buffer. The effect of the final solution's ionic strength (0–100 mM) on the recovery was examined, and an ANOVA test was conducted to verify the statistical significance of the results.

2.8. Optimisation of separation parameters

Optimisation of the experimental conditions was performed with regard to: BLIS's overall activity recovery (%)-the most important parameter; protein recovery (%)-low due to all the other proteins initially present in the broth, and; purification factor-this is also an important parameter which is an indication of the volume reduction from the broth. These parameters were calculated using the following equations:

$$Activity\ recovery\ (\%) = \frac{Total\ activity\ in\ final\ recovery\ solution}{Total\ activity\ in\ original\ crude\ broth} \times 100\% \tag{1}$$

$$Protein\ recovery\ (\%) = \frac{Total\ protein\ in\ final\ recovery\ solution}{Total\ protein\ in\ original\ crude\ broth} \times 100\% \tag{2}$$

$$Purification\ factor = \frac{Specific\ activity\ in\ final\ solution}{Specific\ activity\ in\ original\ crude\ broth} \tag{3}$$

Download English Version:

https://daneshyari.com/en/article/5133404

Download Persian Version:

https://daneshyari.com/article/5133404

Daneshyari.com