Title: The simultaneous production of sphingan Ss and poly(R-3-hydroxybutyrate)
in *Sphingomonas sanxanigenens* NX02

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Abstract

Sphingans and poly(R-3-hydroxybutyrate) (PHB) are both widely used biopolymers produced by bacteria. In the batch fermentation of *Sphingomonas sanxanigenens* NX02 in a 5 L fermenter using glucose as carbon source, ivory colored sphingan Ss production was a growth-associated process with a maximum purified production of 14.88 ± 0.83 g/L, while 6.08 ± 0.23 g/L PHB was simultaneously produced. Sphingan Ss and PHB were separated by a simple dilution, heating and centrifugation or filtration process, and sphingan Ss can be cost-effectively extracted using a small amount of acid rather than multi-fold volumes of alcohols. From ultrathin sections of *S. sanxanigenens* NX02, we found that the interior space of the cells was filled with PHB granules, and the outside was surrounded by abundant Ss. The purified sphingan Ss can be used as an excellent gelling and emulsifying agent in biotechnology applications such as food, personal care and production processes. Proposed pathways of Ss and PHB biosynthesis from glucose are also presented.

Keywords: Simultaneous production; Sphingan Ss; poly(R-3-hydroxybutyrate).

1. Introduction

Bacteria of the *Sphingomonas* genus have been widely used in decomposition of polycyclic aromatic hydrocarbons [1], in production of β -carotene [2] and acidic capsular polysaccharide [3–5]. A number of bacteria of this genus produce polysaccharides called sphingans, which include gellan, welan, S-88, rhamsan and diutan [3, 4, 6]. Sphingans share similar tetrasaccharide backbone structures but have divergent side chains. They are always extracted from fermentation broth with 3-fold volumes of ice-cold ethanol or isopropanol. Because of their excellent properties as a stabilizing, suspending, gelling and thickening agent [3,7], sphingans have been used in a wide range of biotechnological applications including in food, oilfields, pharmaceuticals, personal care, and production processes. Gellan gum, as one of the most extensively studied sphingans, is currently described as a potent multifunctional additive for various pharmaceutical products. Due to the unique structure and specific gelling properties in different media, controlled release forms based on gellan, such as various pharmaceutical solid, semi-solid and liquid formulations, have been developed [8–11].

A kind of ivory colored extracellular polymer called sphingan Ss, composed of carbohydrate, lipid and protein, is synthesized by *Sphingomonas sanxanigenens* NX02 throughout its life cycle [12, 13]. The carbohydrate of Ss has a linear tetrasaccharide repeat unit like other sphingans, consisting of glucose, glucuronic acid,

rhamnose and mannose [13]. Structural details of the tetrasaccharide repeat unit have not yet been published. The protein of sphingan Ss contains 16 types of amino acid, of which the greatest in number are the acidic amino acids Asp and Glu [13]. This may be one of the reasons sphingan Ss can be precipitated when the pH of strain NX02 fermentation broth is adjusted to approximately 3.0. In contrast to other sphingans, this acid precipitation method significantly reduces the cost of production. Sphingan Ss has been used in China for years as a drilling mud and as a thickening agent in the recovery of petroleum by water flooding.

Poly(R-3-hydroxybutyrate) (PHB), an intracellular microbial thermoplastic, is a type of storage compound for carbon and energy produced by many bacteria and some archaea [14]. This biologically produced polyester has received much attention since it is biodegradable, biocompatible and used widely [15]. Numerous strains can produce PHB, including *Alcaligenes eutrophus* H16 [16] and *Bacillus megaterium* SRKP-3 [17]. In the *Sphingomonas* genus, PHB has been an undesirable side-product during gellan and diutan production by *S. elodea* ATCC31461 [18] and *Sphingomonas* sp. ATCC53159 [19] because of its low yield and negative influence on sphingans. In fact, in addition to impairing clarity, the presence of PHB affects the rheological properties of the gums. Therefore, PHB-deficient strains are used to produce clarified sphingans, especially the native or high-acyl gellan (elastic gel), while in the fermentation of *S. elodea* ATCC31461 wild-type strain, only deacylated gellan (brittle

gel) can be clarified [20]. In industrial production, if a large amount of PHB accumulates and the properties of sphingan are not changed after a simple separation, low-cost extraction process, the simultaneous production of two products will be an advantageous fermentation mode.

In this paper, the efficient simultaneous production of extracellular sphingan Ss and intracellular PHB was achieved in *S. sanxanigenens* NX02. The separation and extraction procedure was simple and cost effective. The fermentation kinetics of strain NX02 was studied through batch fermentation in a 5 L fermenter. In addition, the rheological, gel-forming and emulsifying properties of sphingan Ss were analyzed. To date, this is the first report on the simultaneous production of sphingan and PHB in *Sphingomonas* genus. In addition, strain NX02 accumulated the highest amount of PHB yet observed for the *Sphingomonas* genus.

2. Materials and methods

2.1 Culture conditions and materials

S. sanxanigenens NX02 was cultured at 30°C for 24 h on NK medium containing (per liter): glucose 15.0 g, peptone 5.0 g, beef powder 3.0 g, yeast extract, 1.0 g, and agar 15.0 g (pH 7.0). Ten percent (v/v) NK medium was inoculated into the fermentation medium consisting of (per liter): glucose 40.0 g, yeast extract 0.2 g, K₂HPO₄ 1.2 g, NaNO₃ 2.0 g, CaCO₃ 1.0 g, FeSO₄ 0.005 g, NaCl 0.4 g, and MgSO₄ 0.5 g (pH 7.5)

[21]. Peptone, beef powder, yeast extract, agar and other chemicals were purchased from Dingguo Limited (Tianjin, China). Gellan was extracted from the fermentation broth of *S. elodea* ATCC31461 as previously described [7].

2.2 The batch fermentation of S. sanxanigenens NX02

The simultaneous production of sphingan Ss and PHB was studied in a 5 L autoclavable fermenter (Shanghai Bailun Bio-technology Co., Ltd., Shanghai, China) containing 3.5 L medium. The temperature and pH were maintained at 30°C and 7.0, respectively. The dissolved oxygen concentration was set at 50% air saturation by adjusting the agitation speed and aeration rate.

2.3 Separation and extraction of sphingan Ss and PHB after fermentation

To separate sphingan Ss and PHB, the fermentation broth was diluted at least 5-fold with deionized water, then heated at 90–100°C for 10 min, and centrifuged at 12 000 × g for 20 min or filtered to separate the supernatant and cell pellet. Sphingan Ss was dissolved in the supernatant aqueous solution, while a mass of PHB existed in cell fragments. Transparent supernatant was collected, and then the pH of the supernatant was adjusted to 3.0 to precipitate sphingan Ss. Precipitate was dialyzed against deionized water until neutrality. Finally the sphingan Ss was lyophilized and the yield of purified Ss was obtained by weighing. The PHB was extracted with chloroform as previously described [22].

2.4 Analysis of fermentation broth

The viscosity of fermentation broth was measured with a Brookfield viscometer DV_II+ (USA) equipped with a no. 64 spindle at a shear rate of 60 rev/min. A SBA-40C Biosensor equipped with a glucose oxidase electrode (Shangdong Academy of Sciences, China) was used to determine the concentration of glucose in fermentation broth. To measure cell dry weight (CDW), after separation from the supernatant, the cell pellet was washed out of the centrifuge tube into a preweighed centrifuge tube and dried to a constant mass at 60°C. The total precipitable material (TPM) was the yield of fermentation broth after acid precipitation, which contained bacteria, sphingans and culture components.

2.5 Qualitative and quantitative analysis of PHB

PHB was characterized by ^1H -NMR and ^{13}C -NMR with a Bruker AV400 NMR spectrometer. To determine the concentration of PHB, samples were prepared according to Braunegg et al. [23]. The resulting methyl esters of PHB monomers were quantified by gas chromatography (Agilent 6820 equipped with a HP-PONA capillary column and a FID detector, USA) with benzoic acid as the internal standard.

2.6 Analysis of purified sphingans

The purity of sphingan Ss was analyzed by gel permeation chromatography (GPC) using an Shimadzu Prominence LC-20A instrument equipped with a differential refractive index detector and an PL Aquagel-OH MIXED 8 μm column (300 mm $\times$ 7.5 mm). The eluent was HPLC-grade water with a flow rate of 0.8 ml/min. The column temperature was 36°C [24]. The purity was defined as the ratio of peak area of sphingan Ss to total area.

2.6.1 Rheology analysis of sphingan Ss solutions

Sphingan Ss solutions (0.05%–0.25% w/v) were prepared by dissolving purified sphingan Ss in deionized water. The viscosity of the sphingan Ss solutions was measured with a Brookfield viscometer DV_II+ (USA) equipped with a no. 63 spindle at a shear rate of 2–60 rev/min.

2.6.2 Gel-forming properties of purified sphingan Ss.

Sphingan Ss solutions (0.2%–1.2% w/v) were prepared by dissolving sphingan Ss in deionized water under magnetic stirring followed by heating to 80–121°C for 15 min. Sphingan Ss gelation was induced after cooling to ambient temperature without addition of any chemical compounds. Then seven textural parameters (hardness, springiness, cohesiveness, adhesiveness, gumminess, chewiness and resilience) were measured by a Texture Analyzer TA.XT Plus (Stable Micro Systems Ltd., Surrey, United Kingdom) with a cylindrical probe (p/0.5) in the following conditions: data

acquisition rate, 200 pps; pre-test speed, 2.00 mm/s; test speed, 1.00 mm/s; post-test speed, 1.00 mm/s; strain, 75%; trigger type, Auto-4g.

2.6.3 Emulsifying properties of purified sphingan Ss

Volumes of 1.0 mL of each sphingan Ss solution and 1.0 mL diesel oil (-10#, China National Petroleum Corporation) or corn oil (COFCO Limited, China) were mixed together in cylindrical glass vials. Oil-in-water emulsions were then prepared by maximum-vortexing for 2 min [25]. The emulsification index was defined as the ratio of the height of an emulsion layer to the total height of mixed liquid after standing for 24 h.

2.7 Electron microscopy

Different growth phase cells (2 h, 24 h, 48 h and 72 h of growth) were prepared for electron microscopy. After chemical fixation and dehydration, the samples were plastic embedded in Epon-Araldite, sectioned on a Leica EM UC7 microtome, stained with uranyl acetate and lead citrate [26], and examined in a Hitachi HT7700 transmission electron microscope (TEM; Hitachi, Tokyo, Japan).

2.8 Nucleotide sequence accession number

The complete genome sequence of *S. sanxanigenens* NX02 is deposited at GenBank under the accession no. CP006644.

3. Results and discussion

3.1 Co-production of sphingan Ss and PHB in *S. sanxanigenens* NX02

The optimal fermentation conditions for producing sphingan were produced by a nutrient imbalance in the C/N ratio [3]. Considerable amounts of PHB accumulate in the same growth conditions. Both PHB and sphingan were produced from glucose as the carbon source. The simultaneous production indices in different growth periods, the yields of purified Ss and PHB, the concentration of remnant glucose, the viscosity of the fermentation broth, the TPM, and the CDW are shown in Fig. 1.

{Embed Fig. 1}

Sphingan Ss as a primary metabolite was produced with the growth of bacteria. Before the logarithmic growth phase (0–12 h), small amounts of sphingan Ss accumulated, but very little PHB was obtained. Large amounts of sphingan Ss and PHB were produced during the logarithmic growth phase. At the end of batch fermentation (68–72 h), CDW and TPM reached their maximum levels of 9.61 ± 0.14 g/L and 30.35 ± 0.24 g/L, respectively, and strain NX02 effectively produced purified sphingan Ss (14.88 ± 0.83 g/L) and PHB at 63.27 ± 2.4 % of the CDW (PHB yield was 6.08 ± 0.23 g/L). The viscosity of the fermentation broth increased to a maximum 6.52 ± 0.38 Pa s. The remnant glucose reduced to 0 g/L, from an initial 40 g/L, at the

end of batch fermentation. Thereafter, a slight drop in PHB yield was due to use of PHB as a carbon source in energy hungry culture conditions.

PHB production is limited by the bacteria count, which is largely depended on the dissolved oxygen concentration, nutritional utilization (carbon, nitrogen or phosphorus source) and other culture conditions [22]. Efforts to obtain higher PHB production are focused on increasing cell concentration. For example, in *A. eutrophus* species, the PHB production is increased from 13 g/L in batch fermentation [27] to 121 g/L in high cell density fed-batch culture with glucose concentration control [28], and 232 g/L in phosphate limitation cultures [29]. However, in the fermentation of *S. sanxanigenens*, the accumulation of sphingan Ss dramatically reduced the dissolved oxygen concentration of fermentation broth, and the cell density was difficult to be increased in the co-fermentation process. Under this condition, PHB content reached to 63.27 ± 2.4 % of the CDW. In theory, increased PHB production will be obtained in sphingan Ss-deficient strains because of the increased dissolved oxygen concentration and the higher transforming rate of PHB synthetic related substrates.

The TPM production of sphingan Ss (30.35 ± 0.24 g/L) was much higher than gellan or diutan, which were 15.2 g/L and 20.7 g/L, respectively [19, 20]. What's more, *S. sanxanigenens* produced higher yield of purified sphingan Ss. Jin et al. [30] reported that the highest production of purified gellan gum with 2.0% (w/v) glucose and 2.0%

soybean pomace was 7.50 g/L in a 7 l bioreactor. But there has been no report on the purified diutan.

In the patents for strains ATCC 31461 and ATCC 53159 [18–20], PHB-deficient strains were generated by a single mutation in the PHB synthesis pathway in order to produce clarified sphingans, but the production of sphingans decreased in these strains. This may be due to the accumulation of PHB synthesis metabolic intermediates (e.g., organic acids) that has an adverse effect on sphingan synthesis [20]. The PHB-deficient strain LPG-2 (a chemical mutant strain) and strain NPG-1 (a *phaC* gene deletion strain) yielded 14.2 g/L and 7.2 g/L crude gellan, respectively, while the wild-type strain *S. elodea* ATCC31461 produced 15.2 g/L crude gellan [18, 20]. Compared to the crude diutan yield from wild-type *Sphingomonas* sp. ATCC53159 (17.5 g/L), the PHB-deficient strains PDD3 and PDD6 (with uncharacterized spontaneous mutations in *Sphingomonas* sp. ATCC53159) showed lower diutan production (11.4 and 9.4 g/L, respectively). However, the production of crude diutan from wild-type *S. sp* ATCC53159, PDD3, PDD6 increased to 20.7, 21.7 and 17.3 g/L when plasmid pS8 (26kb, *dpsSGRQIKLJFDCEMatrDBdpsBrmlACBD* in pBluescript IIKS+, which overexpressed enzymes related to diutan biosynthesis and gave modest improvement in diutan productivity in wild-type strain) was transferred to each strain [19]. The highest PHB yield, 3.69 g/L, was obtained from the wild-type gellan producing strain *S. elodea* ATCC31461[18], while 0.71–0.87 g/L PHB was

produced from diutan produced strain *S. sp.* ATCC53159 (pS8) [19]. These two strains produced less PHB than *S. sanxanigenens*. The production and comparison of sphingan Ss, gellan and diutan are shown in table 1.

{Embed Table 1 }

Simultaneous production of PHB and gellan or diutan was not cost-effective due to the lower yields of PHB and sphingans and the high cost ethanol extraction process. In addition, the textures of gellan gel depend on the acyl group content, and in the downstream processing of wild-type *S. elodea* ATCC31461, only deacylated gellan can be clarified [20]. However, in the present work, the textures of sphingan Ss gels were not influenced by the separation process (Table 1).

S. sanxanigenens NX02 was an excellent strain for producing sphingan Ss and PHB. These two products can be separated by a simple dilution, heating and centrifugation or filtration process. To obtain the purified sphingan Ss (the purity was over 98%), two important steps were that the fermentation broth should be diluted at least 5-fold with deionized water, and heated at 90–100 °C for at least 10 min. This heating temperature was optimal because sphingan Ss solution showed heat resistance between 20–70 °C, and the viscosity of sphingan Ss solution was significantly decreased when the temperature was up to 90–100 °C [31]. On the other hand, it will

be more difficult to separate supernatant and precipitation when the dilution ratio is smaller. Also the purity of sphingan Ss will be decreased. For example, the purity of sphingan Ss decreased to 89% when the fermentation broth was diluted 3-fold with water, while the purity of sphingan Ss would be over 98% if the dilution ratio was greater than 5-fold. Compared to other sphingans, Ss was composed of carbohydrate, lipid and protein [13], it can be precipitated with acid because of the abundant acidic amino acids. What's more, sphingan Ss is an ivory color sphingan without containing β -carotene or nostoxanthin, which is different from gellan or other sphingans [32], so any organic solvent will not to be required to extract or wash sphingan Ss.

S. sanxanigenens NX02 can simultaneously accumulate a mass of sphingan Ss and PHB in the same culture conditions, which will be more profitable than the separate fermentation of Ss or PHB. In addition, the easy and cost-efficient isolation and acid extraction process will be of tremendous benefit in the application of simultaneous production of PHB and sphingan Ss. Although less or similar amounts of clarified sphingan Ss may be produced by PHB-deficient strains, simultaneous production can accumulate clearer sphingan Ss and additional PHB after a low cost separation process.

3.2 The observation of sphingan Ss and PHB

The growth dynamic of extracellular sphingan Ss and intracellular PHB in different

growth phases (2 h, 24 h, 48 h and 72 h) is shown in Fig. 2 a, b, c and d, respectively. Sphingian Ss was a primary metabolite and fibrous around the cell through the whole growth cycle. With the growth of bacteria, the amount of sphingian Ss significantly increased. PHB granules were white in TEM photographs (Fig. 2 b, c and d) and PHB was a secondary metabolite. PHB production began in the logarithmic growth phase of *S. sanxanigenens* NX02. The PHB granules became larger and their number reduced due to coalescence during the growth of bacteria. At the end of batch fermentation, a bacterial cell was tightly packed with PHB granules, which accounted for more than 80% of cellular space, while the outside space was surrounded by large amount of polymer sphingian Ss (Fig. 2d).

{Embed Fig. 2}

3.3 NMR analysis of PHB from *S. sanxanigenens* NX02

^1H -NMR and ^{13}C -NMR spectroscopies were used to confirm the structure of the PHA biopolymer produced by *S. sanxanigenens* NX02. The ^1H -NMR spectrum of the polymer is shown in Fig. 3 a. Peaks at 1.28, 2.51 and 5.26 ppm corresponded to the methyl group ($-\text{CH}_3$), methylene group ($-\text{CH}_2-$), and methyne group ($-\text{CH}-$), respectively. The ^{13}C -NMR spectrum is shown in Fig. 3 b. Peaks at 19.77, 40.78, 67.61 and 169.15 ppm were regarded as the methyl group ($-\text{CH}_3$), methylene group ($-\text{CH}_2-$), methyne group ($-\text{CH}-$), and carbonyl group ($-\text{C}=\text{O}$), respectively. These

results were consistent with previous reports [33–35].

{Embed Fig. 3}

3.4 Analysis of purified sphingan Ss

3.4.1 Rheology analysis of sphingan Ss solutions

Sphingan Ss solutions possess thermal (20–70°C) and acid stability (pH 4.0–11.0) and high transparency [31]. Moreover, sphingan Ss solutions have good rheological properties at low concentrations. The viscosity of different concentrations of sphingan Ss solutions (0.05–0.25% w/v) at different shear rates is shown in Fig. 4. The sphingan Ss solutions were a type of shear thinning fluid; the viscosity decreased as the shear increased. At a shear rate of 2 rev/min, the viscosity of a 0.25% sphingan Ss solution was >25 pa s (Fig. 4).

{Embed Fig. 4}

3.4.2 Gel-forming properties of purified sphingan Ss

Gel-forming properties are important characteristics of sphingan Ss. At room temperature, 0.1% w/v heated sphingan Ss solution turned into small gel particles, while 0.2% w/v sphingan Ss solution turned into a soft, flexible, elastic, thermally reversible gel without addition of any chemical compounds. The properties of the gels

were influenced by the purity of the sphingan Ss, addition of ions (divalent cations), heating temperature and cooling temperature. Texture profile analysis of Ss gels at different concentrations (0.2–1.2% w/v) is shown in Fig. 5. The hardness, adhesiveness, gumminess and chewiness of Ss gels increased markedly with increased sphingan Ss concentration, and the cohesiveness and resilience remained constant. The springiness of Ss gels significantly increased when the concentrations of sphingan Ss were >0.4% (Fig. 5). Biopolymer Ss is the second sphingan besides gellan that can be used as a gelling agent.

{Embed Fig. 5}

3.4.3 Emulsifying properties of purified sphingan Ss

Sphingan Ss can also be used as an emulsifying agent because of its unique amphipathic structure, composed of polysaccharide, polypeptide and lipid, which is different from polysaccharide gellan, welan and other sphingans [4]. The emulsifying characteristics of other sphingans may depend on their tackifying and suspension performances. The emulsification results showed that 100% emulsion layers started to form stably with diesel or corn oil when the sphingan Ss concentration exceeded 1000 mg/L (0.1% w/v) at room temperature (Fig. 6). With concentrations of sphingan Ss of 100 mg/L (0.01% w/v), a lower emulsification value was observed with diesel (Fig. 6a), while the emulsification value was >60% with corn oil (Fig. 6b). Stable

emulsions could be observed in the presence of as little as 50 mg/L (0.005% w/v) sphingan Ss in diesel or corn oil after 24 h of evaluation. For gellan, stable emulsions (about 60% emulsion layers) were only observed in the presence of 1000 mg/L gellan in diesel, and no emulsion layers were found with 500mg/L gellan (Fig. 6a). Poorer emulsifying abilities were found for gellan with corn oil (Fig. 6b). Sphingan Ss emulsions with corn oil were stable over time, varying only slightly between 24 and 96 h, while the emulsifying activity of 1000 mg/L sphingan Ss on diesel dropped to 70% after 96 h (data not shown). Compared to other biopolymers, xanthan gum and gum arabic, sphingan showed stronger emulsifying activities [36]. Camacho-Chab et al. [36] reported that 1% w/v xanthan gum and gum arabic showed emulsification efficiencies of $90.6\% \pm 0.9\%$ and 0%, respectively, against corn oil.

{Embed Fig. 6}

*3.5 Metabolic flux analysis of sphingan Ss and PHB in *S. sanxanigenens* NX02*

Based on KEGG Orthology (KO) Database analysis, we have determined the metabolic relationship between sphingan Ss and PHB production in *S. sanxanigenens* (Fig. 7). The sphingan Ss and PHB are both biosynthesized from glucose. Intracellular PHB is synthesized from acetyl-CoA, which is produced from pyruvate and fatty acid metabolism, while pyruvate is obtained by the Entner-Doudoroff pathway and glycolysis. The sphingan Ss polysaccharide biosynthetic pathway is divided into three

sequential steps [3]: first, sugar-activated precursors (UDP-glucose, UDP-glucuronic acid, dTDP-rhamnose and GDP-mannose) are synthesized from glucose-6P. Next, tetrasaccharide repeat units are assembled then linked to the inner membrane. Third, the repeat units are translocated to the periplasmic space, followed by polymerization and export through the outer membrane. The production of sphingans Ss and PHB is spatially independent, but both are favored by the same culture conditions. The extracellular polysaccharides are considered a protection against desiccation and predation, and the endocellular PHB provides an internal reserve of carbon and energy.

{Embed Fig. 7}

Interestingly, there were eight proteins functionally related to poly-beta-hydroxyalkanoate synthases (PhaC) in the *S. sanxanigenens* NX02 genome. PhaC is a key enzyme in the synthesis of PHAs, but only two copies were found in the genome of gellan producing strain *S. elodea* ATCC31461 (AGFU000000000.1) [37] and welan producing strain *Sphingomonas* sp. ATCC31555 (ALBQ000000000.1) [38] (Table 1). Perhaps this is a reason why *S. sanxanigenens* NX02 accumulates much more PHB in the cell than other sphingans producing strains.

4. Conclusion

Large amounts of sphingan Ss and PHB were simultaneously accumulated by the fermentation of *S. sanxanigenens* NX02, which increased the efficiency of utilization of the carbon source. The method of separation and extraction of these two products was simple and cost-efficient, and the formation of elastic Ss gel was not affected by the separation process. Therefore, this simultaneous production is an advantageous fermentation mode. Sphingan Ss can be used broadly in biotechnology applications as a gelling, stabilizing, suspending and emulsifying agent.

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Table 1

The production and comparison of sphingan Ss, gellan and diutan.

Production or differences	<i>S. sanxanigenens</i> NX02	<i>S. elodea</i> ATCC31461	<i>S. sp</i> ATCC53159/pS8
Sphingans	sphingan Ss	gellan	diutan
TPM (g/L)	30.35±0.24	15.2	20.7
Purified polymers (g/L)	14.88±0.83	7.33	n.r. ^a
PHB production (g/L)	6.08±0.23	2.03–3.69	0.71–0.87
Extraction methods	acid precipitation	alcohol precipitation	alcohol precipitation
Main characteristics	Elastic gel Tackifier Emulsifier	Elastic or brittle gel	Tackifier
The copy number of PhaC proteins	8 (6 proteins of class I and 2 proteins of class III)	2 (1 proteins of class I and 1 proteins of class III)	n.r. ^a
Accession number	CP006644	AGFU00000000.1	n.r. ^a
References	This study	[18, 20, 30, 37]	[19]

^a not reported.

Figure captions

Fig. 1. The simultaneous production of extracellular sphingan Ss and intracellular PHB in *S. sanxanigenens* NX02 by batch fermentation in 5 L autoclavable fermenter.

Fig. 2. TEM observation of ultrathin sections of *S. sanxanigenens* NX02 in different growth phases. The white fractions were PHB granules and the fibrous polymers were sphingan Ss. (a) 2 h of batch fermentation. (b) 24 h of batch fermentation. (c) 48 h of batch fermentation. (d) 72 h of batch fermentation.

Fig. 3. NMR spectra of PHB from *S. sanxanigenens* NX02. (a) ^1H -NMR spectrum, uppercase letters A, B and C indicate methylene group ($-\text{CH}_2-$), methyne group ($-\text{CH}-$) and methyl group ($-\text{CH}_3$), respectively. (b) ^{13}C -NMR spectrum, lowercase letters a, b, c and d stand for carbonyl group ($-\text{C}=\text{O}$), methylene group ($-\text{CH}_2-$), methyne group ($-\text{CH}-$) and methyl group ($-\text{CH}_3$), respectively.

Fig. 4. Viscosity variation of different concentrations of sphingan Ss solutions at a shear rate of 2–60 rev/min using a viscometer equipped with a no. 63 spindle.

Fig. 5. Texture profile analysis of sphingan Ss gels of different concentrations (0.2%–1.2% w/v). Seven textural parameters (hardness, springiness, cohesiveness, adhesiveness, gumminess, chewiness and resilience) were measured with a Texture

Analyzer TA.XT Plus.

Fig. 6. Effect of sphingan Ss concentration on stability of diesel (a) and corn oil (b) after vortexing treatments. Sphingan Ss in concentrations of (mg/L) 500, 300, 200, 100, 50, 10, 1 and 0 (from left to right) were used to emulsify 1 mL diesel or corn oil after 2 min of vortexing. All samples were maintained at ambient temperature for 24 h before observation.

Fig. 7. Schematic representation of the proposed pathway of sphingan Ss and PHB biosynthesis from glucose in *S. sanxanigenens* NX02. The basic metabolic pathways were identified from the annotated *S. sanxanigenens* genome. Dashed arrows indicate multi-step reactions. Substrates in boxes were sugar-activated precursors. The abbreviations OM and IM indicate the outer membrane and inner membrane, respectively.

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Fig. 1

Fig. 2

Fig. 3

Fig. 3

Fig. 4

Fig. 5

Fig. 6

S. sanxanigenens NX02

D-Glucose