Opportunities and challenges in omics approaches for biosurfactant production and feasibility of site remediation: Strategies and advancements

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ABSTRACT

Biosurfactants are molecules of 21st century. Their application(s) intercedes in daily life of living beings. Major limitation in the wide applicability of biosurfactant(s) is the economicity of production. To overcome this several strategies can be employed. This review is centered on the recent technological advancements in biosurfactant research. The advancement(s) include the use of metabolomic and sequence based omics approaches that has become a high-throughput indispensable tool for the identification of biosurfactant producers. A plethora of microorganisms synthesize biosurfactants, along with other value-added products namely ethanol, microbial lipids, and polyhydroxyalkanoates has been reported. This can significantly improve the economics of the overall process and limitations can further be dealt by employing metabolic engineering approaches. Tailoring strategy enables modification in the composition of congeners produced and improves the yield of biosurfactant. Bio-based surfactants have shown promising results against combating the pollution in terrestrial and aquatic ecosystems either by increasing their bioavailability or aqueous solubility. Owing to the ever-increasing market of biosurfactant(s), this review summarized technologically feasible advancement(s) in biosurfactant research that may enable the researchers to develop more safer and reliable technologies.

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1. Introduction

Surfactants are chemically synthesized or petroleum derived products that exhibit tension-active properties and thus were employed in almost all routine tasks everyday (Mohanty et al., 2021; Varjani and Upasani, 2017). Due to environmental concerns and the advantages of biosurfactants over synthetic surfactants viz., biodegradability, low toxicity, stability over a broad temperature and pH and salt the focus of researchers has shifted to microbial surfactants (Gaur et al., 2020; Liu et al., 2018; Devda et al., 2021). This has led to the wide range application of biosurfactants including in environmental (Sharma et al., 2021; Varjani et al., 2021a; Varjani and Upasani, 2021), food (Gaur et al., 2019b), biomedical (Gaur and Manickam, 2021a,b). The biosurfactants commercial market globally was estimated to rise at CAGR of 5.6% from 2017 to 2022 and may reach to 5.52 billion (Gaur and Manickam, 2021a). In 2016, the biosurfactant market was US$ 30.64 billion which was expected to rise US$ 39.86 billion in the year 2021. The biosurfactant market has a revenue generation of US$ 1.8 billion with an anticipated 8% gain in 2023 to US$ 2.6 billion that led to the generation of 540 kilo tons biosurfactant (Gaur et al., 2021c).

With the growing interest in biosurfactants during the recent decades, yet the market of biosurfactants lacks due to economicity of the production and availability of cheaper raw materials (Helmy et al., 2011). Also, the traditional approaches viz. the screening methods including the blue agar plate assay, drop collapse assay, the hemolytic assay, the oil spreading assay, surface tension measurement, and the emulsification assay for the identification of efficient biosurfactant producers from specific environmental niche is time consuming and labor intensive (Jackson et al., 2015; Markande et al., 2021; Varjani and Upasani, 2016). Thus, the employment of high throughput omics techniques in biosurfactant research has become imperative. Based on the sample studied viz. DNA, RNA, protein, and total macro- and micro-molecules, the omics technique can be metagenomics, metatranscriptomics, metaproteomics and metabolomics respectively (Fig. 1). These techniques provide staggeringly high information for the metabolic profiling, genetic makeup, and the functionally active fraction of an organism (Datta et al., 2020; Maron et al., 2007; Worley and Powers, 2013; Vinayak et al., 2021).

Metabolomic studies have been performed to study the effect of biosurfactants on the bioremediation of petroleum contaminated wastelands (Das et al., 2021). This had revealed the effectiveness of the design process for combating the abiotic stress that has been caused to the plants. The metabolomic approach has identified more than 30 new functional groups containing glycolipids when the strain *Rhodococcus* sp. I2R was grown in 22 different media. The identified active fraction showed anticancer properties against prostate cancer cells and antiviral properties against human coronavirus and herpes simplex virus (Palma Esposito et al., 2021). Furthermore, metagenomic tools have been utilized for the identification of novel biosurfactants (Williams and Trindade, 2017). Genomic analysis has advanced owing to the advancement in the next-generation sequencing platforms along with the decreasing cost of the analysis (Jackson et al., 2015; Patel et al., 2021). The biomolecules of interest were identified through this approach by identifying the sequence homology of the protein coding sequences to the reference database. Several tools have been developed for identifying the secondary metabolites of interest, one such tool is anti-SMASH (the antibiotic and secondary metabolite analysis shell), which annotates and identifies the gene clusters from sequencing data. The antiSMASH tool has been employed to identify the genes related to biosurfactants in *Serratia marcescens* Db11 (Gerc et al., 2014; Jackson et al., 2015).

In addition to this, the tailoring and engineering approaches have significantly improved the yield and nature of the biosurfactant to be produced for distinct applications. In several studies, the non-native producers of biosurfactants were engineered by genome manipulations for the production of biosurfactants (Grosso-Becerra et al., 2016; Roelants et al., 2013). *Pseudomonas aeruginosa* strain ATCC 9027 was reported with low levels of rhamnolipids whereas the introduction of the *rhlAB-R* operon containing plasmid further increased the rhamnolipid titer equivalent to the strain PAO1 (type strain). Further, since the genetic manipulations were done for the synthesis of mono rhamnolipids thus no di-rhamnolipid was produced by the strain ATCC 9027 (Grosso-Becerra et al., 2016). The utilization of these techniques has significantly affected and advanced biosurfactant research in terms of its market acceptability and economicity.

This review has detailed the co-production of biosurfactants with other products of industrial importance such as bioplastics. The next-generation sequencing methods and metabolomics approaches for the identification of biosurfactant
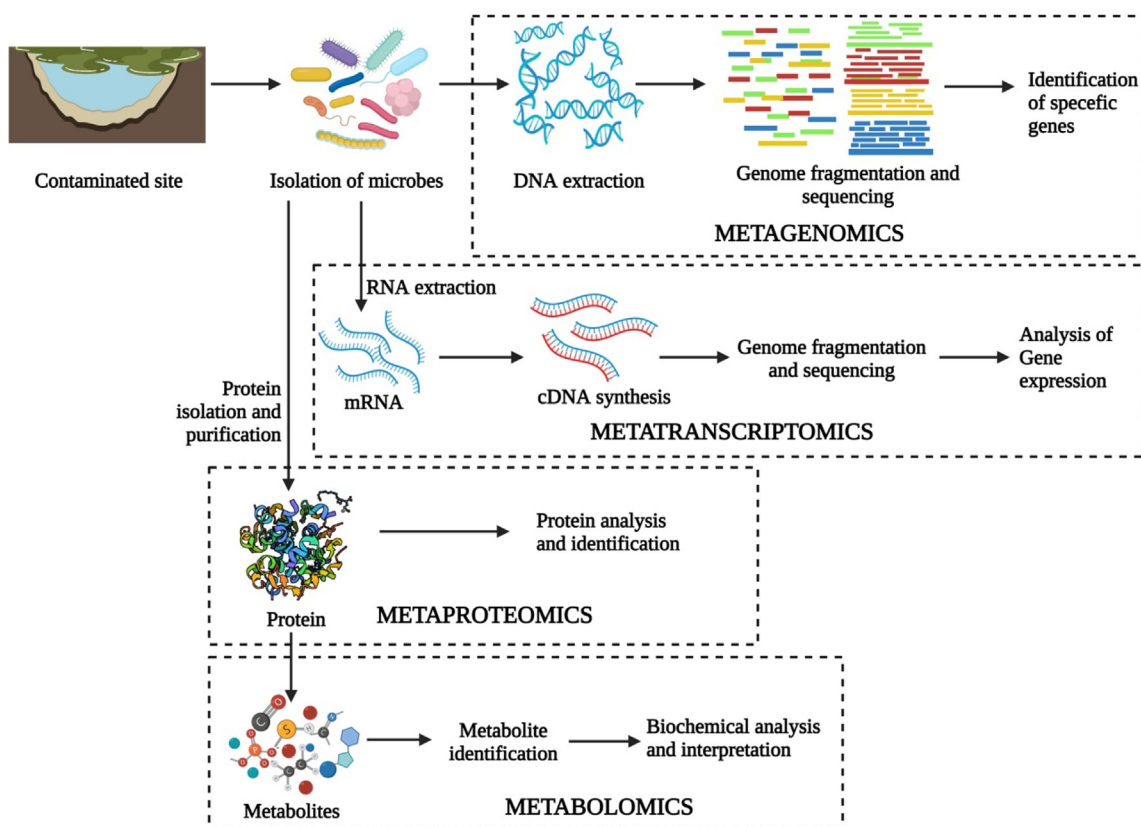
Fig. 1. Omics approaches in biosurfactant research.

producers have been discussed. Furthermore, this review detailed the strategy for enhancing the production of biosurfactant along with the effect of biosurfactants on terrestrial and marine ecosystems. We have identified the bottlenecks and presented the future perspectives and to the best of our knowledge, a compilation study of the advancements in the methods for the identification of biosurfactant producers has not been reported previously.

2. Co-production of biosurfactants with other value-added products

Biosurfactants are structurally diverse amphiphilic molecules having multifunction properties in various fields including cosmetics, food processing, pharmaceuticals, and textile and paint industry (Das and Kumar, 2019; Gupta et al., 2020; Varjani et al., 2021b; Feng et al., 2021). Besides their exclusive synthesis by selective microbes, biosurfactants were reported to be proficiently co-synthesized by a plethora of microbes along with some other value-added products like ethanol, microbial lipids, polyhydroxyalkanoates, etc. Several microorganisms were utilized as microbial biorefinery to convert low-cost substrates into value-added products (Shah et al., 2021). *Bacillus subtilis* BKDS1 was found to produce biosurfactant along with pectinase like pectin lyase (PNL), pectate lyase (PEL), and polygalacturonase (PG), in the same culture medium (Kavuthodi et al., 2015).

Bacillus methylotrophicus DCS1 utilized glutamic acid (5 g/L) and potato starch (10 g/L) as nitrogen and carbon source for simultaneous production of both a unique amylase and lipopeptide biosurfactant after 48 h of incubation at 25 °C and 150 rpm. The obtained amylase enzyme was alkaline in nature, exhibited excellent stability and compatibility with other solid/liquid surfactants. Furthermore, the simultaneously produced biosurfactant was found highly stable at a wide range of salinity, pH, and temperature (Hmidet et al., 2019). In solid-state fermentation *Aspergillus niger* synthesized a crude enzyme complex using rice bran and corn cob as a substrate. Furthermore, *Saccharomyces cerevisiae* and *P. aeruginosa* were witnessed for concomitant production of rhamnolipids and ethanol using sugarcane bagasse. In this fermentation setup 9.1 g/L rhamnolipids with an emulsification index of 84%, and surface tension of 35 mN/m were obtained along with 8.4 g/L ethanol after 86 h of fermentation (Guzmán-López et al., 2021).

A facultative anaerobe *Enterobacter aerogenes* was found to produce polyhydroxyalkanoate (PHA) and rhamnolipid simultaneously. During the stationary phase of growth, it produced β-hydroxyalkanoic acid, a precursor of both rhamnolipid and PHA which aids in the co-production of rhamnolipid and PHA in a single fermentation process. Under the

optimum condition of carbon:nitrogen ratio (5:1) at 6.5 pH, 5.81 g/L of rhamnolipid, and 4.2 g/L or 5% (v/v) of PHA were obtained in this process. *E. aerogenes* is a dark fermentative bacterium therefore, a complete dark period was maintained during the production of PHA and rhamnolipid. A mixture of biogas and some liquid metabolites were also reported to be produced during dark fermentation. During fermentation, PHA was found to be accumulated within the microbial cell while the rhamnolipid was secreted in the fermentation broth. Therefore, there was no hindrance noted during the co-production and downstream retrieval of each product (Shekhar et al., 2015). It was reported that *Thermus thermophilus* HB8 a thermophilic, non-pathogenic microorganism, utilized glucose or sodium gluconate as a carbon source for concurrent production of PHA and rhamnolipids (Li et al., 2017). A monomer of PHA co-polymers namely 3-hydroxyoctanoate accumulates in the microbial cell with a total production of 34.8 wt% while 0.2 g/L rhamnolipids was released into the liquid fermentation media. The initial phosphate concentration (as PO_4^{3-}) in media influences the production of both PHAs and rhamnolipids. In an initial phosphate (PO_4^{3-}) concentration of 25 mM, PHAs and rhamnolipid accumulation of > 300 mg/L and > 200 mg/L respectively were obtained after 72 h of cultivation. The accumulation of 3-hydroxydecanoate, a copolymer of PHAs in the microbial cell was witnessed in gas chromatography (GC) analysis. A plethora of congeners differing in lipid chain length namely mono-, di-rhamnolipid, and di-rhamno-monolipid was analyzed in LCMS results including some saturated or unsaturated fatty acids (Pantazaki et al., 2011; Varjani and Upasani, 2019).

P. aeruginosa IFO3924 yields 36% PHA and 0.4 g/L rhamnolipid synchronously in a single stage batch culture by utilizing 7 g/L hydrolyzed palm oil (glycerol and fatty acids) at 30 °C and 28 °C, respectively. Fatty acid de novo synthesis and β-oxidation were responsible for Mcl-PHA synthesis. (R)-3-hydroxyacyl-ACP, an intermediate of fatty acid de novo biosynthesis transforms into 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), a precursor of rhamnolipid synthesis in the vicinity of HAA synthetase (rhlA) enzyme. HAAs were further converted into mono-rhamnolipid via rhamnosyl transferase I (rhlB) activity and di-rhamnolipid by rhamnosyl transferase II (rhlC) which leads to the cumulative synthesis of rhamnolipids (Hori et al., 2011).

Non-pathogenic strains *Burkholderia thailandensis* utilized used cooking oil as a carbon source to produce rhamnolipids in conjunction with the accumulation of intercellular biopolymers like polyhydroxyalkanoates (PHA), and polyhydroxybutyrate (PHB). *B. thailandensis* was reported to produce up to 2.2 g/L of di-rhamnolipid Rha-Rha-C14-C14, with a capability to reduce the surface tension to 37.7 mN/m and the interfacial tension to 4.2 and 1.5 mN/m against benzene and oleic acid respectively (Kourmentza et al., 2017). *B. thailandensis* possesses a secondary undiscovered PHA synthesis pathway. Recombinant *B. thailandensis* having mutated genes for polyhydroxyalkanoate (PHA) synthesis also manifest increased production of rhamnolipids. Three knockout strains ($\Delta phbA$1, $\Delta phbB$1, and $\Delta phbC$1) were produced to enhance the production of rhamnolipid. In comparison to the wild-type strain (1.28 g/L), $\Delta phbB$1 was found to yield the highest level of purified rhamnolipid (3.78 g/L) having increased mono-rhamnolipid content (Funston et al., 2017).

3. Omics as advance technique for identification of biosurfactant producers

3.1. Metabolomics

Metabolomics is the study of the total metabolic content of a microbial cell. It provides staggeringly high information by capturing a global snapshot of all the metabolites/molecules in the cell, thus reflecting physiological state and biochemical activity (Worley and Powers, 2013). In a study by Floros et al. (2016), an untargeted metabolomics approach based on LC–MS/MS was employed to study the chemistries of various marine organisms. This has led to the identification of diverse molecular families and industrially important molecules viz., desferrioxamine E, actinomycin D, and valinomycin. Interestingly in this study, they have also identified surfactin biosurfactant along with these other metabolites (Floros et al., 2016). This suggested that untargeted metabolic profiling is an efficient tool for the identification of specialized chemistries (Gaur et al., 2021b; Sirohi et al., 2021) and specifically the identification and of biosurfactant producers among a plethora of other non-producers (Adetunji et al., 2021).

A metabolomics study was performed to study the bactericidal effect of *B. velezensis* FZB42 on a phytopathogen *Xanthomonas campestris*. It was found that the bacillibactin, siderophore, and lipopeptides were involved in the killing process. Lipopeptides (bacillomycin, surfactin, and fengycin) and bacillibactin were found in the mono- and co-culture of *B. velezensis*. Mass spectrophotometric analysis revealed that these four compounds were significant amongst the 24 different forms that were identified in the co-culture supernatant. Furthermore, fengycin and surfactin were found to exhibit a difference in their side chain (Mácha et al., 2021). The metabolomics study of more than 250 *Pseudomonas* strains was performed by Nguyen et al. (2016). The strains were ecologically diverse and mass-spectrometric evaluation revealed structural relationships in the molecular networking. Cyclic lipopeptides along and peptide families including putisolvins, tolaasins, orfamides, xantholysins, and massetolides were identified and these were reported to exhibit biosurfactant properties (Nguyen et al., 2016). Furthermore, the molecular networking of *S. marcescens* has led to the identification of lipopeptides with antimicrobial property. These lipopeptides were in the form of serratamolide having two L-serine residues which are linked to two fatty acid chains (with C_{10} to C_{12} chain length) and glucosamine derivatives having butyric acid, fatty acid chain, valine, and glucose (Clements et al., 2021). Media dependent metabolomic investigation of *Rhodotorula mucilaginosa* 50-3-19/20B yeast revealed the presence of anticancerous and antibacterial compounds in different solvent extracts. The manual de-replication against SciFinder and the Dictionary of Natural Products has revealed that glycolipid was the largest molecular family in the molecular network. These glycolipids proposed to be biosurfactants had differential acetylation degrees and belonged to a class of polyol esters of fatty acids (Buedenbender et al., 2021). These studies suggested that metabolomics approaches can be employed to identify biosurfactant producers and/or the biosurfactants produced by the microbial strains.

3.2. Sequencing based

The earth's environment has nurtured the most abundant, diverse, adaptive, and evolutionary form of life i.e., microorganisms. They are essential for maintaining our ecosystem and exploiting these microbes has become an indispensable tool for the welfare of society (Varjani et al., 2015). Conventionally, the familiar and established culturing and identification methods were employed for the growth and study of microbial communities due to which a vast variety of them remained unknown and unidentified (Dhanjal and Sharma, 2018). Therefore, omics technology which includes metagenomics (total DNA), metaproteomics (total protein), and metatranscriptomics (total RNA) was introduced to identify and gain detailed insights into the biosurfactant producing potential of microbial strains (Malik et al., 2021).

3.2.1. Metagenomics

The omics technique employed to identify the undefined microorganisms is known as metagenomics. In this, the genetic material was directly isolated from the environmental samples without any prior enrichment and culturing methods (Datta et al., 2020; Dhanjal and Sharma, 2018). Metagenomics involves DNA isolation, construction of metagenome library, 16S rRNA sequencing, and screening or data analysis (Datta et al., 2020; Dhanjal and Sharma, 2018). The screening in metagenomics is performed in two ways: Sequence based screening which depends on the previously defined sequences i.e., the primers are designed according to the known coding genes whereas, function-based screening is independent of already constructed metagenomics library. In this method, DNA was cloned to obtain sequences with desirable functions which may led to the discovery of novel sequences (Datta et al., 2020; Ngara and Zhang, 2018). It helps in processing and understanding the genetic information of uncultivated microbes, their biochemical pathways, interaction, and functions, thereby, discovering and identifying novel microorganism and their products (Datta et al., 2020; Malik et al., 2021). The discovery of novel biosurfactant producing strains is one such advantage of the metagenomics approach.

Metagenomics technique has proven to be beneficial for researchers to explore and study the microorganisms with more genetic insights. The information collected in the metagenomic libraries helps the scholars to gain insights into the identified microbes, their functions, and interactions between different microbial communities (Dhanjal and Sharma, 2018). However, there are certain limitations of this approach like, the sequence based metagenomics does not identify microorganisms that are scarcely populated and metagenomics does not identify the functional genes i.e., it does not distinguish between expressed or unexpressed genes (Hazen et al., 2013; Malik et al., 2021). Thus, metagenomics is mainly combined with metatranscriptomics to recognize the expression of genes.

3.2.2. Metatranscriptomics

Metatranscriptomics involves the study of mRNA transcripts of microbiome and understanding the activity of the genes. This technique explains the genes that are overexpressed and under-expressed due to environmental alterations (Malik et al., 2021). Direct isolation of RNA from environmental samples and enrichment of mRNA, conversion of mRNA to cDNA, and sequencing using next-gen sequencing are the three main steps involved in metatranscriptomics (Ranjan et al., 2015). da Silva Araújo et al. (2020) extracted DNA from Jundiai River soil samples, Brazil. A metagenome library was constructed, and a clone named 3C6 was identified through functional screening, which contained a novel protein MBSP1 with biosurfactant activity. The sequence of MBSP1 showed the highest similarity to *Haloferax lucentense*, which was known to be hydrocarbon degrader (da Silva Araújo et al., 2020). In a study, Williams et al. (2019) obtained soil samples from Bufflespruit Lake, South Africa. A metagenomic library was constructed within *Escherichia coli, Pseudomonas putida*, and *Streptomyces lividans* fosmid. *P. putida* clone was identified with biosurfactant activity by paraffin spray assay and ornithine acyl-ACP N-acyltransferase (olsB) was found responsible for this activity. Though fosmid was inactive in *E. coli*, yet under the control of the T7 promoter, overexpression of olsB gene was observed resulting in the production of lyso-ornithine lipid with biosurfactant activity (Williams et al., 2019).

Zhou et al. (2019) utilized transcriptomic approach to study the effect of L and D-Leucine on *B. velezensis* BS-37, which produced 1000 mg/L surfactin biosurfactant in the presence of glycerol. On addition of 10 mM L-Leu, the production increased to 2000 mg/L whereas the addition of 10 mM D-Leu reduced the production to 250 mg/L. The RPKM analysis (reads per kilobase per million mapped reads) of strain BS-37 transcriptome revealed that the transcription level of surfactin synthase, branched chain amino acid synthesis pathway, and glycerol utilization pathway genes were at a higher level and L-Leu was utilized as precursor whereas D-Leu behaved as a competitive inhibitor. Thus, metatranscriptomics helped in understanding and formulation of high surfactin producing strains (Zhou et al., 2019). Zhi et al. (2017) compared the efficiency of surfactin production between *Bacillus amyloliquefaciens* MT45 and *B. amyloliquefaciens* strain DSM7[T] using transcriptome analysis approach. The analysis revealed that strain MT45 showed better enrichment of surfactin synthesis pathway, enhanced carbon metabolism, and fatty acid biosynthesis. Also, surfactin synthase was found up-regulated from 9 to 49 fold in strain MT45 as compared to DSM7[T], thereby resulting in high production of surfactin (Zhi et al., 2017). Metatranscriptomics is advantageous as it helps to analyze gene functions and gene regulations of the microbial community but the short half-life, instability, and degradation prone properties of mRNA limits the applicability of this technique (Ranjan et al., 2015; Zhang et al., 2021). Thus, to overcome these limitations, the other omics approach known as metaproteomics comes into play (Malik et al., 2021).

3.2.3. Metaproteomics

Metaproteomics deals with the study of proteins expressed by the microorganisms present in the environmental samples. It analyzes and provides information about the functional genes of the microbial community (Malik et al., 2021; Maron et al., 2007). The data analyzed by metaproteomics serves as a linkage between genetic and functional information of microbial communities (Gaur et al., 2021a; Maron et al., 2007).

Pitocchi et al. (2020) isolated fungal strains namely, *Aspergillus terreus* MUT271 and *Trichoderma harzianum* MUT290 from an oil contaminated marine site to analyze novel biosurfactant proteins. With the help of proteomics, one common protein was detected which belonged to cerato-platanins (CP) family. They observed that the CPs analyzed from both the strains showed biosurfactant and bioemulsifiers properties. However, CP from *T. Harzianum* showed better surfactant activity and can be exploited for industrial uses (Pitocchi et al., 2020). It was reported that glycerol addition to *Burkholderia* sp. C3 enhanced the degradation of dibenzothiophene (DBT) and increased rhamnolipid (RL) biosynthesis. The degradation increased by 25%–30% and simultaneously RL biosynthesis also increased. Proteomics was applied to identify and analyze the upregulation of the enzymes participating in RL biosynthesis and DBT degradation (Ramirez et al., 2020).

Metaproteomics approach benefits researchers to explore novel functional genes and biochemical pathways and to identify the proteins that are expressed due to change in the substrate or environmental conditions (Maron et al., 2007). Even metaproteomics has certain limitations but it provides more information than metagenomics and metatranscriptomics (Malik et al., 2021). The omics approach is beneficial but there are still many challenges that need to be addressed. However, to gather better information and deep insights into genes, the multi-metaomics approach i.e., a combination of two or more approaches must be considered (Malik et al., 2021).

4. Advancements in production strategy

4.1. Tailoring and metabolic engineering approaches

The involvement of chemical or enzymatic catalysis, microbial fermentation in the commercial production of surface-active molecules (biosurfactants) was insufficient to match the market needs and requires researcher's attention (Moutinho et al., 2021). Biosurfactants were reported to exhibit resistance to environmental challenges and extreme conditions. The replacement of synthetic surfactants with microbial surfactants warrants rigorous research at the gene level to enhance the production at a significantly low cost. The development of high yielding strains by employing metabolic engineering has enormous potential to generate a huge number of modified strains (Table 1) (Dobler et al., 2016). Tailoring of the genome, altering metabolic pathways via metabolic engineering are materialized research hotspots (Fig. 2), reported to significantly improve the theoretical yield of biosurfactant (0.47 $g_{biosurfactant}/g_{substrate}$). This revealed that the alternative surfactin pathway was suitable to reduce the imbalance between cofactors and ATP and thereby improve the yield of biosurfactant (Moutinho et al., 2021; Zhang et al., 2020). Genome reduction has emerged as an intensive technique for modeling of an encouraging functional chassis in microbial cell factories. *B. amyloliquefaciens* LL3, contains an intact srfA operon for surfactin synthesis and by slicing/tailoring ∼4.18% futile genomic regions of strain LL3, a new genome-reduced strain GR167 was constructed to harbor a faster growth rate, higher heterologous protein expression capacity, higher transformation efficiency, increased intracellular reducing power, and intensified surfactin production. Further metabolic engineering i.e. slicing iturin and fengycin biosynthetic gene clusters from strain GR167 genome produced strain GR167ID. Strain GR167IDS and GR167IDT were produced after substituting PRsuc and PRtpxi promoters respectively from strain LL3 into strain GR167ID. The strain GR167IDS was found to be the best mutant exhibiting 678-fold improvement at the transcriptional level in srfA operon in comparison to strain GR167ID, and a 10.4-fold increase in surfactant production (11.35 mg/L) as compared to strain GR167 (Zhang et al., 2020).

Becker et al. (2021) combined genes Mac1 and Mac2, coding for acyl-transferases from different fungal species to obtain modified Mannosylerythritol lipids (MELs) producing fungal variants with explicit physical and antimicrobial activity. MELs are glycolipids, carrying sugar moieties with differing in attached acetyl-/acyl-groups side chains. *Ustilago maydis* carries a paralog of acyltransferase Mac2 gene with varying substrate specificity therefore it can participate in the biosynthesis of modified MEL generating variants (Becker et al., 2021). In *U. maydis* a toolbox for glycolipids with predictable fatty acid side chains was successfully developed which showcase that tailoring of Mac enzymes gene repository can result in the production of tailor-made MELs as per specific requirements of the biotechnological or pharmaceutical domain. Rhamnolipid synthesis genes were integrated with the genome of outperformed plasmid-based expression systems to develop an expression cassette and enhanced the genetic stability of the strain by inclusion of an inducible promoter (Tiso et al., 2020). Energy/carbon-consuming traits were further evacuated to strengthen the rhamnolipid synthesis. A maximum of 1.5 g/L of rhamnolipid titer was achieved by recycling the foam without using any antifoaming agent or mechanical foam destructor in a 1 L bioreactor.

A surfactin titer of 0.4 g/L was obtained from a non-surfactant producing *B. subtilis* strain 168 by integrating a complete *sfp* gene into its genome to restore its surfactin biosynthetic activity. Slicing of 3.8% of the total genome including polyketide synthase pathways/nonribosomal peptide synthetase, and biofilm formation-related genes increased the surfactin titer by 3.3-fold (Wu et al., 2019). The surfactin titer can be increased to 8.5-fold by improving the cellular tolerance to surfactins through over-expressing the self-resistance-associated proteins. Furthermore, engineering the
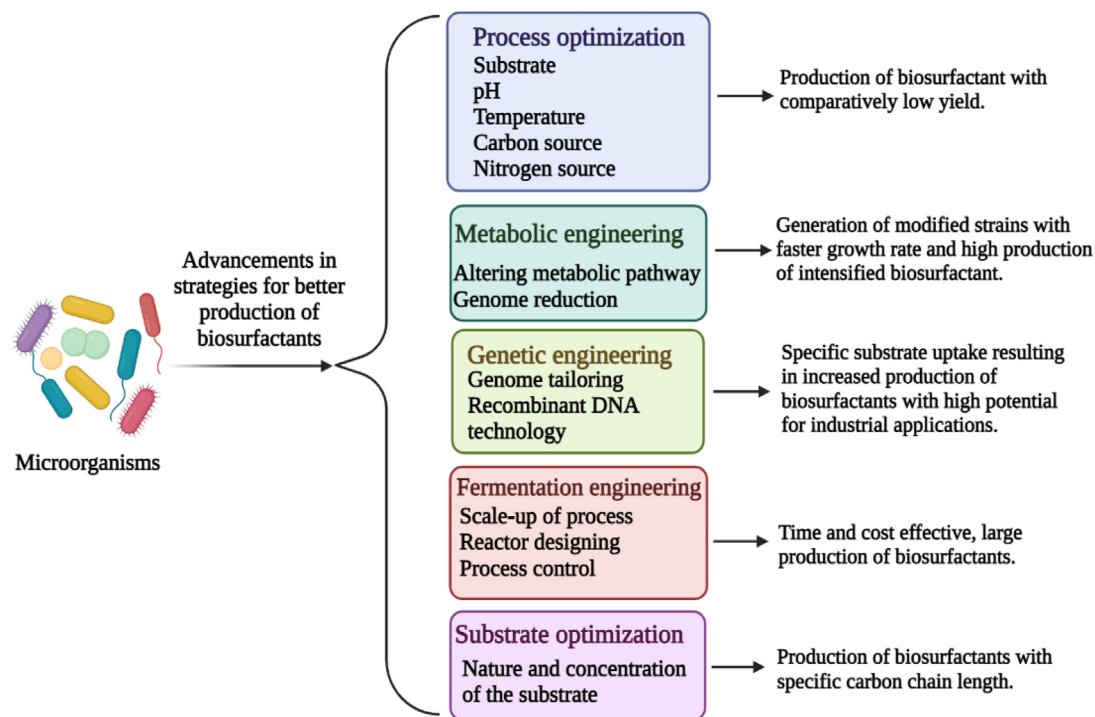
Fig. 2. Advancements in the strategies for biosurfactant production.

Table 1
Metabolic engineering strategies for enhanced production of biosurfactants.

Sr. no.	Organism engineered	Strategy employed (gene inserted or any other thing)	Name of gene	Source organism from where gene was cloned	Name of the biosurfactant produced	Production after engineering	References
1.	*B. amyloliquefaciens* strain GR167	Gene deletion and addition	srfA operon	*Bacillus amyloliquefaciens* LL3	Surfactin	311.35 mg/L	Zhang et al. (2020)
2.	*Ustilago maydis*	Gene addition	acyltransferase Mac1and Mac2 gene	*U. hordei* and *M. aphidis*	Mannosylerythritol lipids	Tailoring of fattyacid side chains	Becker et al. (2021)
3.	Bacillus subtilis strain 168	Gene addition and deletion	sfp gene and biofilm pathway respectively	*B. amyloliquefaciens* MT45	Surfactin	12.8 g/L	Wu et al. (2019)
4.	*P. putida* KT2440	Gene deletion	–	–	Rhamnolipid	700 mg/L	Tiso et al. (2020)
5.	*B. subtilis* (pHT43-comXphrC)	Overexpression	ComX and PhrC	*B. subtilis*	Surfactin	0.135 g/L	Jung et al. (2012)
6.	*Bacillus subtilis*	Gene replacement	PsrfA with Pg3	–	Surfactin	9.74 g/L	Jiao et al. (2017)
7.	*Starmerella bombicola*	Overexpression	lactone esterase (sble)	–	Sophorolipids	99% lactonic form and 1% acidic form	Roelants et al. (2016)
8.	*Candida bombicola*	Knock out knock in strategy	PHAC1co and UGT1co	–	Cellobioselipid and simultaneous poly-hydroalkanoates	2.0% wt/dwt of polyhydroalka-noates	Roelants et al. (2013)

branched-chain fatty acid biosynthesis pathway and enhancing the precursor supply of branched-chain fatty acids has led to an increase in the surfactin titer to 8.5 g/L (20.3-fold increase). In the metabolically engineered strains, the final surfactin titer was reported to be increased up to 12.8 g/L (Wu et al., 2019).

P. aeruginosa PAO1, an engineered strain carrying transposon mediated chromosome integration of *rhlAB* operon showed a maximum yield of 1.819 g/L rhamnolipids using soybean oil as a substrate, with a constant productivity rate of 18.95 mg/L/h. By utilizing glucose as a sole carbon source, the maximum yield of 0.784 g/L was achieved, with a productivity rate of 8.19 mg/L/h. Engineered strain *P. aeruginosa* PG201 while utilizing glycerol (2 g/L) as a substrate yielded 2.2 g/L of rhamnolipids with a productivity rate of 13.1 mg/L/h (Dobler et al., 2016).

5. Case studies

5.1. Terrestrial

The accumulation of a large number of chemical compounds, petroleum hydrocarbons, and heavy toxic metals in soil either by natural calamities or by human activities led to increased soil pollution while simultaneously affecting human and environmental health (Rathankumar et al., 2021). Several approaches such as physio-chemical and thermal processes were developed for the remediation of these polluted contaminated sites, but these processes were ineffective and costly (Bustamante et al., 2012). In this context, the use of microbial biosurfactants for the remediation of the contaminated site offers an effective approach owing to the low toxicity, easy biodegradability, cost-effective production, and eco-friendly nature of biosurfactants (Mulligan, 2021). This has attracted researchers globally and several studies have been published focused on the addition of biosurfactants to the contaminated sites (Table 2). The biosurfactant addition to the soil reduced the surface and interfacial tension by increasing the mass transfer of the contaminants and permitting the entry of hydrocarbons to the microorganisms which is termed as surfactant aided bioremediation or surfactant enhanced bioremediation (Mohanty et al., 2013). It was suggested that for efficient remediation, biosurfactants should be effectively selected as per the pollutant properties and characteristics (Mulligan, 2021).

Ambust et al. (2021) introduced two different processes for the remediation of petroleum hydrocarbon from the contaminated environment. First is the increase in substrate bioavailability and second, the interaction between the bacterial cell surface and hydrophobic substrates by enhancing hydrophobicity and reducing lipopolysaccharides index of the cell wall without causing any damage to the cell membrane. Thus, blocking the hydrogen bond formation and increased hydrophobic–hydrophilic interaction led to the reduction in the surface tension of the water. Biosurfactants play an important role in enhancing bioavailability and biodegradability (Ambust et al., 2021; Souza et al., 2014). Pradeep et al. (2012) demonstrated an experiment to determine the effect of *P. aeruginosa* derived rhamnolipid biosurfactant on the total petroleum hydrocarbon fraction of an artificially oil contaminated soil. Rhamnolipid at two different concentrations viz., 4 g and 8 g per kg of soil was added. It was recorded that the concentration of total petroleum hydrocarbon fraction of the soil reduced from 6% to 1.3%, 2%, and 2.75% in 4 g per kg, 8 g per kg, and non-treated soil respectively. This suggested that the rhamnolipid biosurfactant aids in the removal of petroleum compounds (Pradeep et al., 2012). The surfactin like biosurfactant isolated from an environmental isolate *Bacillus nealsonii* S2MT was studied for its ability to reduce the pollution level of soil contaminated with 10% heavy engine oil. It was estimated that the biosurfactant at a concentration of 10 and 40 mg/L remediate 43.6 and 46.7% of oil whereas the same concentrations of sodium dodecyl sulfate reduced 39.4 and 45.3% as compared to 18.5% in control. This suggested that the microbial derived biosurfactant was more efficient in the removal of contaminants as compared to synthetic ones (Phulpoto et al., 2020). The effect of biosurfactant treatment in oil removal from the petroleum polluted soil was studied by adding 50 mL of crude biosurfactant in combination with cow dung and cow urine. It was recorded that 67% of the oil was reduced as compared to the untreated soil (Das et al., 2021).

Furthermore, sophorolipid derived from *C. bombicola* ATCC 22214 showed improved biodegradation of crude oil and model hydrocarbons in soil. Introduction of sophorolipid at a concentration of 10 g/L increased the biodegradation of pristine, hexadecane, and 2-methylnaphthalene to 85, 97, and 95% in 6, 6, and 2 d respectively. Also, sophorolipid addition resulted in 72% and 80% degradation of aromatics and saturates in 56 d (Kang et al., 2010). In a metagenomic study conducted by Regar et al. (2019) for the comparative microbial dynamics in pesticide contaminated sites, the introduction of rhamnolipid biosurfactant was found effective in increasing the rate of degradation of chlorinated pesticides present in the soil. It was found that the augmentation with *Rhodococcus* sp., *Sphingomonas* sp., and *Pseudomonas* sp., degrades 58% of α-hexachlorocyclohexane, whereas the addition of rhamnolipid JBR 425 (commercial biosurfactant derived from *P. aeruginosa*) at 20–100 μM increased the degradation to 64% in comparison to 48% by Triton X-100 (synthetic surfactant). In the case of endosulfan, maximum degradation of 61% was reported in presence of rhamnolipid as compared to 50% by Triton X-100, and 50% in the absence of any surfactant. Also, for DDT 57% degradation was reported in presence of rhamnolipid, 38% for Triton X-100, and 34% by augmented bacteria alone. This suggested that microbial biosurfactants were more effective as compared to synthetic ones and the addition of biosurfactants significantly affected the degradation potential of degrader strain (Regar et al., 2019).

5.2. Marine/aquatic

The accidental spill of hydrocarbons into aquatic environment such as lakes, ponds, bays, or oceans enormously affects the aquatic food chain, causes death of aquatic species, etc. The marine environment has suffered from constant oil spills, making oil one of the most abundant organic pollutants in the sea (Souza et al., 2014). The petroleum industry generates polyaromatic hydrocarbons, monoaromatic hydrocarbons, and heavy metals as the major toxic pollutants that are released in the environment adversely affecting microorganisms, humans, plants, and animals (Mishra et al., 2019; Varjani, 2017; Nakkeeran et al., 2020). Aiding in the remediation of these pollutants, the biosurfactants enhance the efficiency of biodegradation due to their surface active property (Fenibo et al., 2019; Varjani et al., 2017). Alternatively, use of chemical dispersants imposes toxicity to aquatic lives, and thus replacing them with the bio-based non-toxic product becomes advantageous (Dang et al., 2019; Shindhal et al., 2020). The crude biosurfactant produced by *B. subtilis* and *P.*

Table 2
Biosurfactant and their sources in combating the aquatic and terrestrial pollutants along with their treatment efficacy.

Sr. No.	Biosurfactant	Source of microorganisms	Effective concentration of biosurfactant	Application	Treatment efficiency	Reference
Aquatic application						
1.	Lipopeptide	*Bacillus atrophaeus* 5-2a	Culture supernatant	Microbial enhanced oil recovery	93.9% recovery	Zhang et al. (2016)
2.	Lichenysins	*Bacillus licheniformis*	–	Microbial enhanced oil recovery in-situ application.	16.6% recovery	Karlapudi et al. (2018)
3.	Emulsan Gly-colipopeptide	*Acinetobacter calcoaceticus*	0.5 mg/ml	Microbially enhanced oil recovery	98% reduction	Karlapudi et al. (2018)
4.	Surfactin	*Bacillus coagulans*	–	Increases the recovery of oil	17%–31% recovery	Karlapudi et al. (2018)
5.	Surfactin	*Bacillus subtilis*	1 g/L	Increases the recovery of oil	19%–22% recovery	Pereira et al. (2013)
6.	Glycolipid	*Halomonas* species	25 mL	Remediation of spilled oil in marine ecosystems.	Recovered 62% of crude oil.	Tripathi et al. (2018)
7.	Phospho-lipopeptide	*Marinobacter hydrocarbonoclasticus*	1% (v/v)	Crude oil solubilization	13 folds efficient	Tripathi et al. (2018)
8.	Rhamnolipid	*Rhodococcus soli*	–	Bioremediation of crude oil from the contaminated environment.	85% degradation	Lee et al. (2018b)
9.	Rhamnolipid	*Pseudomonas aeruginosa*	43.73 mg/L	Efficiency for heavy metal removal such as mercury and lead	50.20% mercury and 62.50% lead	Chen et al. (2021)
10.	Rhamnolipid	*Pseudomonas* species	–	Biodegradation of hydrocarbons	92.34% and 95.29% degradation of crude oil and diesel oil	Karlapudi et al. (2018)
11.	Emulsan	*Acinetobacter venetianus*	0.1 mg/mL	Removal of crude oil from the marine environment.	89% removal	Karlapudi et al. (2018)
12.	Sophorolipid	*Candida albicans*	–	Microbial enhanced oil recovery	8.6% recovery	El-Sheshtawy et al. (2016)
Terrestrial application						
13.	Surfactin	*Bacillus nealsonii*	10 mg/mL and 40 mg/mL	Reduction in the pollution level of 10% heavy engine oil contaminated soil	43.6% and 46.7% reduction	Phulpoto et al. (2020)
14.	Surfactin	*Bacillus subtilis*	–	Removal of heavy metals and petroleum hydrocarbons	65% and 46% reduction of petroleum hydrocarbons and heavy metals	Singh and Cameotra (2013)
15.	Surfactin	*Bacillus subtilis*	12.5 and 37.5 mg/L	Remediation of diesel oil contaminated soil	78.5% and 81.8% removal	Phulpoto et al. (2020)
16.	Rhamnolipid	*P. aeruginosa* BS2	0.5%	Removal of heavy metals such as cadmium and nickel	72 and 68% removal of Cd and Ni	Juwarkar et al. (2008)
17.	Lipopeptide	*Bacillus subtilis*	–	Enhancement in oil recovery and remediation of soil contaminated soil.	85% recovery	Mulligan (2021)
18.	Saponin	*Burkholderiacepacia*	2000 mg/L	Desorption of Copper and nickel from kaolin	83%–85%	Mulligan (2021)
19.	Rhamnolipid	*Stenotrophomonas maltophilia*	1 g/L	Removal of crude oil	8.3 folds	Tripathi et al. (2020)
20.	Rhamnolipid	*Lysinibacillus sphaericus*	90 mg/L	Solubilization of hexachlorocyclohexane	1.8 folds high	Gaur et al. (2019a)

aeruginosa has its potential use for the Microbial Enhanced Oil Recovery process (Fenibo et al., 2019). An increase in surface properties of biosurfactant increases solubility and mobility of the hydrocarbon pollutants. The effectiveness of the biosurfactant in marine remediation can be examined with the CMC value i.e., the biosurfactant with a low CMC of less than 1 to 1.5 is highly effective for enhancing the rate of biodegradation (Fenibo et al., 2019). Biosurfactant producing

strains namely *Ralstonia picketti* and *Alcaligenes piechaudii* showed 80% hydrocarbon degradation. The strains were found to produce biosurfactant while simultaneously degrading hydrocarbons. It was suggested that the production of biosurfactant is linked with increased transport and uptake of hydrocarbons which resulted in enhanced degradation (Płaza et al., 2008).

Biosurfactant was reported to play a pivotal role in the remediation of 5.73 million tonnes of oil spills in the ocean worldwide between 1970 and 2016. Biosurfactant was introduced as a dispersing agent that facilitated microbial aided degradation (Tripathi et al., 2018). It was suggested that the biosurfactant produced from the marine microorganisms such as *Halomonas* species can be effectively used for the remediation of spilled oil in marine ecosystems by synthesizing surface active compounds that increase the solubility of hydrocarbon that initiates bioavailability for degradation and oil recovery processes (Tripathi et al., 2018). Nievas et al. (2008) demonstrated an experiment to degrade the hazardous bilge waste (a fuel oil-type residue) that comprises a mixture of seawater, n-alkanes, resolved total hydrocarbons, and unsolved complex mixtures (branched, cyclic and aromatic hydrocarbons) using an emulsifier producing microbial consortium. It was recorded that about 91%, 78%, and 61% of n-alkanes, resolvent hydrocarbons, and unsolvent mixtures respectively were reduced in less than 6 d (Nievas et al., 2008). Karlapudi et al. (2018) reported that biosurfactant produced by marine bacterium reduced the oil slicks from the water surface by promoting oil in water dispersion with a formation of a stable emulsion. Thus, suggesting the use of biosurfactant in the remediation of oil spills on shorelines and in the sea. The marine bacterial isolate with the potential to degrade the hydrocarbon polluted marine environment was categorized as hydrocarbonoclatic bacteria (Karlapudi et al., 2018). It was reported that rhamnolipid biosurfactant enhanced the biodegradation of more than 15 carbon chain alkanes. Many recent studies reported the application of biosurfactant producing bacterial genera namely *Pseudomonas, Bacillus, Acinetobacter, Alcaligenes, Alcanivorax, Rhodococcus,* and *Corynebacterium* in the removal of hydrocarbons from the oil polluted environment with improved remediation rates (Lee et al., 2018a).

Biosurfactant was also reported to exhibit heavy metal remediation potential from aquatic environment (Bustamante et al., 2012; Chen et al., 2021). The rhamnolipid from *Pseudomonas aeruginosa* was reported for its better efficiency (as compared to sodium dodecyl sulfate) in the removal of heavy metals from intertidal sediment. It was recorded that 50.20% mercury (Hg) and 62.50% lead (Pb) were removed by using rhamnolipid at a critical micelle concentration of 43.73 mg/L. It is important to note that the initial concentration of Hg and Pb was 13.15 mg/kg and 520.32 mg/kg suggesting high removal efficiency demonstrated by rhamnolipid. Also, it was shown that the alteration in pH affects the removal competence of rhamnolipid and alkaline medium serves as the favorable condition (Chen et al., 2021). Furthermore, the rhamnolipid was found to exhibit enhanced dissolution of heavy metals from river sediments. The addition of rhamnolipid significantly increased the washing efficiency and 47.85, 86.87, 80.21, and 63.54% of Cr, Cd, Cu, and Pd respectively was removed at 3% rhamnolipid concentration. It was found that high pH and long washing time were optimum for efficient washing (Chen et al., 2017).

6. Bottlenecks and perspectives

Despite the fact that biosurfactants have shown excellent potential in replacing synthetic petroleum derived surfactants and exceptional environmental application(s), yet the market of biosurfactants lacks due to their high production cost (Mishra et al., 2020). Several factors responsible for the high cost of production include cost of substrates, downstream processing, and yield of biosurfactant (Prajapati et al., 2021). The strategy of simultaneous production of other value-added products i.e. co-production could be utilized which may increase the economics of the production process (Adesra et al., 2021; Janani et al., 2022). The major factor that limits this is the product recovery process since it is difficult and costly to purify products having the same polarity against organic solvents. To address this issue existing technologies have been modified in such a way that the substrate manipulation can lead to the expression of one product while inhibiting the other. As in the case where Roelants and co-workers grew engineered *Candida* species in presence of high glucose which increased the production of sophorolipid biosurfactants while suppressing the β-oxidation pathway which decreased and/or completely blocks the polyhydroxyalkanoate production (Roelants et al., 2013). Microbial strains that exhibit the potential to actively grow and produce biosurfactant under anaerobic conditions will effectively serve to enhance the oil removal process. Genetic engineering approaches can aid in the production of modified strains with the capability to secrete biosurfactants that may be applied in injections to recover the remaining oils from reservoirs (Nikolova and Gutierrez, 2021; Quraishi et al., 2021). Furthermore, the laborious and time consuming biosurfactant screening methods can be overcome by utilizing the omics approaches that may led to the identification of new to nature biosurfactants. All these techniques may further aid in increasing the structural variation which was reported as a major drawback of most studied glycolipid biosurfactants (Roelants et al., 2013). This suggests that future research may be centered on the utilization of renewable cheap substrate for biosurfactant production by the engineered strains that will deliver tailored molecules for specific applications.

7. Conclusions

The present review provides information on the advanced techniques for the identification of biosurfactants and their producers. The co-production strategy could be a cost effective strategy for biosurfactant production that may enhance its industrial applicability. Backspace Utilization of metabolomic and metagenomic technologies has made identification

process rapid and more robust with the advantage of identifying novel biosurfactant. This has improved understanding of microbial community structure, dynamics and has made the technology more reliable and safer. Biosurfactants exhibited a significant effect in the reduction of pollutant load from terrestrial and aquatic ecosystems. This review presented a thorough observation on the engineering approaches that would lead to the path towards enhancement in the yield of specific biosurfactant(s) and make the overall process economical. Metabolic engineering of microorganisms for specificity of products can be a future breakthrough in this area of research.

CRediT authorship contribution statement

Vivek K. Gaur: Literature review, Writing – original draft, Data curation. **Poonam Sharma:** Literature review, Writing – original draft, Data curation. **Shivangi Gupta:** Literature review, Writing – original draft, Data curation. **Sunita Varjani:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **J.K. Srivastava:** Supervision, Writing – review & editing. **Jonathan W.C. Wong:** Writing – review & editing. **Huu Hao Ngo:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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