Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Review

Towards bacterial lipopeptide products for specific applications — a review of appropriate downstream processing schemes

Vivek Rangarajan, Kim G. Clarke (Prof.)*

Department of Process Engineering, Stellenbosch University, Stellenbosch, South Africa

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 29 July 2016
Accepted 22 August 2016
Available online xxx

Keywords:
Lipopeptides
Downstream processing
Precipitation
Solvent extraction
Membrane ultrafiltration
Adsorption
Size exclusion
In situ product recovery

ABSTRACT

Lipopeptides are versatile molecules that are viable and potential replacements for synthetic surfactants in agricultural, pharmaceutical, food and cosmetic industries. While process optimization and intensification approaches have significantly improved lipopeptide production in terms of yield and productivity, downstream processing options to produce tailor-made lipopeptide products of different degrees of purity for specific applications have received considerably less attention. The use of conventional downstream methods such as solvent extraction, membrane filtration, adsorption and size exclusion has satisfactorily addressed the demand of lipopeptide mixtures to some extent, but the lack of well-established downstream techniques for these molecules still withholds their complete commercial realization. Moreover, fractionation of lipopeptide mixtures into families or individual isoforms is undeveloped, significantly limiting the use of lipopeptides for high end applications. This review highlights the recent developments in downstream processing of lipopeptides and discusses their pertinence on a case-to case basis in obtaining lipopeptides of appropriate purity for distinct and diverse applications.

Contents

1. Introduction

Lipopeptide biosurfactants, owing to their diverse structural and functional characteristics, hold promising applications in food [1], agricultural [2–4], pharmaceutical and cosmetic [5–9], microbial enhanced oil recovery [10,11] and environmental industries [12–14]. Many microbial species have been identified as having the ability to synthesize different types of lipopeptides [15–17]. However, as lipopeptides are synthesized predominantly by *Bacillus* species, the focus of the current review is on the *Bacillus* lipopeptide families, namely surfactin, fengycin and iturin.

Bacillus lipopeptides are amphiphilic molecules comprising a cyclic peptide moiety linked to a hydrocarbon moiety, and rapidly

* Corresponding author at: Department of Process Engineering, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa.
E-mail address: kclarke@sun.ac.za (K.G. Clarke).

http://dx.doi.org/10.1016/j.procbio.2016.08.026
1359-5113/

self-assemble to form supramolecular structures such as micelles and vesicles above the critical micelle concentration [18,19]. Surfactin has a cyclic lactone ring structure consisting of a C_{12}-C_{16} β-hydroxy fatty acid attached to a heptapeptide with a variable amino acid at positions 2, 4 and 7 [20]. Iturin has a C_{14}-C_{17} β-amino fatty acid moiety liked to a cyclic heptapeptide moiety with Asp or Asn at position 1 [20]. Fengycin consists of a β-hydroxy fatty acid chain linked to a decapeptide, which also forms a cyclic lactone ring. Fengycin A and fengycin B are the two variants with Val and Ala respectively, at position 6 [21,22]. While a structural diversity of these molecules leads them towards antimicrobial and therapeutic applications [3,7,23,24], functional properties such as surface activity and micelle forming ability, also qualify them for microemulsion related applications particularly in food and cosmetic industries [1,25].

In general, the economy of bio-production relies strongly upon the meticulous and efficient design of downstream purification operations, which may account for nearly 70% of the total production cost. However, the purification of biosurfactants, particularly lipopeptides, unlike other biological compounds such as proteins and antibiotics whose downstream purification is well established, remains a concern. This is primarily due to the subtle variations observed in the lipopeptide chemical structures, owing to the presence of different isoforms, which have posed significant challenges for the design of effective purification techniques. The difficulty involved in their purification is reflected in the relatively low purity levels of lipopeptide standards offered by M/S Sigma-Aldrich, with approximately only 90%, 95% and 98% for fengycin, iturin and surfactin respectively.

On the other hand, similarities in properties among different isoforms of the same lipopeptide family, such as uniform polarity and hydrophobicity, enable their separation as families using less selective purification procedures such as macro-porous resin chromatography [26]. Also, the fact that the functional traits of lipopeptides can be readily altered by changing pH and ionic strength of the solution [26,27] has facilitated uncomplicated separation of these compounds from other contaminating impurities. Nevertheless, the choice of purification procedure that would result in the desired product purity will ultimately be determined by the end application of the product, and to some extent the inherent ability of organism to synthesize preferred lipopeptide ratios.

The use of conventional downstream unit operations like precipitation, solvent extraction, membrane ultrafiltration, adsorption and size-exclusion chromatography techniques are limited to the removal of impurities such as proteins, salts and other media components, leading to partially purified or purified lipopeptide mixtures. The fractionation of lipopeptides into their individual families, or individual isoforms, inevitably relies upon high-end purification tools such as reverse phase high pressure liquid chromatography (RP-HPLC). Thus, a partially purified lipopeptide mixture suitable for general environmental applications could be obtained directly from the culture supernatant by precipitation, while a pure mixture of lipopeptides catering to the food and cosmetic industries may require techniques such as extraction, membrane filtration, and adsorption or size exclusion. For an ultrapure form of individual isoforms for say therapeutic applications, techniques such as RP-HPLC would need to be considered.

While there are a number of reviews portraying the recent improvements on lipopeptide production and purification [28–31], the application of appropriate downstream processing schemes leading to specific tailor-made lipopeptide products of different degrees of purities has not previously been considered or evaluated. In view of the importance and the considerable range of degrees of purity of lipopeptides required for numerous and wide-ranging applications, and the key role of the downstream operations in the total bioprocess, this review systematically examines and evaluates the purification operations suitable for lipopeptide concentration, purification and fractionation and categorizes these operations according to the level of purity of the lipopeptide product obtainable. Thus this review provides a convenient generic base for the judicious choice of appropriate purification operations in the development of a lipopeptide downstream processing program for distinct and diverse applications.

2. Downstream processing of lipopeptides: general considerations

Initial downstream operations in the sequence should be designed to achieve maximal concentration, preferably while still effecting some degree of separation from other culture constituents, so that subsequent purification operations can be conducted at a reduced scale. These subsequent downstream operations in the program should then effect the necessary degree of isolation of the lipopeptides from the other culture impurities.

For the successful design of downstream operations, insight into the constituents of the culture supernatant of *Bacillus* spp. is essential. Mulligan and Gibbs [32] have identified macromolecules like lipopeptide micelles, polysaccharides, peptides and proteins as the main constituents in the *Bacillus* culture, in addition to unutilized substrates and other metabolic products such as alcohols and acids. Where impurities such as protein, amino acids and other unconsumed substrates are present in the lipopeptide mixture, the purity level can be categorized as partial. Purification is achieved when the product comprises a mixture of lipopeptide families with their numerous isoforms, together with impurities in low quantities. Ultra- purification refers to a single family of isoforms or, in the purest form, to individual isoforms of a particular lipopeptide family. A number of downstream processing operations have been employed to obtain lipopeptides at these different purity levels (Table 1).

3. Recovery of partially purified lipopeptide mixtures

Acid precipitation, the most widely employed method for initial recovery of lipopeptides, serves as an effective method for the removal of low molecular weight impurities such as substrates and products of metabolism (Table 1). This procedure results in a lipopeptide recovery of about 90–95%, with purity close to 55% [33].

Acid precipitation involves the drop-wise addition of acid (usually 4 N HCl) into a continuously stirred culture supernatant until the pH drops to 2. The acid destabilizes the micelles and induces their aggregation to form an insoluble yellowish precipitate containing lipopeptides and other macromolecules. The co-aggregation of other macromolecules is minimized by overnight settling at 4 °C. The concentrate is then centrifuged and lyophilized to obtain a crude form of lipopeptide which can be readily solubilized by changing the pH to alkaline using NaOH. The lipopeptides can be extracted from the resolubilised precipitate (liquid–liquid extraction) or directly from the crude lipopeptide powder (solid-liquid extraction) obtained after acid precipitation.

The use of ammonium sulfate precipitation, the most commonly used method for precipitation of proteins, has not been reported for lipopeptide purification. In the case of lipopeptides, this method is discouraged as it can co-precipitate other macromolecules. Also, the need for subsequent steps to eliminate the salt increases the number of purification procedures required and consequently, the overall cost of the downstream processing.

Please cite this article in press as: V. Rangarajan, K.G. Clarke, Towards bacterial lipopeptide products for specific applications — a review of appropriate downstream processing schemes, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.08.026