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Review

# Promoting efficient synthesis and customization of sphingans based on metabolic engineering and synthetic biology strategies

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ABSTRACT

Sphingans are important exopolysaccharides due to their unique functional characteristics and potential application prospects in various fields. In recent years, the chemical structure, biosynthesis and function of sphingans have been studied extensively. With the development of metabolic engineering and synthetic biology, problems that restricting the production capacity and the design of sphingans, such as complex synthetic path and unclear research background of the wildtype strain, would be expected to be solved to some extent. This review describes the structure and biosynthetic pathways of different sphingans, analyzes the feasibility of obtaining high-performance sphingans-producing strains via classical mutagenesis combined with high-throughput screening techniques and chassis cells construction, and focuses on discussing how to efficiently synthesize and customize sphingans based on metabolic engineering and synthetic biology strategies. These strategies include using highly effective tools like genomic metabolic network models (GSMM) and CRISPR to regulate metabolic pathways, as well as customizing sphingans with different molecular weight through molecular weight regulation and controllable substituent modification based on genetic engineering. At last, the main challenges and prospects are discussed.

## 1. Introduction

Sphingans are a general class of natural microbial polysaccharides synthesized by *Sphingomonas*, which includes gellan, welan, rhamsan, diutan, S-88, S-7, among others (Fialho et al., 2008). Due to their short production cycle, unique physical and chemical properties, safety, and non-toxicity, sphingans have found widespread applications in industrial production and daily life. They were used as microbial flocculants, oil displacement agents, food additives, anti-cancer medications, packaging materials, etc. (Chang et al., 2022; Huang et al., 2022).

As scientists deepen their understanding of the microbial polysaccharide biosynthesis pathway and the associated genes, researches into the metabolic engineering of polysaccharide synthesis have made significant advancements. The objectives of microbial polysaccharide synthesis can be categorized into two main types: increasing yield and modifying structure. The former is closely linked to improve efficiency and reduce production costs. To enhance the efficiency of target complex synthesis in microbial cell factories, traditional regulatory strategies are typically employed, which include directly regulating, downregulating, and knocking out genes in the metabolic pathway. However, the high complexity of sphingan metabolic pathways often results in undesirable metabolic phenotypes. For instance, gene overexpression may lead to the accumulation of toxic intermediate metabolites, while downregulation and gene knockout may result in a deficiency of metabolites essential for cell growth (Zhu et al., 2011). The structure of polysaccharides can also be modified through metabolic engineering, potentially altering their physicochemical properties and biological activities, thereby enhancing their utility in industrial, medical, and other applications (Paliya et al., 2023). Consequently, the molecular weight and composition of polysaccharides are targeted for modification to obtain materials with new applications, which have attracted the attention of researchers, such as sphingan oligosaccharides

*Abbreviations:* PCP, polysaccharide copolymerase; OPX, outer membrane polysaccharide export; EMS, ethyl mesylate; NTG, *n*-methyl-n′-nitro-n-nitrosoguanide; ARTP, atmospheric and room temperatureplasma; NIRS, near infrared spectroscopy; DMFS, droplet microfluidic high-throughput screening; MOFs, metal organic frameworks; GTs, glycosyltransferases; GFP, green fluorescent protein; DBTL, design-synthesis-test-learn; HA, hyaluronic acid; GSMM, Genome-Scale Metabolic Model; PGM, phosphoglucomutase; PHB, poly-β-hydroxybutyrate; VHb, vitreoscilla haemoglobin; PgdS, γ-PGA hydrolases; EPS, extracellular polysaccharides.

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and their derivatives.

Profiting from the rapid advancement of synthetic biology, a variety of genetic engineering techniques, metabolic engineering methods, and engineering splicing-assembly technologies are maturing (Cameron et al., 2014). These developments enable researchers to design metabolic synthesis pathways tailored to specific user requirements and to produce targeted products using microbial cells. Although the research background of the *Sphingomonas* strains were not as well-established as that of model strains, recent progress in synthetic biology underscores the clear advantages and necessity of applying these techniques to biosynthesize sphingans (Su et al., 2015). Based on the chemical structure and biosynthetic mechanisms of sphingans, we aimed to demonstrate the feasibility of obtaining high-performance strains by combining classical mutagenesis with high-throughput screening and chassis cell construction. Additionally, we reviewed strategies for efficiently improving the yield of sphingans based on recent advancements in metabolic engineering and synthetic biology. We also analyzed the customization of sphingans in terms of molecular weight regulation and controllable substituent modification. Furthermore, we discussed existing challenges and potential research directions in the microbial synthesis of sphingans.

## 2. Chemical structure of sphingans

Sphingans are primarily acidic heteropolysaccharides characterized by a similar yet distinct structure. Their backbone predominantly consists of tetrasaccharide building blocks, specifically two glucose units, one rhamnose, and one glucuronic acid. This structure can be represented as [→4) α-L-Rha-(1 → 3)-β-D-Glc (1 → 4)-β-D-GlcA (1 → 4)-β-D-Glc (→1], which repeats (Schmid et al., 2014) (see Fig. 1). The types and positions of the side chains exhibit significant diversity, making sphingans a valuable natural polymer resource rich in structural and functional variety. This diversity also imparts unique physical properties to each member of the sphingan family. The carboxyl side chains of sphingans molecules repel each other due to electrostatic interaction, which prevents the tight aggregation of the helix. The intervention of cations can shield the electrostatic repulsion, so the strength of sphingan increases with the increase of the mass fraction of cations (Li, Liu, et al., 2020). The solubility of sphingan was also significantly affected by carboxyl groups, and their cross-linking ability was related to cis-OH groups (the configuration of hydroxyl (—OH) in some organic molecules in a cis-arrangement) (Luo et al., 2025). In aqueous solutions, interactions between the main and side chains (van der Waals forces and hydrogen bonds) affected the conformation of the sphingans, and the strength of these interactions depended on the side chain length (Xu et al., 2019). Side chains folded back onto the main chain can form hydrogen bonds with carboxylate groups, thus enhancing molecular stability.

### 2.1. Gellan (S-60)

Gellan is a linear tetrasaccharide polymer with a repeating unit and an average molecular weight of 5 × 10^5 Da, produced by *Sphingomonas paucimobilis* ATCC 31461. Although no sugar side chains have been

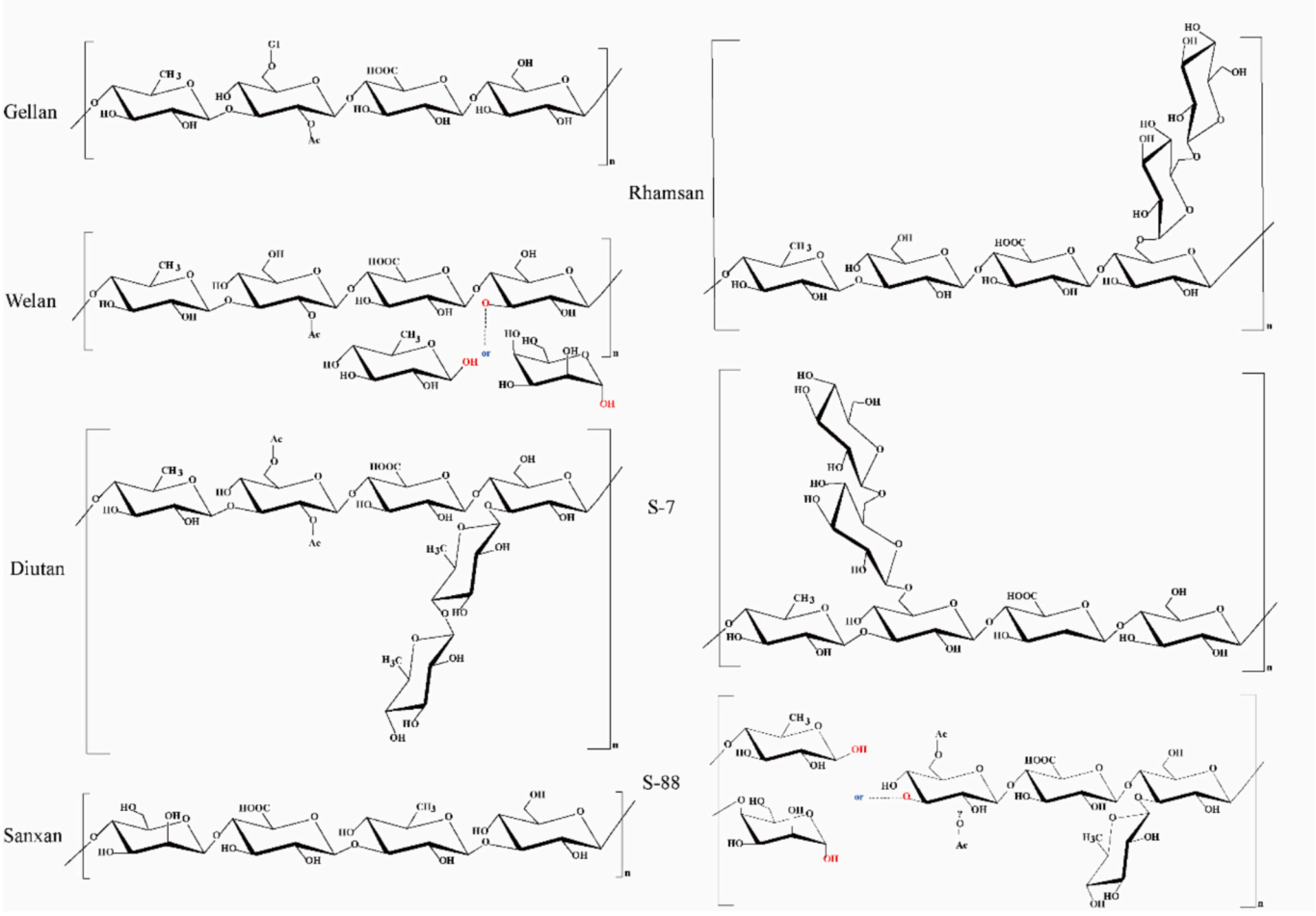


**Fig. 1.** Structure of different sphingans. Their backbone predominantly consists of tetrasaccharide building blocks, specifically two glucose units, one rhamnose, and one glucuronic acid. This structure can be represented as [→4) α-L-Rha-(1 → 3)-β-D-Glc (1 → 4)-β-D-GlcA (1 → 4)-β-D-Glc (→1], which repeats. And the difference between sphingans is that they have different side chain substituents.

identified, there are esterified residues at the second glucose of the tetrasaccharide unit. Based on the degree of esterification, gellan exists in two primary forms: high acyl gellan (natural gellan) and low acyl gellan, which has been physically and chemically deacylated and contains virtually no acyl groups. High acyl gellan contains glyceryl acyl and acetyl groups, typically both attached to the same glucose residue (Diener et al., 2020; Kuo et al., 1986). Generally, each tetrasaccharide repeating unit contains one glyceryl group and 0.5 acetyl groups. Therefore, gellan represents the simplest form of sphingans.

### 2.2. Welan (S-130)

Welan is a high molecular weight polymer synthesized by *Sphingomonas* sp. ATCC 31555, typically ranging from 10^6 to 10^7 Da. As a type of sphingan, the skeletal structure of welan is similar to that of gellan; however, it features side chains composed of L-rhamnosyl or L-mannosyl pyranopyranose. Additionally, the C-2 position of the second glucose residue is linked to an acetyl group, which constitutes approximately 2.8% to 7.5% of the polymer. The content of glucuronic acid in the main chain is about 11.6% to 14.9%, and the molar ratio of mannose, glucose, and rhamnose is approximately 1:2:2 (Jansson & Widmalm, 1994; Luo et al., 2025).

### 2.3. Diutan (S-675)

Diutan is primarily produced by the *Sphingomonas* sp. ATCC 53159 strain. The backbone structure of diutan is similar to that of gellan, consisting of a conserved tetrasaccharide repeating unit and a dimeric L-rhamnose side chain, which is connected to the first glucose residue of the repeating unit. An *O*-acetyl group is added to the $C_2$ and $C_6$ positions of the second glucose in each repeating unit, respectively (Coleman et al., 2008).

### 2.4. Rhamsan (S-194)

Rhamsan is produced by *Sphingomonas* sp. ATCC 31961 and features a conserved tetrasaccharide repeating unit in its skeletal structure. Each D-glucosyl residue adjacent to the L-rhamnosyl residue in rhamsan is substituted at the O-6 position by an α-D-glucosyl-(1 → 6)-β-D-glucosyl disaccharide side chain (Bian et al., 2002).

### 2.5. Sanxan

Sanxan is a novel type of biological gum produced by *S. sanxanigenens* NX02. The tetrasaccharide skeletal structure of sanxan is distinct from that of most sphingans, with its repeating unit consisting of [→4) β-D-Man-(1 → 4) β-D-GlcA (1 → 3) α-L-Rha (1 → 3) β-D-Glc (→1] (Huang et al., 2016).

### 2.6. S-88

Sphingan S-88, typically produced by *Sphingomonas* sp. ATCC 31554, consists of a conserved skeletal backbone with side chains of L-rhamnose or L-mannose. Approximately 50% of the repeating units feature a branched L-rhamnopyranyl. The frequency of acetyl substituents at the skeletal glucose unit is estimated to be around 5%; however, the precise proportion remains challenging to determine (P E Jansson, 1986; Tamaki, 2005).

### 2.7. S-7

S-7 is produced by *Sphingomonas* sp. ATCC® PTA-2175. Its chemical structure differs from that of other sphingans, as it contains 2-deoxy-glucuronic acid in the main chain instead of the more common glucuronic acid (Thorne et al., 2000). Additionally, it has been observed that two D-glucose units are attached as side chains to the second glucose of the tetrasaccharide repeating unit. To date, no other substituents, such as acetyl or glycerol, nor as many applications as those of other sphingans, have been associated with S-7.

## 3. Synthesis pathway and key gene cluster of sphingans

The biosynthesis pathway of sphingans primarily consists of three components: (1) the synthesis of intracellular nucleotide precursors; (2) the assembly and connection of tetrasaccharide repeat units at the inner membrane; and (3) the transfer of these repeat units into the periplasmic space for polymerization, followed by their export across the outer membrane.

The synthesis pattern of certain common sphingans by *Sphingomonas* is illustrated in Fig. 2a. In the synthesis of nucleotide precursors, such as UDP-glucose, UDP-glucuronic acid, dTDP-L-rhamnose, GDP-mannose, and 2-deoxyglucuronic acid, glucose-1-phosphate serves as the primary intermediate product. The enzyme responsible for catalyzing the synthesis of glucose-1-phosphate plays a crucial role in this process. The polymerization and export of sphingans occur via the *Wzx/Wzy*-dependent pathway, which has been documented for several enterobacterial capsular polysaccharides (Rehm, 2010). The assembly of the conserved tetrasaccharide repeating unit is facilitated by the continuous addition of nucleotide sugars to undecyl phosphate vectors anchored in the inner membrane, with the assistance of various glycosyltransferases (Whitney & Howell, 2013). The assembled repeating units are subsequently transported across the inner membrane by a flipping enzyme (*Wzx*-like) that spans it. The regulation of sphingan chain length is achieved through the action of a polymerase (*Wzy*-like). Final secretion across the outer membrane and cell wall is accomplished through the protein translocation pathways of polysaccharide copolymerase (PCP) and the outer membrane polysaccharide export (OPX) family.

Genes involved in the biosynthesis of sphingans S-88 (Yamazaki et al., 1996), S-7 (Thorne et al., 2000), gellan (Harding et al., 2004; Rocha et al., 2010), diutan (Coleman et al., 2008), and welan (Li et al., 2010) have been identified and are presented in Table 1. Several glycosyltransferase-coding genes are responsible for forming the skeletal structure (P Videira, 2001; Pollock et al., 1994; Pollock et al., 1998), as well as a number of genes potentially involved in secretion and chain length determination (e.g., *gelC* and *gelE* in gellan), have been biochemically analyzed (Moreira et al., 2005). Furthermore, genes responsible for the synthesis of glyconucleotide precursors have also been clarified (ElisabeteSilva, 2005; Sa-Correia et al., 2002). However, the genetic mechanisms governing polymerization, secretion, and chain length control of polysaccharide molecules for most sphingans remain poorly defined, particularly regarding the synthesis of side chain substituents. Typically, the genes responsible for the assembly, aggregation, and secretion of repeat units are tightly clustered in the genome, while those required for the synthesis of nucleotide precursors are dispersed throughout the genome (Schmid et al., 2014). Notably, the gene for dTDP-L-rhamnose synthetase resides within the same gene cluster as the glycosyltransferase gene responsible for the assembly of repeat units. In addition to the aforementioned genes involved in the sphingan biosynthetic pathway, a gene encoding acetyltransferase (O-AT), which is responsible for the acetylation of gellan, has been located approximately 20 kb upstream of *gelG* (as shown in Fig. 2b) (Aylward et al., 2013). Additionally, a gene responsible for regulating gellan production has been described and designated as *gelA*, which has also been identified in other sphingan gene clusters (Harding et al., 2004). Furthermore, the *atrD* and *atrB* genes, which encode enzymes associated with ABC transporters, were found in the gellan gene cluster, although the relationship between these transporters and gellan synthesis remains unclear (Yamazaki et al., 1996). The roles of the genes *urf31*, *urf31.4*, and *urf34* remained inadequately characterized, and it was possible that these genes existed simultaneously or manifested in pairs within the gene clusters of various *Sphingomonas* species.

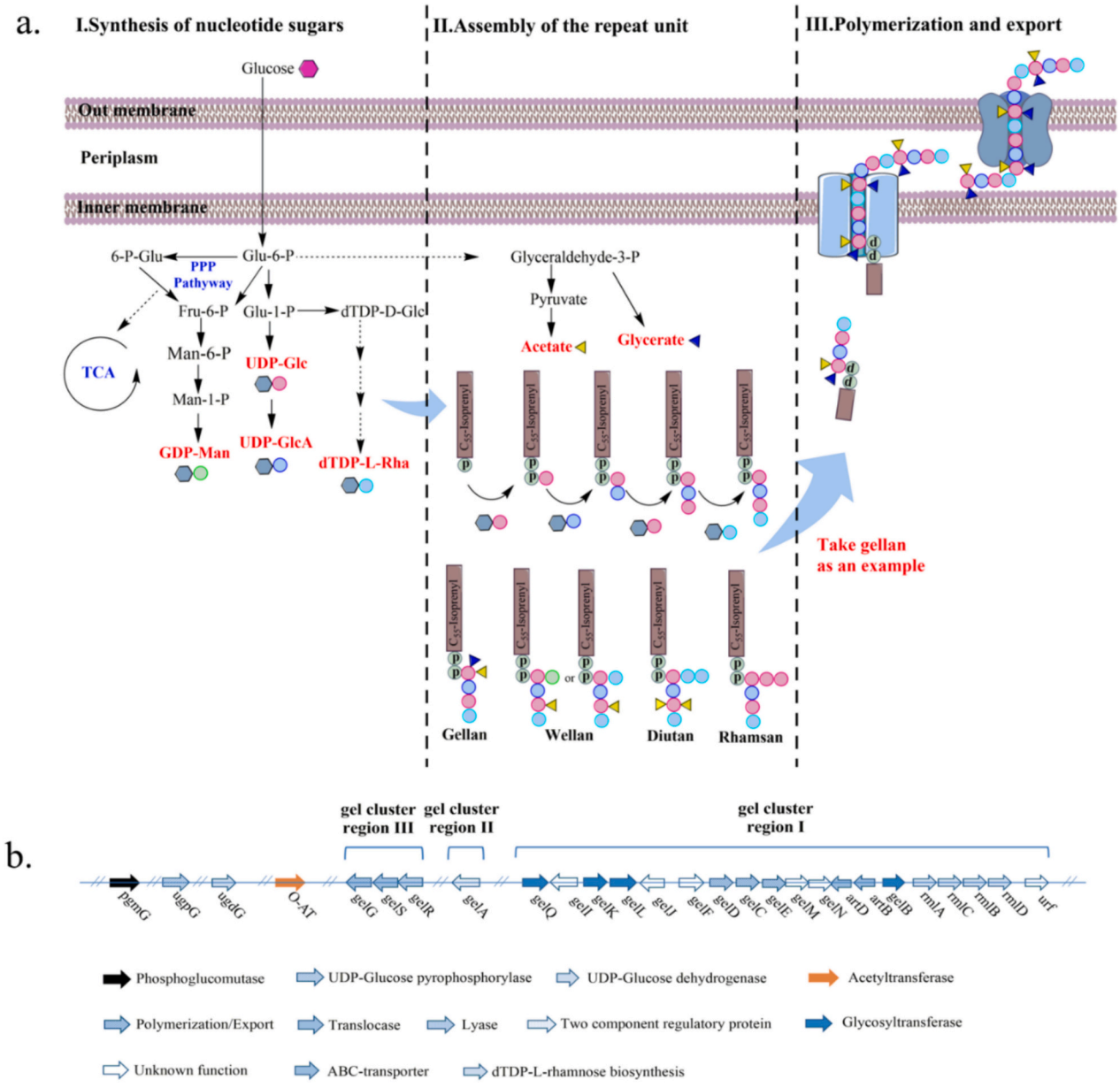


**Fig. 2.** a. Biosynthesis mechanism of several common sphingans. The biosynthesis pathway of sphingans primarily consists of three components. b. Gene cluster related to the biosynthesis of gellan in *Sphingomonas paucimobilis* ATCC 31461 and their distribution.

## 4. Acquisition of dominant sphingans producing strains

### *4.1. Mutation combined with high-throughput screening strategy*

#### *4.1.1. Mutation breeding*

The increased yield capacity of original strains is hindered by the factor of low substrate conversion efficiency, high by-product formation, and poor stress resistance. Therefore, the key to improve the microbial factory is to obtain superior mutant strains with desired traits by technical means. Mutagenesis breeding technology, a form of gene mutation technology, typically employs physical or chemical methods to perform single or multiple mutagenesis treatments on the original strain, thereby altering its genetic material to produce strains with specific traits. Commonly used chemical mutagens include ethyl methanesulfonate (EMS) and *N*-methyl-N′-nitro-N-nitrosoguanidine (NTG), while ultraviolet (UV) light is widely utilized as a physical mutagen. The current cases of increasing yield of sphingans through different mutagenesis methods are shown in Table 2. Previous studies have shown that when ethyl methanesulfonate was used for mutagenesis, the yield of gellan could be about 40% higher after 72 h of fermentation (Dev et al., 2024; West, 2002). Ke et al. developed a high-yielding mutant, FM01-S09, through UV mutagenesis technology, optimizing the yield of welan to 21.67 g/L, which was higher than the control group yield of 10.97 g/L, in conjunction with the fermentation process (Ke et al., 2021). In recent years, efficient technologies and devices have been developed to facilitate effective mutations, with atmospheric and room temperature plasma (ARTP), plasma, and lasers serving as typical examples. Zhu et al. obtained the high-temperature-resistant strain *Sphingomonas* sp. HT-1 through plasma mutagenesis, achieving a welan concentration of 26.8 g/L at 37 °C (Zhu et al., 2014). Additionally, different mutation methods can be combined to treat strains, resulting in higher levels of strain diversity (Zheng et al., 2022). The rationale behind this approach is that mutants produced by a single mutagen are often unstable, with a high likelihood of reverse mutations and a low positive mutation rate. Interestingly, Sun et al. utilized ARTP in combination with near-infrared spectroscopy (NIRS) to efficiently mutate high-yield gellan mutants,

**Table 1**
Overview of gene clusters involved in sphingans biosynthesis.

| Sphingans | Strain | Gene name | Gene function | Physical-chemical characteristics | Reference |
|---|---|---|---|---|---|
| gellan | Sphingomonas elodea ATCC 31461 | *gelB gelK*<br>*gelL gelQ*<br>*gelG gelC*<br>*gelE*<br>*gelS gelD*<br>*gelM gelN*<br>*gelI gelJ gelF*<br>*gelA*<br>*rmlA-D*<br>*atrD artB*<br>*urf34* | Assembly of repeating unit<br>Polymerization of repeating units<br>Export<br><br>Unknown<br>Regulation<br>Rhamnose synthesis<br>ABC transporter<br>Putative branching | Adjustable elasticity and hardness, rheology, emulsivity, suspension, thickening, low-pH stability, high transparency, biocompatibility, biodegradability, non-toxicity, pseudoplasticity | (Harding et al., 2004; Rocha et al., 2010) |
| welan | Sphingomonas ATCC 31555 | *welS welD*<br>*welM welN*<br>*welG welC*<br>*welE*<br>*welS welD*<br>*welM welN*<br>*welI welJ*<br>*welF*<br>*welA*<br>*rmlA-D*<br>*atrD artB*<br>*urf31.4*<br>*urf31 urf34* | Assembly of repeat unit<br>Polymerization of repeating units<br>Export<br><br>Unknown<br><br>Regulation<br>Rhamnose synthesis<br>ABC transporter<br>Putative branching | Thermal stability, salt-tolerance, high-viscosity, rheology, pseudoplasticity, wide-pH tolerance, emulsivity, suspension, thickening, non-toxicity | (Jansson & Widmalm, 1994) |
| diutan | *Sphingomonas* sp. ATCC 53159 | *dpsB dpsK*<br>*dpsL dpsQ*<br>*dpsG dpsC*<br>*dpsE*<br>*dpsS dpsD*<br>*dpsM dpsN*<br>*dpsI dpsJ*<br>*dpsF*<br>*rmlA-D*<br>*atrD artB*<br>*urf31.4*<br>*urf31 urf34* | Assembly of repeat unit<br>Polymerization of repeating units<br>Export<br><br>Unknown<br><br>Rhamnose synthesis<br>ABC transporter<br>Putative branching | Thickening, pseudoplasticity, viscoelasticity, thermal stability, salt-tolerance, rheology | (Coleman et al., 2008) |
| S-88 | *Sphingomonas* sp. ATCC 31554 | *spsB spsK*<br>*spsL spsQ*<br>*spsG spsC*<br>*spsE*<br>*spsS spsD*<br>*spsM spsN*<br>*spsI spsJ*<br>*spsF*<br>*rmlA-D*<br>*atrD artB*<br>*urf31 urf34* | Assembly of repeat unit<br>Polymerization of repeating units<br>Export<br><br>Unknown<br><br>Rhamnose synthesis<br>ABC transporter<br>Putative branching | | (Pollock et al., 1998; Yamazaki et al., 1996) |
| S-7 | *Sphingomonas* sp. ATCC® PTA-2175 | *spnB spnK*<br>*spnL spnQ*<br>*spnG spnC*<br>*spnE*<br>*spnD spnM*<br>*spnN*<br>*spnI spnJ*<br>*spnF*<br>*rmlA-D*<br>*atrD artB*<br>*urf34* | Assembly of repeat unit<br>Polymerization of repeating units<br>Export<br><br>Unknown<br><br>Rhamnose synthesis<br>ABC transporter<br>Putative branching | | (Thorne et al., 2000) |

resulting in a 133.5% increase in gellan production compared to the original strains (Sun et al., 2022). By employing UV-ARTP combined mutagenesis, they obtained low molecular weight gellan from *Sphingomonas paucimobilis* ATCC 31461, which exhibited a 44.6% reduction in molecular weight compared to the initial gellan, while the yield of gellan increased by 24% (Sun et al., 2023). The high yield of the welan mutant screened by UV-ARTP compound mutagenesis was 83.54% higher than that of the original strain (Wei et al., 2022). It is believed that natural mutagenesis therapy can expand the range of mutations to some extent, leading to significant changes at the gene level.

#### 4.1.2. High throughput screening of high sphingans-producing strains

In general, the amelioration of original strains through random mutagenesis techniques often exhibits low repeatability, and the process can be labor-intensive and costly. Therefore, random mutagenesis for strain evolution necessitates a combination of high-throughput screening techniques to potentially obtain mutants with the desired yield. The variety and scale of superior strains are largely determined by the techniques employed for screening. An effective signal detection strategy is crucial for establishing screening methods, and currently, the most commonly used detection method in mutation library screening primarily relies on fluorescence signals. Fluorescence detection of target products offers sensitive and reliable quantitative analysis (Chiu & Stavrakis, 2019). This capability presents an opportunity to enhance the screening capacity for directed evolution in synthetic biology. However, one of the main challenges currently faced is the lack of an appropriate

**Table 2**
The effect of different mutagenesis methods on the yield of sphingans.

| Strains | Sphingans | Mutation method | Yield (g/L) | | Reference |
|---|---|---|---|---|---|
| | | | Before | After | |
| *Sphingomonas trueperi* | Gellan | EMS | 6–7 | 10–13 | (Dev et al., 2024) |
| *Pseudomonas* sp. ATCC 31461 | Gellan | EMS | 1.98 | 4.49 | (West, 2002) |
| Sphingomonas sp. FM01 | Welan | UV | 10.97 | 21.67 | (Ke et al., 2021) |
| Sphingomonas sp. HT-1 | Welan | ARTP | 21.4 | 26.8 | (Zhu et al., 2014) |
| *Sphingomonas paucimobilis* ATCC 31461 | Gellan | ARTP | 4.45 | 9.427 | (Sun et al., 2022) |
| *Sphingomonas paucimobilis* ATCC 31461 | Gellan | UV-ARTP combined | 4.56 | 5.65 | (Sun et al., 2023) |
| Sphingomonas sp. FM01 | Welan | UV-ARTP combined | 8.22 | 17.40 | (Wei et al., 2022) |

high-throughput screening strategy to optimize sphingan production capacity.

Glycosyltransferases (GTs) play a crucial role in polysaccharide synthesis. Under the influence of various GTs, nucleotide precursors and side chain substituents are linked to form repeat units. As our understanding of the functional mechanisms of GTs deepens, the development of GT mutants with enhanced efficiency or novel substrate specificity has gradually become a viable approach to boost polysaccharide productivity and broaden the range of usable polysaccharide structures (Boltje et al., 2009; Wang et al., 2020). Liu et al. discovered and guided the evolution of enzymes that participated in polysaccharide biosynthesis, such as GTs, by using the strategy of combining high-throughput screening with fluorescent probes labeling of key enzymes (Liu et al., 2021). This approach has become increasingly significant in the identification of polysaccharide-synthesizing strains. Han et al. fused the 1,3-β-glucanosyltransferase GAS1 from *Saccharomyces cerevisiae* with the red fluorescent protein (RFP) (Han, Hui, et al., 2022). The expression level of the gene and the production of β-glucan were assessed by measuring fluorescence intensity, leading to the development of a rapid screening method for high-yield glucan-producing yeast strains. GTs are also closely associated with the yield of sphingans. Sá-Correia et al. showed that the overexpression of *gelK* gene, which encoded β-1,4-glucuronosyltransferase, resulted in an increased gellan production (Sa-Correia et al., 2002). Additionally, Li et al. reported that strains overexpressing the *welK* gene, also encoding β-1,4-glucuronosyltransferase, produced 32.65 g/L of welan after 60 h of fermentation, representing a 134.1% increase compared to wild-type strains (Li, Guo, et al., 2021). Li et al. further found that strains overexpressing the *welB* gene, which encoded glucosyl-isoprenylphosphate transferase, also enhanced the yield of welan by 19% (Li, Chen, et al., 2022). By using high-throughput screening of sphingan-producing strains (as illustrated in Fig. 3), GTs can serve as key enzymes in the biosynthetic pathway of sphingans. Fluorescent proteins, such as GFP, were labeled on key enzymes, allowing for the assessment of changes in fluorescence intensity, which can indicate sphingan production in mutant strains.

In addition to identifying key enzymes in the sphingan systhesis pathway, we can also consider combining these enzymes with fluorescent probes to develop a biosensor that can indirectly respond to sphingans. Biosensors recognize and respond to specific substances within the cell or those catalyzed by enzymes, converting them into distinct output signals. Consequently, the levels of cellular metabolites or enzymatically catalyzed products can be reflected in the signal strength (Michener et al., 2012). The synthesis pathway of bacterial polysaccharides involves successive multi-enzyme reactions, and the level of product synthesis can be represented by the content of particular substances in the cell, such as energy substances or intermediates (Li, Wang, et al., 2020; Li et al., 2015). It is known that the activation and energization of nucleotide precursors are prerequisites for glycosyltransferases (GTs) to identify and facilitate monosaccharide transfer, making bacterial polysaccharide synthesis an energy-intensive process. For each hyaluronic acid (HA) disaccharide repeating unit, cells consume two NADH molecules, five ATP molecules, and one acetyl-CoA molecule; for each welan tetrasaccharide repeating unit, they consume fourteen NADH molecules, eight ATP molecules, one acetyl-CoA

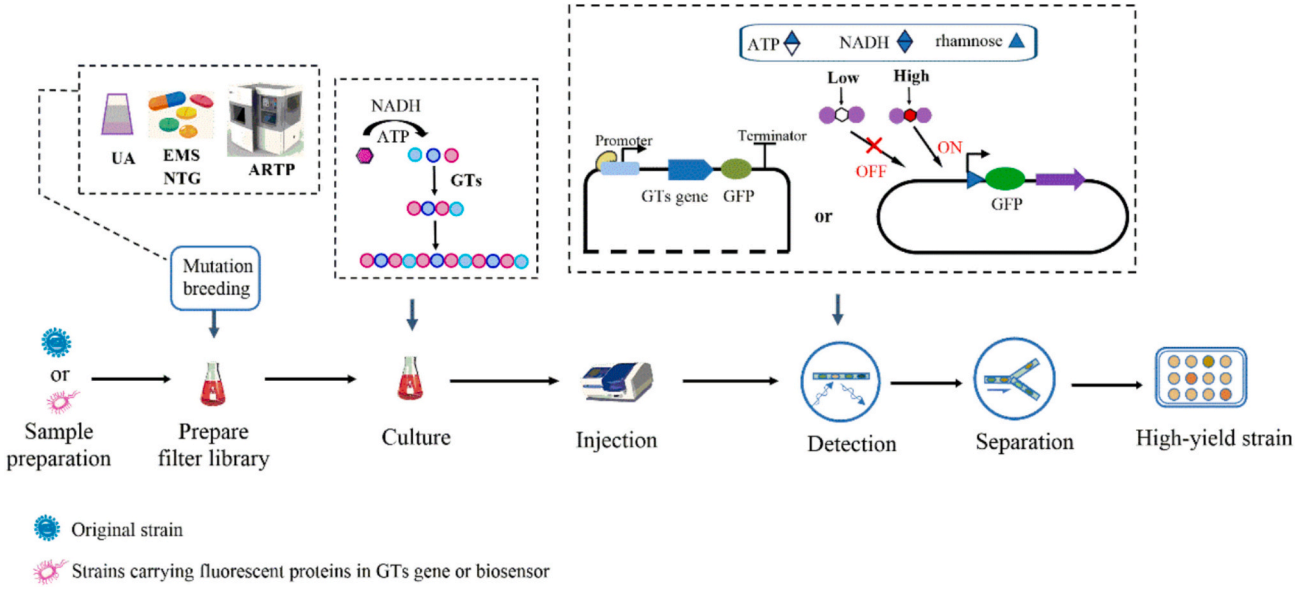


**Fig. 3.** Principle of mutagenesis combined with high-throughput screening for high production strains of sphingans. The original strain is mutagenized single or multiple times using physical or chemical methods to alter its genetic material to produce strains with specific traits. Additionally, by combining glycosyltransferases with fluorescent proteins and reflecting enzyme expression through fluorescence intensity, the synthesis of the product can be characterized. However, the synthesis pathway of bacterial polysaccharides involves successive multi-enzyme reactions, and the level of product synthesis can be represented by the content of particular substances in the cell, such as energy substances or intermediates. Therefore, the construction of biosensors using ATP, NADPH and rhamnose as response factors is expected to promote the high-throughput screening of sphingans-producing strains.

molecule, and half a glycerol acyl molecule (Li et al., 2010; Weigel & DeAngelis, 2007). Therefore, in polysaccharide biosynthesis, we should place special emphasis on biosensors that monitor substances associated with polymer chain synthesis and energy metabolism. A novel method for the detection of biomolecules was made possible by Wu et al.'s SERS biosensor, which was very sensitive to ATP levels and can smartly connect DNA structures with metal organic frameworks (MOFs) (Wu et al., 2020). Additionally, by combining reporter systems with promoters that react to variations in the reduction/oxidation coenzyme ratio, biosensors can be made to monitor NADH concentrations. Based on the electrocatalytic oxidation of reduced NADPH produced by an enzyme reaction, Du et al. created a glucose biosensor that could be used to measure the amount of glucose in practical clinical samples (Du et al., 2008). To regulate the biosynthesis of welan, Li et al. discovered a hybrid sensor histidine kinase/response regulator called WelA (Li, Li, et al., 2022). Furthermore, sphingans treated with lyases (such as gellan lyase and welan lyase) could produce rhamnose, which could activate the rhamnose promoter. Thus, if a rhamnose-responsive biosensor is developed by linking the rhamnose promoter to a fluorescent protein, the rhamnose concentration can be quantified based on fluorescence intensity, which in turn reflects the yield of sphingans. Building on this, a biosensor capable of responding to sphingans or their synthetic intermediates (such as ATP, NADH, and rhamnose) is anticipated to facilitate high-throughput screening of high-yielding sphingan strains. Therefore, biosensors hold significant potential for applications in microbial polysaccharide synthesis, extending to the screening of high-yield strains and the exploration and directed evolution of highly efficient enzymes.

### *4.2. Feasibility of constructing chassis cells for de novo synthesize sphingans based on synthetic biology strategy*

Like programming, synthetic biology adheres to the design-synthesis-test-learn (DBTL) cycle principle. In this process, engineered strains are tested, and the design of the microscopic "cell factory" is continuously modified. Data is synthesized, retested, and analyzed until a suitable strain is developed (Yang et al., 2021). Such a strain is often referred to as a "chassis cell". With advancements in genetic engineering, numerous microorganisms have been transformed into chassis cells, such as *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae*, and these chassis cells have been widely used in the synthesis of various high-value products. Furthermore, with the development of synthetic biology strategies, sphingan polysaccharides can also be derived from non-native sphingan-producing strains.

When selecting a suitable chassis cell, priority should be given to its ability to provide sufficient nucleotide precursor molecules for the synthesis of the target natural product. The natural strain *Sphingomonas sanxanigenens* NX02 (deposit number: CGMCC 1.6417) is a thermophilic, gram-negative, rod-shaped bacterium isolated from corn field soil, and it can provide nucleotide precursors such as UDP-Glc, UDP-GlcA, UDP-Man, dTDP-Rha, and UDP-GlcNAc. These nucleotide precursors encompass the major requirements for polysaccharide biosynthesis in common sphingans, glucans, xanthan, and HA. The xanthan operon has been introduced into the genome of the chassis strain *Sphingomonas sanxanigenens* NXdPE as a module (Wu et al., 2024). This chassis strain can produce a substantial amount of activated precursors for polysaccharides and optimize the transcription levels of genes by screening for a more suitable promoter, $P_{916}$, thereby increasing the synthesis yield of xanthan. Therefore, by adjusting the expression of different key genes based on the structural variations among different sphingans, the natural strain *Sphingomonas sanxanigenens* NX02 may also serve as a chassis cell for most sphingans. Wang et al. successfully knocked out genes in *Escherichia coli* that compete with the synthesis of oligosaccharides in *Bacillus pertussis* (Wang, Luo, et al., 2022). Then, they heterologously supplemented the necessary gene clusters for oligosaccharide synthesis in *Bacillus pertussis*, ultimately achieving efficient production of oligosaccharides containing multiple trisaccharide units. Currently, it has been found that three nucleotide precursors—UDP-glucose, UDP-glucuronic acid, and dTDP-L-rhamnose—can be synthesized from glucose in *Escherichia coli* and *Bacillus subtilis* strain 168 (Belda et al., 2013; Guo et al., 2004; Jin et al., 2021; Pei et al., 2019). For instance, if these two strains are utilized as chassis cells for the de novo synthesis of low-acyl gellan (which lacks acyl substituents), gellan could be produced following the exogenous introduction of a repeat unit polymerization module (including the *gelB, gelK, gelL, gelQ* genes) and a polysaccharide output module (including the *gelS, gelG, gelC, gelE, gelD* genes). Overall, existing researches have provided a theoretical foundation for the de novo synthesis of sphingans, and we may attempt the synthesis using natural *Sphingomonas sanxanigenens* NX02, *Escherichia coli*, and *Bacillus subtilis* as chassis cells.

## 5. Efficient synthesis of sphingans based on metabolic engineering strategy

Since the beginning of the 21st century, synthetic biology has emerged as a powerful technical approach for the design and engineering of biological systems. It integrates various fields, including genetic engineering, metabolic engineering, and systems biology (Clarke & Kitney, 2020). Metabolic engineering, in particular, serves as a robust tool for synthesizing desired products by optimizing metabolic pathways, successfully enabling the microbial synthesis of numerous natural compounds (Volk et al., 2023). However, the effective application of metabolic engineering also necessitates the use of advanced genetic tools. Currently, there are examples of optimizing sphingans through metabolic engineering strategies, but the availability of highly efficient genetic tools remains limited. In this context, we analyzed the effects of combining metabolic engineering regulation with genetic engineering and systems engineering on sphingan biosynthesis.

### *5.1. Efficient genetic tool*

DNA cloning technology is an essential tool for molecular biology and genetic engineering research. However, traditional cloning methods are insufficient to meet the demands of the rapidly evolving field of synthetic biology. In recent years, in-fusion cloning and CRISPR technology have advanced significantly, enabling the knockout, replacement, and transcriptional regulation of one or more genes. These innovations play a crucial role in biological research and have greatly accelerated the development of synthetic biology (Li, Zhang, et al., 2020; Liu et al., 2019). Zhang et al. successfully introduced three foreign genes into *Pichia pastoris* through seamless cloning, which led to the preliminary synthesis of chondroitin (Zhang et al., 2022). Furthermore, the discovery of the CRISPR system has broadened the scope of metabolic engineering. In complex synthesis pathways involving multiple genes, the coordinated interaction of these genes is fundamental to achieving the desired phenotype. The application of CRISPR interference (CRISPRi) has been shown to decrease the expression of either *pfkA* or *zwf* in *Bacillus subtilis*, resulting in improved production of HA with a reduction in molecular weight (Westbrook et al., 2018). Zhang et al. utilized CRISPRi to optimize cell growth and central carbon metabolism, thereby enhancing the production of *N*-acetylglucosamine (Zhang et al., 2020). Consequently, several research groups have successfully developed CRISPR systems aimed at the production of desired exopolysaccharides, which may offer valuable insights for their application in sphingan synthesis. Currently, it is possible to design the gRNA of the target gene in *Sphingomonas elodea* ATCC 31461 using the CHOPCHOP online tool. It is anticipated that the knockout efficiency would continue to improve through the adaptive modification of the CRISPR plasmid for this strain. Therefore, although the CRISPR system is infrequently employed in sphingan production, the adaptation of efficient genetic tools in *Sphingomonas* will facilitate the regulation of sphingan metabolism.

### 5.2. Transformation of metabolic pathways

The synthesis of polysaccharides by microorganisms can be summarized using the following metabolic engineering strategies.

Firstly, an adequate supply of nucleotide-linked sugars and increased substrate conversion result in a higher anabolic flow of polysaccharides (Cimini et al., 2023). Previous research indicated that the simultaneous increased in the copy number of the *pgmG* gene, which encoded phosphoglucomutase, and the *gelK* gene, which encoded glycosyltransferase, enhanced the expression of the gellan biosynthesis gene, leading to a 20% increase in the production of gellan (Sa-Correia et al., 2002). Additionally, a newly identified strain, *S. sanxanigenens*, has been reported to enhance sanxan production by 17% through the overexpression of the *pgmG* gene (Huang et al., 2013). Lee et al. demonstrated that the *gelA* and *gelN* genes were involved in the positive regulation and extracellular secretion of metabolites in gellan biosynthesis, and the *gelA* and *gelN* genes increased the secretion of gellan by 21.2% and 48.3%, respectively (Lee et al., 2017). Han et al. transferred two glycosyltransferase genes, *welB* and *welK* into *Sphingomonas* sp. WG for overexpression (Han, Wang, et al., 2022). The results showed that while the yield of welan did not change significantly, the viscosity exhibited a noticeable difference. Li et al. found that strains with overexpressed *atrB* and *atrD* genes increased the yield of welan (Li, Zhang, et al., 2022). In conclusion, the overexpression of genes related to the biosynthetic pathway of sphingans is beneficial for improving production.

Secondly, the inhibition of competitive metabolic pathways reduces the expression of genes related to central metabolic pathways (glycolysis and TCA cycles) or product competition pathways. This shift results in an increased carbon flux directed towards exopolysaccharide synthesis pathways. In addition to sphingans, *Sphingomonas* fermentation also accumulates significant amounts of yellow carotenoids and poly-$\beta$-hydroxybutyrate (PHB) as metabolic byproducts, which might affect yield and increase processing costs. Nevertheless, Li et al. knocked out the *crtI* gene in the carotenoid biosynthesis pathway and the *phaC* gene, which encodes PHB polymerization enzyme (Li et al., 2019). However, only 0.56 g/L of gellan was produced in the double-gene knockout mutant. Therefore, blocking the synthesis of PHB and carotenoids may influence the entire glucose metabolic network, leading to the accumulation of pyruvate from metabolism and consequently reducing gellan production (Schmid et al., 2014). Wu et al. reported that the blockage of PHB in *S. sanxanigenens* NX02 might disrupt the equilibrium of NADPH and $NADP^+$ within the entire metabolic network, leading to a reduction in extracellular polysaccharide production (Wu et al., 2018). In another study, after directly knocking out the *pig* gene cluster responsible for bacterioflavin synthesis in *Xanthomonas campestris*, the strain ceased to produce bacterioflavin; however, its capacity to produce xanthan decreased as well (Poplawsky & Chun, 1997). Interestingly, further investigations revealed that *xanK* gene (which encodes glycosyltransferase) within the *pig* gene cluster negatively regulated xanthan production, and xanthan production increased following the knockout of the *xanK* gene (Cao et al., 2018). These findings suggest that, to mitigate the competition of carotenoids for carbon source substrates, it is essential to identify the negative regulatory genes associated with carotenoid synthesis in the gellan production pathway. To enhance the product conversion rate in *Sphingomonas*, Vartak et al. inactivated the *zwf* gene, which encodes 6-glucophosphate dehydrogenase in the pentose phosphate pathway, through site-directed mutagenesis (Vartak et al., 1995). They hypothesized that this would redirect carbon flow towards gellan production; however, the anticipated results were not achieved. Despite some progress has been made in elucidating the biosynthetic pathway of sphingans, a deeper understanding of the less-known steps, as well as the regulatory mechanisms and bottlenecks within this pathway, is critical for the ultimate success of metabolic engineering in gellan production.

### 5.3. Genome-scale metabolic network model (GSMM)

However, the efficiency of enhancing strain yield through the aforementioned metabolic modification strategy is relatively low in strains with complex metabolic networks. With advancements in systems biology, phylogenetic engineering based on genome-scale metabolic network models (GSMM) has emerged as a rational and effective strategy for strain modification. By utilizing genome sequence and annotation information, GSMM could simulate the metabolic process of organisms through the reconstruction of metabolite-protein-reaction equation relationships. This approach enables researchers to systematically understand the physiological and metabolic functions of microbial cells and provides a platform for designing metabolic engineering strategies, significantly improving the efficiency of microbial manufacturing (Ankrah et al., 2021; Henson, 2015). GSMM has been successfully utilized in the synthesis of various carbohydrates to obtain the key genes and optimal culture conditions. Xu et al. integrated GSMM with the knockout site prediction algorithm OptKnock to determine the engineering gene target based on GSMM yeast model *8.4.036*, aiming to maximize the metabolic flux towards rubusoside synthesis (Xu et al., 2022). Wang et al. reconstructed *iYLW1028*, a genome-scale metabolic model of *Actinoplanes* sp. SE50/110, which identified genes critical for acarbose synthesis and cell growth on sucrose medium, and comprehensively elaborated the acarbose biosynthesis pathway in SE50/110 (Wang et al., 2015). Cheng et al. conducted flux balance analysis on the HA metabolism pathway using the $OptForce_{MUST}$ algorithm, based on the genome-scale metabolic model *iCW773* of *Corynebacterium glutamicum* to determine potential genetic intervention (Cheng et al., 2019). Ultimately, the highest titer of HA reported in the literature to date reached 28.7 g/L in a 5 L fed-batch culture. Currently, the gellan-producing strain *Sphingomonas paucimobilis* ATCC 31461 and the welan-producing strain *Sphingomonas* sp. ATCC 31555 are the most extensively studied strain within the *Sphingomonas* genus, and its whole genome data is accessible from National Center for Biotechnology Information. By compiling genomic and literature data for this strain, a preliminary metabolism network model of the *Sphingomonas* was constructed using software such as *Carveme* or various online websites. Subsequently, a series of algorithms (including OptKnock, OptForce, and OptDesign) were integrated with software such as MATLAB and Python for flux balance analysis (FBA) throughput analysis, as well as for predicting target knockout and up/down regulation of the target reactions, with the goal of enhancing sphingan synthesis through metabolic regulation.

Metabolic engineering strategies have been extensively employed to enhance the yield and regulate the molecular weight of microbial polysaccharides. As illustrated in Fig. 4, a combined approach that integrates genetic engineering, metabolic engineering, and systems biology is expected to optimize the conversion of carbon flux into sphingans, which is anticipated to become a central trend. Therefore, it is essential to develop potential strain resources capable of industrially and efficiently producing sphingans.

## 6. Customized synthesis of sphingans

The current requirement for functional polysaccharides requires the discovery of a broader range of these compounds, which has triggered widespread interest in biotechnology. In addition to established genetic engineering techniques, glycosyltransferase protein engineering aims to enhance the diversity of sugar donors and receptors through domain swapping. This approach has the potential to greatly expand the portfolio of polysaccharide variants. Successful engineering could generate different monomer compositions, resulting in new features of the final polysaccharide product. A deeper insight into functions of the several polymerases and co-polymerase enzymes will make the repetitive units with slender or truncated side chains or different substituent modifications to be connected with each other. Therefore, the customization of

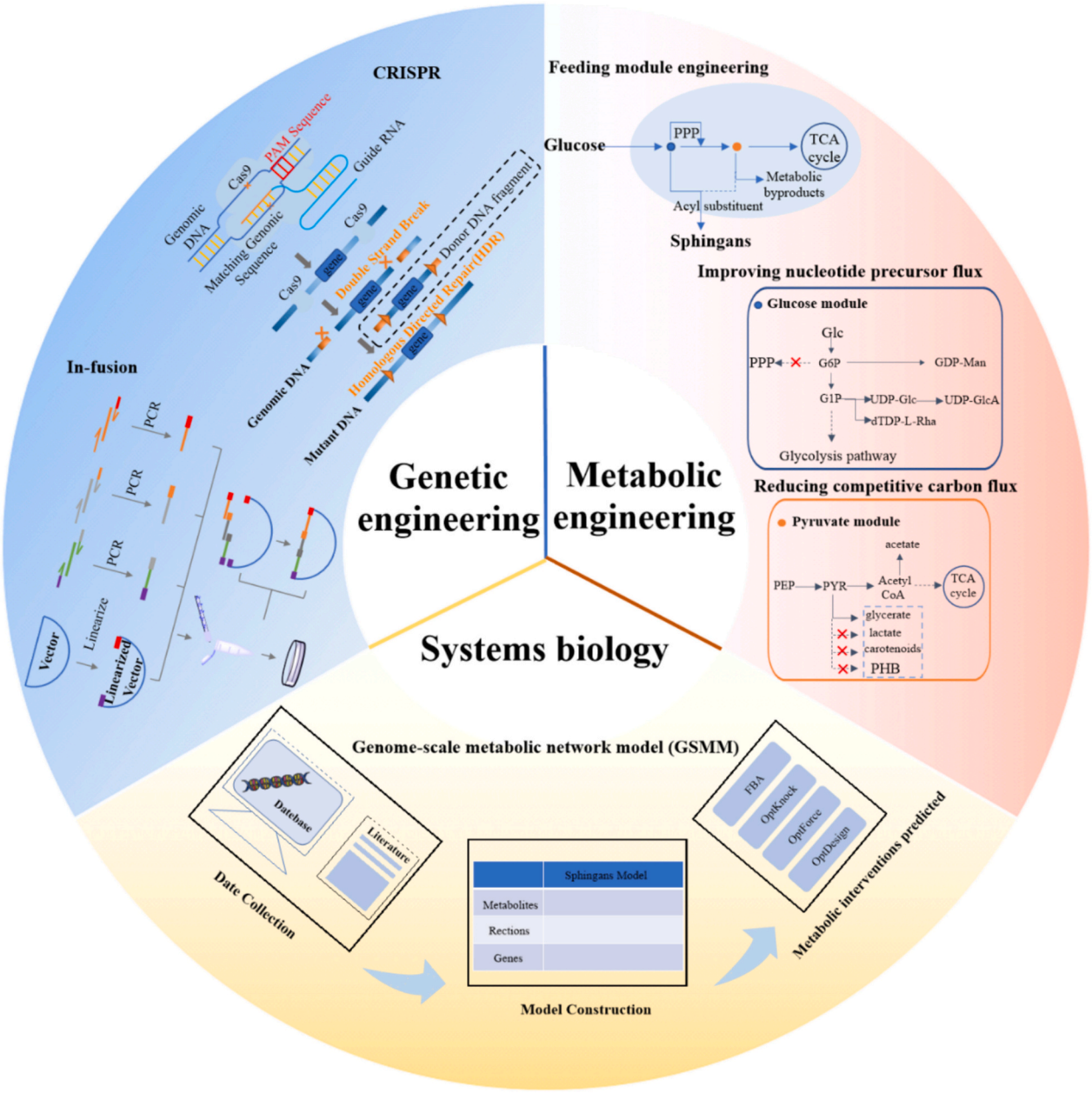


**Fig. 4.** Enhanced the yield of sphingans based on synthetic biology strategy. A combined approach that integrates genetic engineering, metabolic engineering, and systems biology is expected to optimize the conversion of carbon flux into sphingans. Firstly, by optimizing the efficient genetic tools in *Sphingomona*, such as in-fusion cloning and CRISPR, the efficiency of gene editing in strains can be improved. Secondly, based on metabolic engineering strategies, promoting sufficient supply of nucleotide linked sugars and increasing substrate conversion leads to an increase in the synthetic metabolic flow of sphingosine gum, and inhibiting competitive metabolic pathways reduces the expression of genes related to central metabolic pathways (glycolysis and TCA cycle) or product competition pathways. Constructing a genomic metabolic network model (GSMM) using genome sequence and annotation information, and simulating the metabolic process of organisms by reconstructing the metabolite-protein-reaction equation relationship, thereby improving the efficiency of sphingans synthesis.

microbial polysaccharide polymers may play a role in a bio-based future.

### 6.1. *Synthesis of sphingans with different molecular weight*

Polysaccharides and their corresponding oligosaccharides may exhibit different biological activities, among which the degree of polymerization is considered to be one of the most influential parameters on the functional properties. Sphingans are widely used in various fields of industrial production and daily life, and sphingans oligosaccharides also have various active functions, such as antibacterial, antiviral, prebiotic (Salachna et al., 2018; Xu et al., 2021). Therefore, the targeted synthesis of sphingans with varying molecular weight holds significant promise. Natural sphingans are polymers with molecular weights ranging from hundreds of thousands to millions daltons (Da). In order to obtain efficient sphingans with an appropriate molecular weight distribution, besides using common physicochemical methods and enzymatic method for degradation, it can regulate molecular weight during the polymerization process of polysaccharide units within the cell (as illustrated in Fig. 5).

In the process of polysaccharide biosynthesis, polysaccharide lyases may be involved in controlling the molecular weight of polysaccharide to regulate the viscosity of the medium. Duan et al. successfully expressed the gellan lyase gene *gelR* in *Pichia pastoris* for the first time and utilized the enzyme for gellan degradation to produce gellan

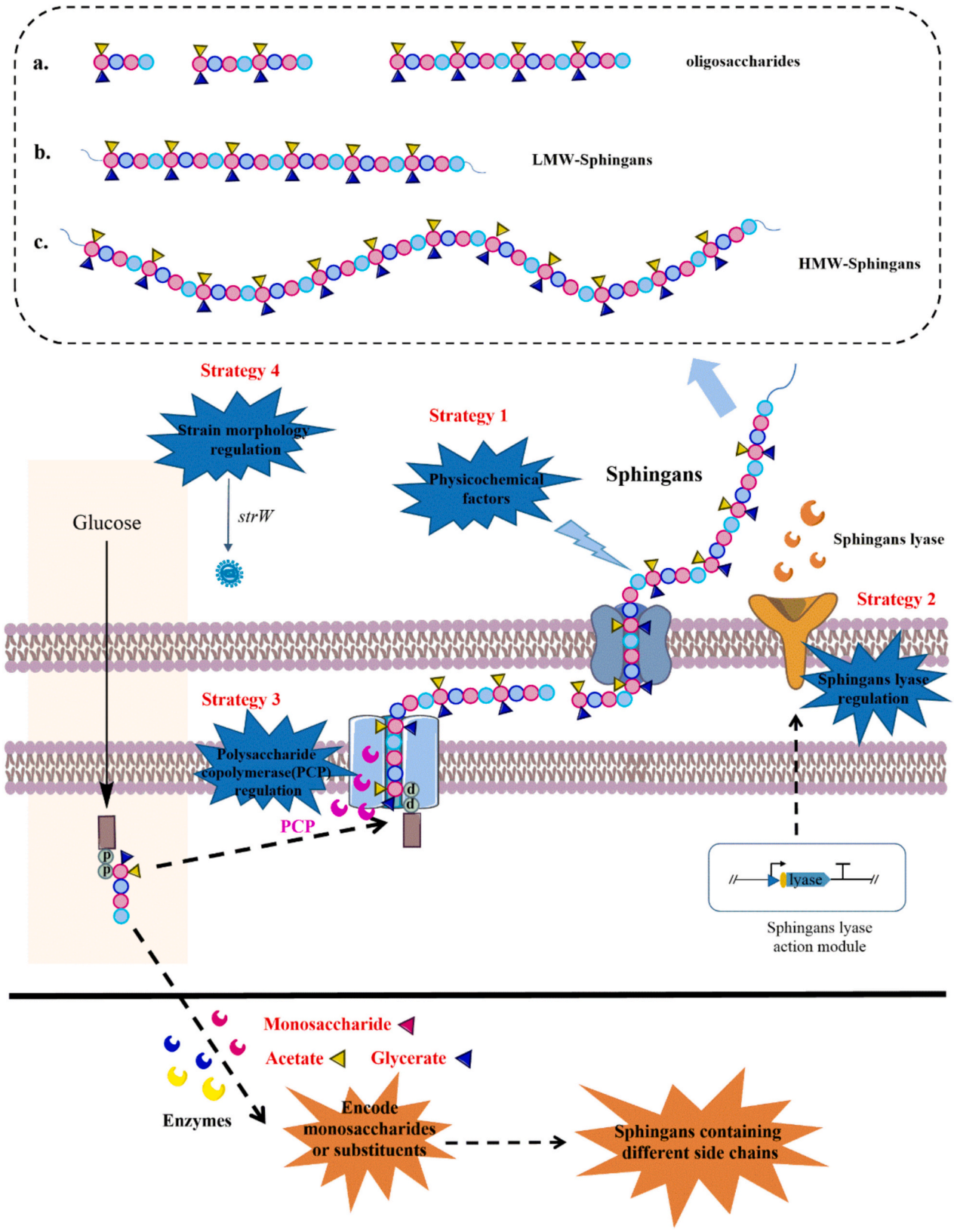


**Fig. 5.** Schematic diagram of currently available directional synthesis strategies for the regulation of the molecular weight and modification of sphingans. Sphingans with different molecular weight variants was expanded by four strategies. a. Use common physical and chemical methods for degradation; b. The molecular weight of sphingans was controlled by polysaccharide lyase of sphingans; c. Polysaccharide copolymerase (PCP) family proteins were used to regulate chain length; d. Sortase was used to change the capsule shape of Sphingomona and affect the molecular weight of sphingans. In addition, the acetyl and pyruvate groups of sphingans were modified to improve their physicochemical properties.

oligosaccharides with a polymerization degree of 4 (Duan et al., 2022). By employing lyase WelR as an effective tool, Li et al. established a strategy for controllable molecular weight degradation (Li, Li, et al., 2022). However, using enzymatic degradation of sphingans to produce oligosaccharides poses problems of prolonged processing times and high costs. Consequently, an increasing number of studies are focusing on sequencing the gene clusters involved in polysaccharide biosynthesis to identify key regulatory genes that determine the chain length of polysaccharides during polymerization (Nickerson et al., 2014). According to reports, the alginate polymerase Alg8 was overexpressed in *Azotobacter vinelandii*, which led to the production of alginate variants with high molecular weight (Díaz-Barrera et al., 2012). Nowadays, HA synthases have been considered to be the optimal enzyme to regulate the molecular weight of HA products (Qiu et al., 2021). EM Galvan et al. conducted relevant studies in *Xanthomonas campestris*. In the biosynthesis process of xanthan, the chain length is regulated by both the polysaccharide copolymerase (PCP) family protein GumC and the outer membrane polysaccharide export (OPX) family protein GumB, with the resulting chain length is positively correlated with the expression of GumC and GumB proteins (Galván et al., 2013). It is believed that the determination of the length of polysaccharide chains in gellan is controlled by the PCP protein family (Fialho et al., 2008; Tocilj et al., 2008). The PCP protein effectively determines the length of the intermediate polymer linked to the lipid carrier Und-PP. Besides the membrane or periplasmic domains, the protein structure of PCP also contains a cytoplasmic domain of protein tyrosine kinase (Whitfield, 2006). Previous studies have shown that tyrosine kinases played a crucial role in determining the chain length of polysaccharides in certain gram-positive bacteria. In *Sphingomonas*, the activation domain and kinase domain of tyrosine kinase are encoded by the *gelC* and *gelE* genes, respectively. Therefore, *gelC* and *gelE* genes are presumed to be participated in the regulation of gellan chain length. By regulating the gene that encodes polysaccharide polymerase, sphingans with different molecular weight may be obtained. Additionally, Zhao et al. altered the capsule morphology of *Sphingomonas* strains by knocking out the sortase coding gene (*srtW*), resulting in the production of welan with a significant reduction in molecular weight (approximately 68 kDa) (Zhao et al., 2021). This research provides valuable references for the application of low molecular weight welan and may be applicable to the production of other sphingans.

### 6.2. Synthesis of sphingans with different substituent modifications

The structural characteristics of polysaccharides, such as composition and spatial conformation, can be changed by adjusting the modification groups of polysaccharide polymer, thereby enhancing its properties for various applications. To regulate polysaccharide polymers, the polysaccharide structure can be modified by varying the proportion of substituents or monomeric sugars in the side chains. Xanthan is one of the most extensively studied biopolymers and offers valuable insights for sphingans. Schmid et al. obtained a modified strain of xanthan with excellent rheological properties by modifying the genes related to pyruvylation and acetylation identified in xanthan production (Gansbiller et al., 2019). Liang et al. constructed a recombinant strain with acetyl transferase deletion by knocking out *nat* gene, and the acetyl group content in the gellan synthesized decreased by 32.79% (Liang, Liu, et al., 2023). Therefore, we can consider modifying the acetyl and pyruvate groups of sphingans to enhance their apparent viscosity, gel strength and structural stability. Salecan is a soluble glucan produced by *Agrobacterium* sp. ZX09. Knocking out the *sleA* gene resulted in the loss of succinyl substituents on salecan, which altered its rheology, particularly decreasing its intrinsic viscosity (Xu et al., 2017). Currently, the gene encoding acetyl transferase in gellan has been identified, allowing us to obtain gellan with varying acetyl groups by regulating the expression of this gene (Schmid et al., 2014). Unfortunately, the gene encoding glyceryl transferase remains unknown. We propose the idea of customizing different sphingan modifications based on the known synthetic gene cluster of sphingans; however, no studies have been published to confirm this hypothesis. Today, significant advances have been obtained in genome sequencing, functional genomics, computer simulation analysis, and gene editing technology, providing a solid technical foundation for the modified synthesis of sphingans.

## 7. Application prospect of sphingans

Sphingans are promising extracellular polysaccharides (EPS) produced by natural microorganisms, characterized by their non-toxic and harmless nature. Due to their superior gel properties and unique physical and chemical characteristics, sphingans can be utilized as gels, stabilizers, thickeners, and film-forming agents. They have a wide range of applications in the food, medical, chemical, and other industrial fields (see Table 3). Currently, the most successful company in the commercial development and large-scale production of sphingans is CP Kelco, based in the United States. Gellan and welan are representative products of sphingans, notable for their large production scale and extensive application range. The application scope of diutan, rhamsan and sanxan was followed. In addition, the current application of S-88 and S-7 are relatively few, and it is believed that their future use may be gradually

**Table 3**
Overview of gene clusters involved in sphingans biosynthesis.

| Application area | Major product category | Typical product/ formulation | Reference |
|---|---|---|---|
| Foods | Confectionaries<br>Jams and jellies<br>Water-based gels<br>Meat<br>Icings and frostings<br>Dairy products<br>Beverages<br>Food film or food coating | Fillings, marshmallow<br>Reduced calorie jams, jellies<br>Dessert gels, aspics, canned puddings<br>Minced fish, fat substitute<br>Bakery icings, canned frostings<br>Ice cream, yogurt, milkshakes<br>Fruit, milk-based, carbonated drink<br>Batters, fibrous casing, coatings | (Li, Guo, et al., 2021)<br>(Cong et al., 2004; Petcharat & Benjakul, 2018)<br>(Cong et al., 2004; Liu et al., 2009) |
| Medical and beauty | Tablets<br>Capsules<br>Filler<br>Films/coatings<br>Nano fibers<br>Tissue engineering<br>Medical beauty material | Sustain release tablets of gastric acid<br>Prolonged release GG-alginate microspheres<br>Dressing for postoperative wound repair<br>Corneal tissue<br>Sustain release delivery<br>Oral colonization, cartilage tissue engineering<br>Facial mask, injectable biomaterial | (Chang et al., 2022)<br>(Lee et al., 2010)<br>(Ribeiro et al., 2020; Seo et al., 2023)<br>(Busto et al., 2023; de Paula et al., 2007) |
| Chemical | Adhesive<br>Cleaning agent<br>Film<br>Rheological regulator | Toothpaste, body wash, hair spray, deodorant, cosmetics<br>Paper cleaning hydrogels<br>Waterproof coating<br>Ink manufacturing | (Zhang et al., 2017)<br>(Kaur et al., 2014)<br>(Ji et al., 2011) |
| Petroleum | Oil drilling fluid | Displacing agent, drilling additive | (Kaur et al., 2014) |
| Microbial | Culture media<br>Micro-bioreactors/ cell immobilization | Plant tissue culture, microbial plating<br>Bioethanol production, bioremediation of coking wastewater | (Shi et al., 2014) |
| Agricultural | Agricultural production supplies | Insecticide, fertilizer, slow-release fertilizer | (Wang et al., 2023; Xu et al., 2020) |

popularized with the deepening of research.

### 7.1. Food industry

Sphingans provide the desired texture, rheological properties, appearance, and moisturizing characteristics required in contemporary food products. In beverage applications, gellan serves as the primary suspension agent. Its incorporation into suspended beverages containing fruit particles and acidic milk drinks ensures that the fruit is evenly distributed and remains suspended stably, thereby maintaining the product's stability throughout its shelf life (Li, Li, et al., 2021). Welan exhibited excellent thickening properties and was unaffected by pH and temperature, allowing the pulp to retain its superior taste over an extended period, making it a highly promising additive in the juice and beverage industry (Kaur et al., 2014). Incorporating welan into cream and acidic dairy products helped maintain optimal water retention during the freeze-thaw cycles of cream, prevented protein flocculation in dairy products, and resulted in a solid product structure with a delicate and smooth taste (Cong et al., 2004). Liu et al. developed an ideal ice cream formula by adjusting the ratio of gellan, taking advantage of its ability to bind $Ca^{2+}$ more effectively (Liu et al., 2009). For meat products, gellan could be used as a thickening agent, significantly enhancing the gel strength and whiteness of minced fish (Petcharat & Benjakul, 2018). Furthermore, to meet consumers' increasing demand for high-quality and safe food, the global market demand for natural foods is steadily growing. Numerous studies have demonstrated that the performance of sphingans-based edible films could be enhanced by incorporating functional ingredients into the sphingans matrix, such as plasticizers (which improve performance) and emulsifiers (which increase stability and adhesion). Fonseca employed microfluidic technology to produce nanocomposites using hydrolyzed chitosan and gellan for improved caffeine encapsulation, showcasing significant potential for food and drug packaging applications (Fonseca et al., 2022). Due to its high viscosity, water solubility, and stable structure, welan could be combined with konjac gum to create a composite film that was safe, nontoxic, edible, and biodegradable, thereby reducing environmental pollution from food packaging (Wu et al., 2016). Various hydrogel systems have been developed using gellan or its derivatives, either alone or in combination with other substances, to address numerous challenges related to the delivery and stability of food and nutritional compounds. Oxidized gellan hydrogel exhibited higher loading and encapsulation efficiency for resveratrol, and the release of resveratrol could be regulated by adjusting calcium ion concentrations and oxidation levels (Wang, Fan, et al., 2022).

In addition, diutan, rhamsan, and sanxan have also been approved as food additives, and their use in the food industry is gradually gaining popularity. Phycocyanin was utilized in combination with diutan to prepare emulgels formulated with avocado oil (Tello et al., 2024). Due to its environmentally friendly and sustainable properties, the phycocyanin-diutan complex has emerged as a promising alternative for stabilizing dispersion systems in the food industry. Currently, sanxan is primarily used as a food thickening agent, stabilizer, and coagulant. Sanxan was an effective additive that enhanced the quality of salt-free noodles (SFNs), serving as a substitute for salt in certain characteristics (Liang, Zhang, et al., 2023). Furthermore, a type of edible emulsion gel with sanxan as a stabilizer has been studied and developed (Shi et al., 2020). At present, emulsions with enhanced physical stability can be obtained by combining sweet fennel oil with biological macromolecule rhamsan, so rhamsan can be developed as a stabilizing emulsion and latex for the food industry (Báez et al., 2019). In addition, sphingans such as S-88 and S-7 are relatively few in the food industry, and it is believed that their use in the food industry in the future may be gradually popularized with the deepening of research.

### 7.2. Medical and beauty industries

Sphingans are promising biological material, and it is widely used in the medical field. It is mainly divided into the following four categories. (1) Applied to tissue engineering as a hydrogel. Seo et al. prepared methyl acylated gellan gel by mixing methacrylic acid with gellan to give corneal tissue the appropriate mechanical properties and it can be used as a promising cell carrier in corneal tissue engineering (Seo et al., 2023). (2) As a carrier system. Camargo Ribeiro et al. found that gellan was a promising biomaterial that could be used as a carrier system to promote oral colonization of probiotics and prevent oral candidiasis (Ribeiro et al., 2020). (3) Controlled drug release. Gel carriers of natural polysaccharides have been extensively studied for their potential to provide slow and controlled release. Gellan could be used as a general encapsulation agent and active raw material to control drug release at many diseased sites (Ding et al., 2020). Welan could be used for sustained release control of drugs by forming a viscous film on the surface of the drug to achieve sustained release (Chang et al., 2022). The crosslinked gel prepared by sanxan-$Ca^{2+}$ also has slow-release properties (Lu, Li, et al., 2023). (4) Film materials used as wound dressings. Lee et al. used a thin film material made of gellan as a dressing for postoperative wound repair (Lee et al., 2010). Compared with other wound dressings, the gellan membrane produces more collagen and has a better effect on hemostasis and repair. The combination of sanxan, bacterial cellulose and usnic acid has been designed to create a hydrogel film with accelerated wound healing and enhanced bactericidal efficacy (Zhao et al., 2024). (5) As a medical beauty material. The hydrogel blended with low-acyl gellan and sodium alginate might be used as a facial mask as an innovative treatment against photoinduced skin aging (Busto et al., 2023). As a good anionic water-soluble EPS, rhamsan could be used as an injectable biomaterial for plastic reconstruction in plastic surgery (de Paula et al., 2007). Sphingans provide an in-depth analysis of the latest discoveries and advances in these fields, making it a valuable resource for researchers and professionals in these fields.

### 7.3. Chemical industry

The application of sphingans in the chemical industry mainly revolves around daily necessities and cosmetics. Gellan can be added in toothpaste production as a gel, as well as in body wash, hairspray, deodorant and cosmetics to provide proper consistency, good stability and excellent lightness. Zhang et al. found that mixing sodium alginate with gellan improved the water resistance of paper cup film, which could be used as a waterproof coating for hot drink paper cups (Zhang et al., 2017). Welan can be used in the manufacturing process of shampoo, cosmetics, etc. Welan could improve the fluidity of shampoo, suspend active particles and beneficial substances that were easy to precipitate, stabilize foam, and improv the washing ability of shampoo (Kaur et al., 2014). Welan could also be used to manufacture ink. In a stationary state, welan could act as a thickener and has strong thixotropy to ensure the effectiveness of ink use (Ji et al., 2011).

### 7.4. Petroleum industry

At present, many oilfields face the problem of high-water content and low recovery rate after 30–40 years of mining, which has increased the production cost and difficulty. Currently, by applying biotechnology, many high-performance biopolymers have been discovered and applied in the development of petroleum industry. Compared with xanthan, the excellent viscosity and temperature resistance of welan made it promising as an oil displacement agent and new type of drilling fluid additive, and its strong viscoelasticity could be applied in tertiary oil recovery (Kaur et al., 2014). Moreover, the gellan agent and gellan-xanthan mixture induced by brine could be used as plugging agents for high permeability channels in oil reservoirs and as a shut-off agent in polymer flooding technology (Nurakhmetova Z., 2018). Due to its

excellent physical and chemical properties, diutan had the potential for reservoir oil displacement applications under extreme reservoir conditions (high salt and temperature, strong acid and alkali), and could be used as a biopolymer oil displacement agent under extreme reservoir conditions (Gao et al., 2023).

### 7.5. Microbial industry

Studies have proved that gellan is a gel with better performance and it can replace agar in the process of microbial culture. In addition, gellan also exhibits good bioremediation activity. Shi et al. chose gellan as the optimal immobilization carrier, and the results showed that the toxicity of magnetically immobilized cells containing activated zeolite in treating coking wastewater was 128% lower than that of untreated wastewater (Shi et al., 2014).

### 7.6. Agricultural industry

In agriculture, gellan could be used as an insecticide, leaf fertilizer, slow-release fertilizer, etc. for agricultural production (Wang et al., 2023). Research has found that welan could promote nitrogen absorption and metabolism in rice seedlings, thereby increasing their biomass (Xu et al., 2020). This indicated that welan might become a promising fertilizer in the future. Furthermore, sanxan is an ideal carrier for managing water and urea. By adding urea to sanxan, a new effective sanxan fertilizer was produced, which had the ability to retain water and slowly release nutrients (Lu, Zhang, et al., 2023).

## 8. Outlook and perspectives

With the continuous expansion of application fields and the increasing demand from consumers, the commercial potential for the safe and efficient production of sphingans remains enormous. In recent decades, research and development in sphingans have made significant progress, with gellan and welan being the most successfully developed types. This paper first introduces the chemical structures and biosynthetic pathways of sphingans, primarily focusing on gellan and welan. It then analyzes the feasibility of obtaining dominant strains of sphingans through classical mutagenesis combined with high-throughput screening and the construction of chassis cells. Additionally, it elaborates on the efficient synthesis and customization of sphingans, emphasizing metabolic engineering and synthetic biology strategies to propose promising methods for enhancing sphingan production and optimizing performance.

The economic value generated by microbial polysaccharides largely depends on the cost of precursor substrates, the productivity of the host strain, the processing costs of both upstream and downstream operations, and the application value of the polysaccharides. The rapid advancement of synthetic biology and metabolic engineering has established a solid foundation for the design and construction of sphingan-producing strains; however, several challenges remain. For instance, *Sphingomonas* lacks efficient genetic modification methods and encounters difficulties in preserving and maintaining strain stability. Furthermore, some chassis cells with significant production potential, such as *Escherichia coli* and *Bacillus subtilis*, have rarely been reported for sphingan production. With ongoing advancements in genome integration and modification technologies, there is potential to transform sphingan-producing strains by exploring new modification techniques, including de novo synthesis of metabolic pathways, conversion of existing metabolic pathways, and the synthesis of sphingans with specific structures. These efforts aim to facilitate the industrialization and broader application of sphingans. Consequently, metabolic engineering and synthetic biology strategies are expected to receive increasing attention in the biological production of sphingans.

Currently, there is limited research on sphingans, aside from gellan and welan. The following strategies have been proposed to enhance the research and development of sphingans: (1) Expand the diversity of *Sphingomonas* species to discover novel sphingan polymers. (2) Conduct a systematic investigation of the biosynthesis pathway of sphingans, clarifying the key regulatory steps and identifying metabolic bottlenecks in polymer synthesis at the molecular level. (3) Modify the biosynthesis pathway of sphingans through genetic engineering, which includes blocking competing pathways and enhancing the activity of pathway enzymes to promote sphingan biosynthesis. (4) Optimize the downstream extraction process of sphingans, such as using lysozyme and alkaline protease treat sphingans to reduce the use of organic solvents, and adding electrolytes or polymer flocculants could cause cell and colloid aggregation in the broth, and ultimately obtaining clear products free of cell and protein impurities in an efficient and environmentally friendly manner. (5) Increase the production of sphingans with specific molecular weight distributions; certain molecular weight regulation strategies, such as adjusting precursor ratios, regulating polymerase activity, and employing a combination of enzymatic methods, merit further consideration. (6) Enhance the properties of sphingans through chemical modification and explore new medical applications, including tissue engineering.

## CRediT authorship contribution statement

**Yujia Zhou:** Writing – original draft, Visualization, Conceptualization. **Jielun Hu:** Supervision. **Yadong Zhong:** Writing – review & editing. **Shaoping Nie:** Project administration, Funding acquisition.

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## Declaration of competing interest

The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

## Data availability

No data was used for the research described in the article.

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