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## EDITED BY

Pattanathu K. S. M. Rahman,  
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United Kingdom

## REVIEWED BY

Stephen Robert Euston,  
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Rajasekar Aruliah,  
Thiruvalluvar University, India

## \*CORRESPONDENCE

Bryan W. Berger,  
✉ bwb2k@virginia.edu

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# Biosurfactants as templates to inspire new environmental and health applications

Toriana N. Vigil<sup>1</sup>, Samantha M. Felton<sup>2</sup>, William E. Fahy<sup>1</sup>,  
Mason A. Kinkeade<sup>1</sup>, Alexandra M. Visek<sup>3</sup>, Abigail R. Janiga<sup>1</sup>,  
Sarah G. Jacob<sup>4</sup> and Bryan W. Berger<sup>1,2\*</sup>

<sup>1</sup>Department of Chemical Engineering, University of Virginia, Charlottesville, VA, United States,

<sup>2</sup>Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, United States,

<sup>3</sup>Department of Chemistry, University of Virginia, Charlottesville, VA, United States, <sup>4</sup>Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD, United States

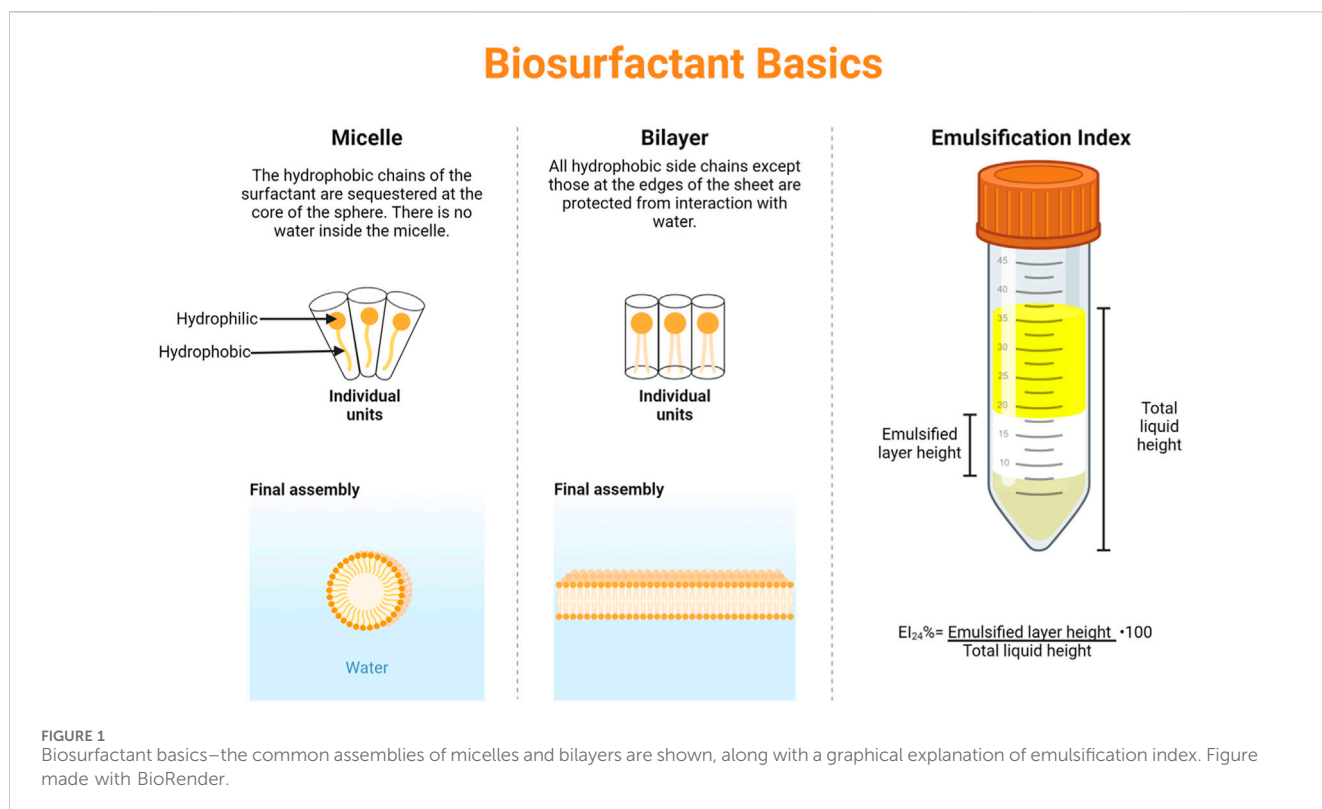
Life exists at an interface. One of the key characteristics of biological cells is compartmentalization, which is facilitated by lipids that create a water-impenetrable barrier to control transport of materials across the hydrophilic-hydrophobic interface. Microbial systems utilize a rich diversity of surfactants beyond lipids to adapt to an environmental niche, modify the properties of an interface, facilitate solubilization of nutrients for metabolism and as antimicrobials. As such, they are a fascinating class of biomolecules to study in terms of how effectiveness in an application or niche environment depends on sequence, structure and chemical properties. Moreover, there is increasing appreciation of the negative health and environmental impacts petrochemical-based surfactants can have, such as soil erosion and toxicity to plants and aquatic life, as well as the carbon footprint and associated greenhouse gas emissions associated with petrochemical surfactant manufacturing. In this review, we discuss the properties of biosurfactants and applications, and highlight key glycolipid-, protein- and peptide-based surfactants described in literature as examples of biosurfactants with unique potential and applications. As society looks towards the transition to a circular bioeconomy, we are excited by the potential of synthetic biology to develop new materials such as biosurfactants to facilitate this important transition.

## KEYWORDS

biosurfactant, lipoprotein, hydrophobin, emulsification, formulation, foam fractionation, antimicrobial, sustainability

## 1 Introduction

Solubility and interfacial interactions are a chemical challenge that impact nearly every sector, including food, medicine, and the environment (Shaban et al., 2020; Konwar, 2022; Nagtode et al., 2023). In many cases, insolubility and limited surface interactions function to decrease or prevent reaction activity from occurring. This is true for the case of oil-soluble and water-soluble compounds. In order to maximize conversion of the reaction between compounds in these two immiscible phases, the surface area of interaction between the two must be maximized. With consideration to the hydrophilic (water) and hydrophobic (oil) properties of the two compounds, amphiphilic molecules combine the properties of both. An amphiphilic molecule has a hydrophilic head group and a hydrophobic tail, creating a bridge between the hydrophilic and hydrophobic compounds. These molecules are often



called surfactants, which reduce surface tension by adsorbing to interfaces and displacing interfacial water molecules back to the bulk phase (West, 2018).

The name “surfactant” itself is a portmanteau of surface-active agent, highlighting the role of these molecules at interfaces. A household example is soap, commonly sodium stearate, whose amphiphilic character functions to solubilize greasy molecules to be washed away with water. Some specific examples of synthetic surfactants commonly used in scientific laboratories or in industry are sodium dodecyl sulfate (SDS), TWEEN, and Triton-X (Domínguez et al., 2011; Parra et al., 2020; Knoch et al., 2021).

The hydrophilic head and hydrophobic tail of surfactants are both variable in terms of chemical structure. For example, the head of the surfactant can have different ionic charges ranging from anionic, to cationic, to nonionic, or even zwitterionic (W.-C. Chen et al., 2015). In the case of sodium stearate, the negative charge associated deprotonated stearic acid means it is an anionic surfactant. Not only can surfactants align across a flat interface, they also commonly form micelles (Figure 1). A micelle is a spherical group of surfactants with hydrophilic heads facing outwards and the hydrophobic tails occluded within the sphere. The propensity for micelle formation, and a measure of surfactant activity, is quantified as critical micelle concentration, or CMC, which will vary depending on the specific surfactant (Czajka et al., 2015). When micelles form, the hydrophilic head groups form hydrogen bonds with aqueous solvent, stabilizing the solution (Rana et al., 2017). Although CMC is a commonly used metric for surfactants, there is some concern regarding the stringency of its definition (Knoch et al., 2021), however, in the absence of a better alternative, we will compare CMCs in our discussion of emerging biosurfactants.

Currently, many synthetic surfactants are petroleum-derived, meaning that extensive chemistry with petroleum-based compounds is required to produce these small molecules (Czajka et al., 2015). Another class of common synthetic surfactants are poly- and perfluoroalkyl substances, which are nearly impossible to break down (Mann et al., 2022; Trinh et al., 2022). In both cases, the production and use of synthetic surfactants involve many hazardous and environmentally harmful chemicals at massive scales. A promising alternative is the use of biosurfactants, *i.e.*, surfactants that are naturally occurring or derived from biology, which includes small-molecule, lipid- and protein-based molecules (Antonioli Júnior et al., 2022; Konwar, 2022). This review will present the current usage of biosurfactants including hydrophobin, and examine other promising emerging biosurfactants.

## 2 Current applications of biosurfactants

### 2.1 Research towards future food and consumer products

The food industry has an ever-growing need for surfactants as emulsifying agents, food additives, anti-adhesives, and antimicrobial agents, to improve food quality and extend shelf life. Synthetic surfactants may have all of these properties, but are often toxic, difficult to degrade, and lack stability in complex environments (Kiran et al., 2017). Therefore, biosurfactants are gaining popularity in many industries, including food and agriculture, due to their increased biodegradability, non-toxicity, stability in various temperatures, pHs, and salinity conditions, as well as their environmentally and economically friendly production methods

(Kiran et al., 2017; Thraeib et al., 2022). Two popular biosurfactants are surfactin, a lipopeptide from *Bacillus subtilis*, and rhamnolipid, a glycolipid from *Pseudomonas aeruginosa*. Both have emulsifying properties, but more strikingly, also have the ability to inhibit adhesion of pathogenic bacteria, such as *Listeria monocytogenes* and *Staphylococcus aureus* which contributes to improvement of food quality and safety (Zezzi Do Valle Gomes and Nitschke, 2012). Additionally, biofilm-forming bacteria pose a large threat in the food industry; Kiran et al. describes lipopeptide MSA31 from *Nesterenkonia sp.* not only has anti-biofilm activity against *S. aureus*, but is also “halo-alkali and thermal tolerant” for optimal use in the food industry and is shown in the laboratory to improve the texture of muffins (Kiran et al., 2017). To add, another laboratory study has shown that an unidentified biosurfactant from *Saccharomyces cerevisiae* URM 6670 with emulsification and antioxidant properties was found to be a non-toxic and non-product altering food additive that has potential to serve as a replacement for egg yolk (Ribeiro et al., 2020). One challenge to the adoption of biosurfactants in the food industry may be uncertainty and fear of utilizing products from pathogenic bacteria such as *P. aeruginosa*. Addressing that concern, it has been discovered that non-pathogenic strains such as *P. fluorescens*, *P. chlororaphis*, and *Pseudomonas putida* BD2 are also all able to produce rhamnolipids (Thangavel and Sridevi, 2015). Additionally, highlighting the use of rhamnolipids in industry, Evonik has recently begun commercial scale production of rhamnolipids for use in cleaning equipment used in food and consumer product manufacturing (Evonik Builds World's First Industrial-Scale Production Plant for Rhamnolipids - Evonik Industries, 2024). Another way to alleviate the concerns surrounding biosurfactant production from pathogenic bacteria is to apply synthetic biology for the production of these biosurfactants in non-pathogenic hosts such as *Escherichia coli*. Interestingly, Kleetz, et al. show that *E. coli* can be engineered to produce non-native lipids via recombinant protein production (Kleetz et al., 2021). Alternatively, the protein-mediated production of biosurfactants via cell-free systems, such as those pioneered by the Jewett Lab, are promising for the high-throughput production of microbially-based molecules (Rasor et al., 2022). Biosurfactants serve as a natural, safe, and economically friendly approach to improving the needs of the food industry.

## 2.2 Medicine

The pharmaceutical industry similarly looks to biosurfactants for their desirable antimicrobial, anti-adhesive, antiviral, anticancer, anti-inflammatory, and immune system promoting properties that they possess (Inès and Dhouha, 2015; Bjerck et al., 2021; De Giani et al., 2021). Glycolipids, including the commonly known rhamnolipids produced by *P. aeruginosa*, have the ability to permeabilize bacterial cell membranes resulting in antibacterial effects, with the added benefit of likely being more biocompatible than synthetic surfactants (Inès and Dhouha, 2015). For example, Saravanakumari and Mani describe a xylolipid from probiotic bacteria *Lactococcus lactis* proven to be safe for oral and dermal use with antibacterial activity against pathogenic *E. coli* and *S. aureus* (Saravanakumari and Mani, 2010). De Giani, et al. provides a

comprehensive review of other biosurfactants with antimicrobial activity that could be beneficial to human health (De Giani et al., 2021). In addition to antimicrobial use, some biosurfactants have shown promising anticancer effects. Sophorolipid, produced by yeast strain *Wickerhamiella domercqiae*, has been shown to cause morphological changes such as shrinkage and blebbing in H7402 human liver cells which ultimately leads to apoptosis supporting anticancer activity (Chen et al., 2006). With their aforementioned properties, biosurfactants offer a wide range of therapeutic potential and show promise for improving healthcare. As with the food industry, the origin of bacterially produced biosurfactants may be a barrier to their implementation and use. It is worth noting that recombinant protein expression, gene-knockouts, domestication of pathogenic bacteria, and other additional synthetic biology techniques may be helpful to increase acceptance and use of biosurfactants. For example, recombinant DNA technology and protein expression allows for the introduction of a biosurfactant gene sequence into a desired host organism for recombinant protein production (Jimoh et al., 2021). Studies have shown that incorporating the *P. aeruginosa* rhlAB operon into non-pathogenic hosts, such as *P. putidia* KT2440 and *E. coli*, serves as a safer production method for rhamnolipid (Setoodeh et al., 2014; Jimoh et al., 2021). To date, explicit implementation of synthetic biology to address these issues is a limited, but active area of research.

## 2.3 Environment

Pollutant sequestration with the use biosurfactants can be an effective method of bioremediation. Hydrocarbons that are difficult to degrade often accumulate in waste sites along with heavy metals. These pollutants can be extracted from soil with the use of biosurfactants, which effectively act to decrease surface tension and increase the solubility of contaminants (Ben Ayed et al., 2015). Some biosurfactants capable of pollutant degradation are produced by various bacteria including *B. subtilis* A21, *P. aeruginosa* PP3 and PP4, *P. aeruginosa* S5, *B. subtilis* B30, and others (Singh and Cameotra, 2013; Al-Wahaibi et al., 2014; Sun et al., 2019; Muthukumar et al., 2022; 2023). Biosurfactants including glycolipids, lipolipids, and rhamnolipids are particularly important for environmental applications as they reduce pollutants that would otherwise be added to existing contamination when using synthetic detergents or chemical solvents. This makes them environmentally sustainable solutions for removal of environmental contamination caused by hydrocarbons, heavy metals, oil, etc (Selva Filho et al., 2023).

Removal of polycyclic aromatic hydrocarbons (PAHs) from soil can be costly and ineffective due to the high hydrophobicity of PAHs and their strong affinity for soil (Singh and Cameotra, 2013). However, PAH removal can be facilitated by biosurfactants as discussed in Sun et al., where *P. aeruginosa* S5 was isolated from coking wastewater (Sun et al., 2019). Further examination of *P. aeruginosa* S5 showed the production of a glycolipid biosurfactant that decreased the surface tension enabling the enhanced the removal of PAHs (Ben Ayed et al., 2015). Similarly, Bezza and Chirwa identified biosurfactant producers *Bacillus stratosphericus*, *B. subtilis*, and *Bacillus megaterium*, whose biosurfactants increased the degradation potential of creosote in contaminated soil (Bezza and Chirwa, 2016). Conventional methods of remediation from oil spills generally yield a maximum of 15%

degradation, but biosurfactants produced by *P. aeruginosa* often result in crude oil degradation of 50%–89% (Thavasi et al., 2011; Muthukumar et al., 2023). Likewise, *Bacillus amyloliquefaciens* An6 produces a biosurfactant with low toxicity that decreased the interfacial tension between diesel oil and water, enhancing bioavailability of diesel oil for degradation and making it a useful tool for bioremediation (Ben Ayed et al., 2015). Investigations of highly polluted soil continually reveal bacterial and fungal species that exhibit biosurfactant production (Singh and Cameotra, 2013; Janek et al., 2021; Yasmin et al., 2022), perhaps as a potential survival mechanism amongst hydrocarbon and heavy metal pollutants. Here, the evolution and adaptations of microbes for the natural production of biosurfactants may serve as a foundation for directed evolution or rationale-based design of improved or novel biosurfactants. This approach is discussed in further detail in Section 4, with directed evolution-derived biosurfactant MBSP1 (Araújo et al., 2020).

### 3 Frequently used biosurfactant: hydrophobin

One family of protein biosurfactants called hydrophobins has been extensively studied for their wide-reaching applications (Berger and Sallada, 2019). Hydrophobins are small proteins (<20 kDa) with high surface activity secreted by filamentous fungi for a variety of vital functions (Wösten, 2001). Biosurfactant activity is created by an amphiphilic structure stabilized by four disulfide bonds among eight conserved cysteine residues in a characteristic pattern (Hakanpää et al., 2004). This family can be further divided into two classes based on self-assembly structures at air-water interfaces: Class I hydrophobins create amyloid-like fibrils independent of disulfide stability, while Class II hydrophobins create highly ordered films when disulfide bonds are intact (Sallada et al., 2018; Paananen et al., 2021). Naturally occurring hydrophobins within each class are very structurally similar with sequence variations indicative of uniquely evolved application (Hakanpää et al., 2004). This genetic flexibility has led to many engineered applications for hydrophobin in industry (Akanbi et al., 2010; Wang et al., 2017).

The pharmaceutical industry in particular has capitalized on the versatility of hydrophobins for drug delivery. Water-insoluble drugs such as cyclosporine A and nifedipine can be suspended in aqueous solution and made up to 500% more bioavailable by introduction of the hydrophobin SC3 (Akanbi et al., 2010). Fang et al. utilized Class I hydrophobin HPB to solubilize the lipophilic chemotherapy drug docetaxel, demonstrating high drug loading with delayed drug release (Fang et al., 2014). Valo et al. similarly suspended an insoluble corticosteroid in aqueous solution with a GFP-fused hydrophobin HFBII, allowing further study of the robust self-assembled micelle structure with fluorescence imaging (Valo et al., 2010). The retained functionality of these fusion proteins opens the door for various other surface modifications (Valo et al., 2010). For example, itraconazole was solubilized in micelles of synthesized hydrophobin HFBI fused to two cellulose binding domains and stabilized for 10 months by binding onto cellulose nanofibrils for storage (Valo et al., 2011). Chimeric hydrophobins have also been applied, seen in work by Vejnovic et al. improving permeability of terbinafine through human nails via a hydrophobin blending the

N-terminal section of Class I hydrophobin SC3 with the C-terminal section of Class II hydrophobin HFBII (Vejnovic et al., 2010). Even without forming micelles, hydrophobins can be used to inhibit crystallization of drugs such as flufenamic acid (Sallada et al., 2021). The disulfide-dependent amphiphilic structure of hydrophobins can even be intended for intentional compromise *in vivo* as a drug release mechanism, as demonstrated by hydrophobin HFBII-stabilized gold nanoparticles created by Maiolo et al. to release drug payloads upon disulfide reduction by cytoplasmic glutathione (Maiolo et al., 2017).

The amphiphilic structure of hydrophobins has been utilized for wettability and dispersion applications outside of pharmaceuticals. Hydrophobins SC3 and HFBII, representatives of Class I and Class II respectively, were both shown to assemble on Teflon surfaces drastically reducing water contact angle, as well as facilitate wet-in of Teflon particles into water (Lumsdon et al., 2005). Reger and Hoffmann utilized hydrophobin HPB as an emulsifier, stabilizing boehmite particles in a toothpaste-like emulsion for use in cosmetics (Reger and Hoffmann, 2012). Water-dispersible suspensions of graphene and graphite can be created in one step for electrochemical applications with hydrophobins, and engineered hydrophobin variants can be substituted for modified surface properties (Laaksonen et al., 2010). As hydrophobin HFBII is not toxic, cytotoxic, or immunogenic (Shokribousjein et al., 2011), laboratory studies have shown that it can be used in food products to stabilize foams for significantly longer times than traditionally used emulsifying agents like milk proteins (Cox et al., 2009). This biocompatibility allowed Zhang et al. to design bioactive poly ( $\epsilon$ -caprolactone) grafts coated in self-assembled hydrophobin HFBI films as a scaffold for immobilized anti-CD31 antibody to promote endothelialization (Zhang et al., 2011). Similar immobilization strategies were used on polydimethylsiloxane (PDMS) for microfluidics applications (Hou et al., 2009; Wang et al., 2007). Wang et al. utilized hydrophobin HFBI to reverse the hydrophobicity of PDMS, creating a bioactive surface which could then be robustly patterned with chicken IgG for immunoassays (Wang et al., 2007). Hou et al. iteratively improved the stability of this model, instead utilizing hydrophobin HGFI to create wetted PDMS surfaces able to withstand hot SDS washes (Hou et al., 2009). This immobilization strategy has been used to create a wide array of biosensors and antimicrobial coatings stable in various conditions by engineering or selecting hydrophobins with specific enzyme-binding properties (Wang et al., 2017; Zhao et al., 2009).

Production and purification methods of hydrophobins are as widely varied and interesting as their applications. Fungal host strains which naturally produce hydrophobins can be genetically engineered to overproduce, such as a *Trichoderma reesei* strain containing three additional copies of the HFBII gene fermented by Bailey et al. (Bailey et al., 2002). Grown in lactose-enriched media, this strain is capable of producing 240 mg/L HFBII in 92 h, a five-fold increase over the single-copy parent strain (Bailey et al., 2002). Both *E. coli* and yeast expression systems have been utilized as well (Kirkland and Keyhani, 2011; Sallada et al., 2019; Winterburn et al., 2011). Kirkland et al. designed a recombinant hydrophobin mHyd2 for expression in *E. coli*, and produced yields from 7–10 mg/L in less than 24 h (Kirkland and Keyhani, 2011). Although fermentation times increase from hours to days, *S. cerevisiae* yeast can also be used to express high yields of hydrophobins



TABLE 1 A comparison of emulsification index, surface tension reduction, and CMC for emerging biosurfactants (most of which are unspecified as of yet), Triton-X, and hydrophobin. In cases where CMC was reported as mg/L, we performed a basic conversion to weight percent for ease of comparison.

	Emulsification index	Surface tension	CMC
<i>Fusarium fujikuroi</i> Reis et al. (2018)	30% (toluene) Sena et al. (2018)	20.1 mN/m Reis et al. (2018)	30 mg/L (Reis et al. (2018)) or 0.003%
<i>Penicillium</i> 8CC2	54% (toluene) Sena et al. (2018)	-----	----
<i>Trichoderma citrinovide</i> Piegza et al. (2021)	30% (toluene) Sena et al. (2018)	32 mN/m Piegza et al. (2021)	----
<i>Mucor circinelloide</i> Marques et al. (2019)	66% (crude oil) Zadeh et al. (2018)	26 mN/m Zadeh et al. (2018); Marques et al. (2019)	1.5% Marques et al. (2019)
<i>Candida lipolytica</i> Rufino et al. (2014)	60% (motor oil)	25 mN/m Rufino et al. (2014); Santos et al. (2017)	0.03% Rufino et al. (2014)
	58% (corn oil)		
	40% (soy oil)		
	30% (kerosene) (dos Santos et al., 2021)		
<i>Candida bombicola</i> Pinto et al. (2022)	----	29 mN/m Pinto et al. (2022)	0.5% Pinto et al. (2022)
<i>Klebsiella</i> sp. Ahmad et al. (2021)	50% (toluene) Ahmad et al. (2021)		124 mg/L Ahmad et al. (2021) or 0.0124%
TritonX	35% (toluene) Ahmad et al. (2021)	33 mN/m Technical Data Sheet, (2024)	150 mg/L Ahmad et al. (2021) or 0.015%
	21% (dectol) Blesic et al. (2018)		189 ppm Technical Data Sheet, (2024) or 0.019%
Hydrophobin	74% (dectol) Blesic et al. (2018)	30 mN/m Cox et al. (2007)	0.5 mg/L Mancipe, (2019) Or 0.00005%

without need of structural modification (Winterburn et al., 2011). Sallada et al. introduced chaperone co-expression to a multi-copy HFBI *Pichia pastoris* strain, generating a 30-fold increase in protein production (Sallada et al., 2019). Regardless of production method, purification of interfacially-active hydrophobins is a challenge of its own, as necessary air supply can create large amounts of hydrophobin-sequestering foam (Winterburn et al., 2011). Winterburn et al. employed foam fractionation as a method to capitalize on this surface activity, continuously harvesting hydrophobin-rich foam throughout fermentation (Winterburn et al., 2011). Utilization of a biofilm reactor instead of traditional bioreactors and shake flasks can improve hydrophobin recovery by keeping the air inlet above the liquid volume (Khalesi et al., 2014). Hydrophobins can also be extracted from culture supernatants via phase separation utilizing nonionic surfactants, then recovered out of the non-ionic surfactant phase by addition of alcohol (Linder et al., 2001). Instead of extraction, cultures of purification tagged hydrophobins can be purified through immobilized metal affinity chromatography (IMAC) and eluted with imidazole (Sallada, 2020).

## 4 Promising emerging biosurfactants

Many diverse fungi produce glycolipid biosurfactants under harsh or starvation conditions, and in combination with fungal hydrolases, chitinases, and glutinases are effective for biocontrol (Jeziarska et al., 2018; Zadeh et al., 2018; Da Silva et al., 2021). Yeast strain *P. churashimaenius* OK96, originally isolated from sugarcane, can produce mannosylerythritol lipid (MEL), whose hydrophilic head and hydrophobic tail impart surface active and self-assembling properties (Arutchelvi et al., 2008), resulting in activity as an antitumor agent and

usefulness as an emulsifier in various cosmetics (Morita et al., 2013). *P. churashimaenius* OK96 has also shown adaptability to different sole carbon sources, including cuttlefish oil, for the production of MEL (Morita et al., 2013). This adaptability is promising as a method for sustainable and economical production of useful biosurfactants with alternate carbon sources. While these biosurfactants have shown effectiveness, the implementation of synthetic biology tool and approaches may further improve their activity.

Other fungal species, including *Fusarium*, *Penicillium*, and *Trichoderma* have been identified as biosurfactant producers through exploratory research in regions of high biodiversity, such as the Amazon rainforest (Sena et al., 2018; Piegza et al., 2021; Chotard et al., 2022). Along with *Mucor*, *Candida*, and *Klebsiella* species, these biosurfactants are compared in Table 1. In some cases, such as with *M. circinelloide*, the emulsification index (66%) suggests high surface activity, while the CMC does not (1.5%) (Zadeh et al., 2018; Marques et al., 2019), suggesting that the active biosurfactant has unique structure or specificity and that further studies will be imperative. Furthermore, it may be illuminating to consider other factors, such as ease of production, for which case *Mucor circinelloide* has a biosurfactant yield of 6 g/L whereas *C. sphaerica* yields 4.5 g/L when cultured on corn steep liquor (Marques et al., 2019). Interestingly, genome editing of *Mucor circinelloide* with a plasmid-free CRISPR-Cas9 system for the modification of metabolic pathways shows differential production of *M. circinelloide* natural products, thus suggesting that CRISPR-Cas9 targeting of the biosurfactant production pathway is feasible to increase biosurfactant yields even further (Nagy et al., 2017).

Altogether, while many of these species have high biosurfactant activity *in vivo*, the targeted, recombinant production of biosurfactants *in vitro* may be faster and more economical.

One exciting biosurfactant that can be recombinantly produced in *E. Coli.*, is the protein ransaspumin-2 (RSN-2). RSN-2 is one of six proteins that comprise foam nests of the túngara frog (Fleming et al., 2009). RSN-2 has an amphiphilic amino acid sequence and simple tertiary structure, where-in the C-terminal is analogous to the hydrophilic head of a surfactant, and the N-terminal analogous to the hydrophobic tail, protecting the eggs or sperm of the frog (Fleming et al., 2009). Work done by Morris *et. al.* confirmed a clam-shell model of adsorption at the interface and a two-step absorption process (Morris et al., 2016). Molecular dynamic simulations show that the removal of the hydrophobic N-terminus inhibits the protein from adsorbing to the interface while deleting the hydrophilic C-terminus only affected adsorption of RSN-2 in one scenario (Brandani et al., 2017). This suggests that the N-terminus is critical for adsorption while the C-terminus may aid in orienting the protein properly at the surface (Brandani et al., 2017). Another intriguing application of RSN-2 involves using it as a fusion tag for the foam fractionation of downstream enzyme processing (Krause et al., 2022).

Ranasmurfin is a protein similar to RSN-2, and is a biofoam produced by the *Polypedates leucomystax* tropical frog to protect their offspring (Oke et al., 2008; Cooper et al., 2017). The protein has a novel cross-link between two subunits of indophenol-like groups, which may cause the protein to have a blue color upon binding with zinc (Oke et al., 2008; Cooper et al., 2017). These groups could serve as targets for rational modification with the goal of modulating detection of other transition metals via coordinated cross-links. Oke, *et al.* postulate that RSN-2 and Ranasmurfin likely evolved in different and independent phylogenetic lineages due to their limited amino acid similarity. In spite of this, both these frog foams are able to protect embryonic staged eggs in harsh environments from many microbes, suggesting that these foams may have potential medical applications with anti-microbial properties.

Latherin is a mammalian produced biosurfactant protein found in the sweat and saliva of horses, that aids in evaporative cooling and acts as a microbial agent (McDonald et al., 2009). Latherin produces a significant reduction in water surface tension at low concentrations (less than 1 mg/mL). Structurally it has a predominantly polar outer surface with the non-polar residues buried. At the air-water interface the protein self-assembles to form a 10 Å layer which contributes its detergent-like properties (McDonald et al., 2009). Latherin represents the first mammalian surfactant protein with a known mechanism of action and structure, having potential uses in veterinary and medical science as well as nanotechnology processes (Vance et al., 2013).

In a completely genomic approach, MBSP1 is a biosurfactant protein derived from a metagenomic library derived from a soil sample taken from the Jundiaí River in Brazil. The protein of interest was shown to have a high similarity to hypothetical proteins from the Halobacteriaceae family. This protein has the ability to emulsify toluene and xylene, and yields positive results for drop collapse and oil dispersion assays (Araújo et al., 2020). The emulsification index, the height of the emulsion layer after a 24 h rest period in comparison to the original height of the liquid column (Figure 1), of purified MBSP1 with substrates including toluene, kerosene, and diesel was greater than 50%, with further studies showing long-term emulsion behavior even after 1 year (Araújo et al., 2020). MBSP1 is a promising example of implementing synthetic biology techniques to create novel biosurfactants, and similar strategies will likely have equally promising results.

As society continues to look for green alternatives to petrochemical surfactants, companies like Evonik, Locus Fermentation Solutions, BASF, AGAE Technologies, Glycosurf, Tensiogreen, Stepan Company, Holiferm, TeeGene Biotech Ltd., TARA Biologics Ltd., and Jeneil Biotech aim to produce biosurfactants as alternatives (BIOSURFACTANTS BY EVONIK ENTERING A NEW ERA OF SURFACTANTS, 2024; THE BIOSURFACTANT COMPANY ENABLING AN ECONOMIC TRANSITION TO A CLEANER WORLD, 2024). Additionally, as more biosurfactants are discovered and implemented in industrial processes, there is always room for improvement and diversification. Using synthetic biology tools, current biosurfactants can be adapted to improve their performance in their current roles as well as expanding their application to more diverse fields that also rely on chemical surfactants.

In conclusion, this mini-review has discussed the properties of key glycolipid-, protein-, and peptide-based biosurfactants described in literature along with their unique potential in a society moving towards a circular bioeconomy. This mini-review highlights the potential of synthetic biology to develop new materials such as biosurfactants to facilitate this important transition.

## Author contributions

TV: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Supervision, Writing—original draft, Writing—review and editing. SF: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. WF: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. MK: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. AV: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. AJ: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. SJ: Conceptualization, Data curation, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing. BB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing—original draft, Writing—review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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