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A carotenoid-free mutant strain of *Sphingomonas paucimobilis* ATCC 31461 for the commercial production of gellan

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ABSTRACT

Gellan, an exopolysaccharide produced by *Sphingomonas paucimobilis* ATCC 31461, is used in the food, pharmaceutical, and other industries. During the fermentative production of gellan, strain ATCC 31461 also accumulates large amounts of yellow carotenoid pigments, thereby increasing processing costs. A carotenoid-free mutant strain of ATCC 31461 was isolated by ethyl methanesulfonate mutagenesis combined with ultraviolet irradiation. A new gellan purification method which reduced the consumption of isopropanol was developed based on this mutant strain. The final gellan production of the mutant strain was 13% higher than that of the parent strain. The glycerate and acetate substituents of gellan produced by the mutant strain were 17% and 65% higher than those produced by gellan from ATCC 31461, respectively. Stability tests suggested that the broth produced by the mutant strain was more stable during storage at room temperature compared with that produced by the parent strain. The mutant strain seems to be an ideal strain for use in the commercial production of gellan.

1. Introduction

Gellan is a new microbial exopolysaccharide with an average molecular mass of about 0.5 MDa (O'Neill, Selvendran, & Morris, 1983) produced after aerobic fermentation using the Gram-negative bacterium strain *Sphingomonas paucimobilis* ATCC 31461 (formerly classified as *Pseudomonas elodea*) (Kang & Veeder, 1983). The repeating tetrasaccharide unit of this linear heteropolysaccharide is composed of β-D-glucose, β-D-glucuronic acid, and α-L-rhamnose in a molar ratio of 2:1:1. The native gellan is partially esterified with the 1,3-D-glucose residue linked to L-glycerate at C-2 and to the acetate at C-6. Due to its unique structure and excellent physical properties, gellan has applications in food, pharmaceutical, and other industries as stabilizing, emulsifying, thickening, suspending, texturing and gelling agents (Banik, Kanari, & Upadhyay, 2000).

Gellan, unfortunately, has the highest per pound price of any food hydrocolloid at $18–20 lb^{-1} (Seisun, 2002), and this has limited its widespread application. Thus, improving gellan production by increasing the efficiency of the industrial process is of considerable importance. Previous studies have attempted to optimize culture conditions, such as the composition and proportion of carbon and nitrogen sources (Banik, Santhiagu, & Upadhyay, 2007; Kanari, Banik, & Upadhyay, 2002; Wang et al., 2006; West & Fullenkamp, 2000; West & Strohfus, 1998, 1999), other additional substrates (Arockiasamy & Banik, 2008; Bajaj, Saudagar, Singhal, & Pandey, 2006), pH (West & Fullenkamp, 2001), temperature (West, 2003), agitation (Giavasis, Harvey, & McNeil, 2006), and dissolved oxygen (Banik & Santhiagu, 2006). Modification of different culture conditions and various analysis methods showed different gellan yields, from 3.2 (West, 2003) to 43.6 $g\,l^{-1}$ (Bajaj, Saudagar, Singhal, & Pandey, 2006). In current industry practices, gellan yield is about 14–15 $g\,l^{-1}$. Satisfactory results have also been acquired by strain improvement. Significant progress in studies of the gellan biosynthetic pathway (Fialho et al., 2007; Fialho et al., 2008; Harding, Patel, & Coleman, 2004; Sá-Correia et al., 2002) and genetic engineering have increased gellan production over the last several years (Armentrout, Mikolajczak, Thorne, Yamazaki, & Pollock, 1998; Thorne, Mikolajczak, Armentrout, & Pollock, 2000; Vartak, Lin, Cleary, Fagan, & Saier, 1995). The best results, however, were acquired by traditional strain mutation. For instance, Lobas et al. isolated a mutant strain from plates solidified with gellan instead of traditional agar after treating with ethyl methanesulfonate (EMS). Total gellan production from this method was 8.73 $g\,l^{-1}$, about four times higher than that produced from the parent strain (Lobas, Schumpe, & Deckwer, 1992). Similarly, West isolated a mutant strain with a twofold higher yield (4.49 $g\,l^{-1}$) than its parent strain using a procedure involving chemical mutagenesis and screening for resistance to ampicillin (West, 2002, 2005). All these research studies, however, focused on improving gellan production and provided little information about increasing purification efficiency and reducing processing costs. During the synthesis of gellan, the

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fermentation broth accumulates large amounts of the bacterial metabolite poly-β-hydroxybutyrate (PHB) and yellow carotenoid pigments, mainly nostoxanthin (Jenkins, Andrewes, McQuade, & Starr, 1979), both of which are major impurities. Baird and Cleary (1994) and Bower et al. (2006) simplified the purification process by using PHB-deficient strains isolated by random mutagenesis and gene knockout, respectively.

None of the above studies focused on other metabolite carotenoids, which are yellow pigments that compete with gellan for carbon sources. To remove the pigments, large amounts of organic solvents, such as ethanol or isopropanol, are needed. In this study, a carotenoid-free strain with a null mutation that deactivated carotenoid biosynthesis was isolated by combining chemical and ultraviolet mutagenesis. Then, experiments were carried out to examine the gellan production of this mutant strain and further develop a low-cost method for gellan recovery. The results indicated that the mutant strain could be used to synthesize gellan with better storage stability against gellan lyase and higher acyl content which are counter for better application properties.

2. Materials and methods

2.1. Strains and media

The strain utilized in this investigation was *S. paucimobilis* ATCC 31461 (Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982), and the mutant strain βmw007 was isolated in this study. The strains were maintained in slant with YPG agar medium (glucose 20 g l^{-1}, yeast extract 3 g l^{-1}, peptone 5 g l^{-1}, agar 15 g l^{-1}) at 30 °C for 3 d and stored in a refrigerator at 4 °C. The preculture medium was as follows: NaCl 5 g l^{-1}, yeast extract 1 g l^{-1}, peptone 5 g l^{-1}, beef extract 3 g l^{-1}, and sucrose 5 g l^{-1}. A loop of cells maintained in slant was inoculated into 100 ml of the sterile medium described above in 500 ml Erlenmeyer flasks, incubated for 24 h at 30 °C, and shaken at 200 rpm on a rotary shaker. The gellan production medium contained the following: K_2HPO_4 1.5 g l^{-1}, KH_2PO_4 1 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.6 g l^{-1}, yeast extract 0.2 g l^{-1}, soy protein 2 g l^{-1}, and sucrose 30 g l^{-1}. For fermentation, the appropriate preculture was inoculated at a volume ratio of 1:10 into 100 ml of the production medium in 500 ml Erlenmeyer flasks. The inoculated flasks were kept on a rotary shaker at 200 rpm at 30 °C for 48 h.

Batch fermentations were studied in a 6.7-l stirred bioreactor (BioSCADALab R'ALF plus, Bioengineering AG, Switzerland) with a 4.5-l working volume. The process set points were pH, 7.0 (±0.1); temperature, 30 °C; agitation rate, 400 rpm; and aeration rate, 1 vvm. Both agitation and aeration were kept constant throughout the entire process. The pH was controlled by the automatic addition of 3 M NaOH and 3 M HCl.

2.2. Mutant isolation

The mutant strains were isolated by a combination of conventional chemical mutagenesis and ultraviolet (UV) irradiation. Exponentially growing YPG cultures of *S. paucimobilis* ATCC 31461 cells were treated with 1% (v/v) EMS at 30 °C for 60 min without agitation (West, 2002), followed by UV irradiation (30 W, 30 cm distance) for 45 s under magnetic stirring. Then, the cells were spread onto YPG agar plates at 30 °C after suitable dilutions of the cultures. After 72 h, the colonies were visually inspected for color. Among the 8651 colonies, 5 white colonies, indicative of carotenoid-deficient strains, were detected and checked for gellan production in 500-ml Erlenmeyer flasks at 30 °C and 200 rpm for 48 h. One mutation, designated as βmw007, had much higher gellan production and viscosity compared with the parent strain after 48 h. Subsequent batch fermentations of strain ATCC 31461 and βmw007 were studied to analyze their gellan production, viscosity, total residual sucrose, total carotenoids, and pyruvate content.

2.3. Recovery of gellan

The broths were purified by isopropanol according to the method described by Kang, Veeder, and Colegrove (1983). In addition, another aliquot of βmw007 broth was recovered by mixing with 2% $CaCl_2$ (m/v) pre-dissolved in an appropriate amount of ddH_2O followed by the addition of 30% isopropanol (v/v). The total amount of impurities in the recovered gellan, such as protein and ash, was determined by the method of Kang, Veeder, Mirrasoul, Kaneko, and Cottrell (1982).

2.4. Stability test of the fermentation broth

Two hundred milliliters of each fermentation broth were stored in sterile 500 ml Erlenmeyer flasks at room temperature without agitation, after which the broth viscosity was measured.

2.5. Sequence analysis of acetyl transferase gene and its flanking sequences

The acetyl transferase genes of βm007 and ATCC 31461 were cloned by PCR using the primers 5′-GTTTTTCGGCCCTAGACA-3′ and 5′-ATCCAGCGATAGGTGAGC-3′ and then sequenced. Site finding-PCR was employed to retrieve the flanking sequence, as previously described (Tan et al., 2005). Specific primers were designed according to the 3′-end and 5′-end sequences of the acetyl transferase gene. The primer sequences were as follows: 5′GSP1, 5′-CGAAGGTGGAACAGAACGA-3′; 5′GSP2, 5′-GCCGAAAAACGTAGGCCTGACTTT-3′; 3′GSP1, 5′-CCCTCTCCTACTCGATCTACA-3′; and 3′GSP2, 5′-TGTTCGGCGCTCACCTATCGC-3′.

2.6. Analysis method

To analyze gellan production, the fermentation broths were diluted 10 times with distilled water because of the high viscosity of the broths, heated for 15 min in a boiling water bath, and then centrifuged at 15,000 × *g* for 45 min at 25 °C (Wang et al., 2006). A volume of isopropanol equal to thrice the volume of the supernate was to precipitate gellan, and the solution was kept overnight at 4 °C. After several washings with isopropanol, the polymer was separated by centrifugation at 10,000 × *g* for 45 min, dried at 80 °C, and weighed (Banik, Santhiagu, & Upadhyay, 2007). The viscosity of the fermentation broth, which was expressed in centipoises (cP), was measured by a Brookfield viscometer (model RVDV-II Pro; Brookfield Engineering Laboratories, Stoughton, MA), using spindle No. 6 at 60 rpm and 25 °C. The total sucrose content was estimated by the Fehling method after acid hydrolysis with 1 M HCl at 75 °C for 10 min. The concentrations of glucose and rhamnose, as well as the glycerate content of the gellan were determined after hydrolysis with 0.5 M H_2SO_4 (100 °C for 16 h) by HPLC (Agilent 1200) with an Aminex HPX-87column (300 mm × 1.8 mm internal diameter; Bio-Rad) (Martins & Sa-Correia, 1994) at 40 °C. The glucuronic acid and acetate content of the gellan were determined by the methods described by Bitter and Muir (1962) and McComb and McCready (1957), respectively. The relative molecular weight was measured by gel permeation chromatography as described by Bezanson et al. (2006). The total carotenoid content of the fermentation broth was determined spectrophotometrically as described by Maziya-Dixon, Dixon, and Ssemakula (2008). The pyruvate content was measured according to the method described by Johnson and Edwards (1937).

Table 1
Gellan production of carotenoid-deficient strains.

Strains	48 h gellan yield ±SD ($g l^{-1}$)
ATCC 31461	11.89 ± 0.46
βmw003	8.73 ± 0.53
βmw004	10.78 ± 0.59
βmw005	11.27 ± 0.61
βmw006	7.30 ± 0.37
βmw007	13.15 ± 0.65
βmw007(G10)[a]	12.95 ± 0.67
βmw007(G20)[a]	13.07 ± 0.54
βmw007(G30)[a]	13.20 ± 0.55

[a] Indicates the number of passages.

Table 2
Recovery of gellan from broth.

Sample	Method	Consumption of isopropanol (v/v)	Total impurities content, % (w/w)
βmw007	$CaCl_2$	30%	13.70
βmw007	Isopropanol	2 Times	13.45
ATCC 31461	Isopropanol	2 Times	14.31

3. Results

3.1. Screening carotenoid-deficient strains by EMS mutagenesis in combination with UV irradiation

Five carotenoid-deficient mutant strains that gave rise to white colonies on the medium were isolated from about 8600 colonies by a combination of EMS mutagenesis and UV irradiation. A subsequent comparison of gellan production by these five white colonies (designated as βmw003–βmw007) and the parent strain ATCC 31461 in Erlenmeyer flasks was made (Table 1). Among these strains, βmw007 showed about 11% higher gellan production than that of its parent strain, and exhibited a stable phenotype with respect to pigment deficiency and gellan production after 30 passages in the preculture medium (Table 1).

3.2. Recovery and the properties of gellan

The significant advantage of the βmw007 strain for gellan production was that it was non-pigmented. Thus, batch fermentation was conducted in a 6.7-l fermentor, and a new recovery method based on the non-pigment broth of βmw007 was developed (Table 2). To remove the carotenoid pigments of ATCC 31461, two full volumes of isopropanol were needed. In contrast, only a 30% volume of isopropanol was consumed for gellan recovery from βmw007 using the method with $CaCl_2$. In addition, no obvious difference in purity was observed in the gellan recovered from the two different methods.

To characterize further the properties of gellan from the mutant strain, the chemical composition of gellan produced by βmw007 was compared to that of its parent strain (Table 3). The carbohydrate backbones of gellan produced by both strains were almost the same, whereas differences in the acyl content, especially the acetate content, and the relative molecular weight were significant. The glycerate substituent of gellan produced by βmw007 was 17% higher than that produced from ATCC 31461. An obvious difference was also found in the acetate substituent of the gellan produced from both methods: gellan from βmw007 had 65% more acetate substituent than did gellan from ATCC 31461.

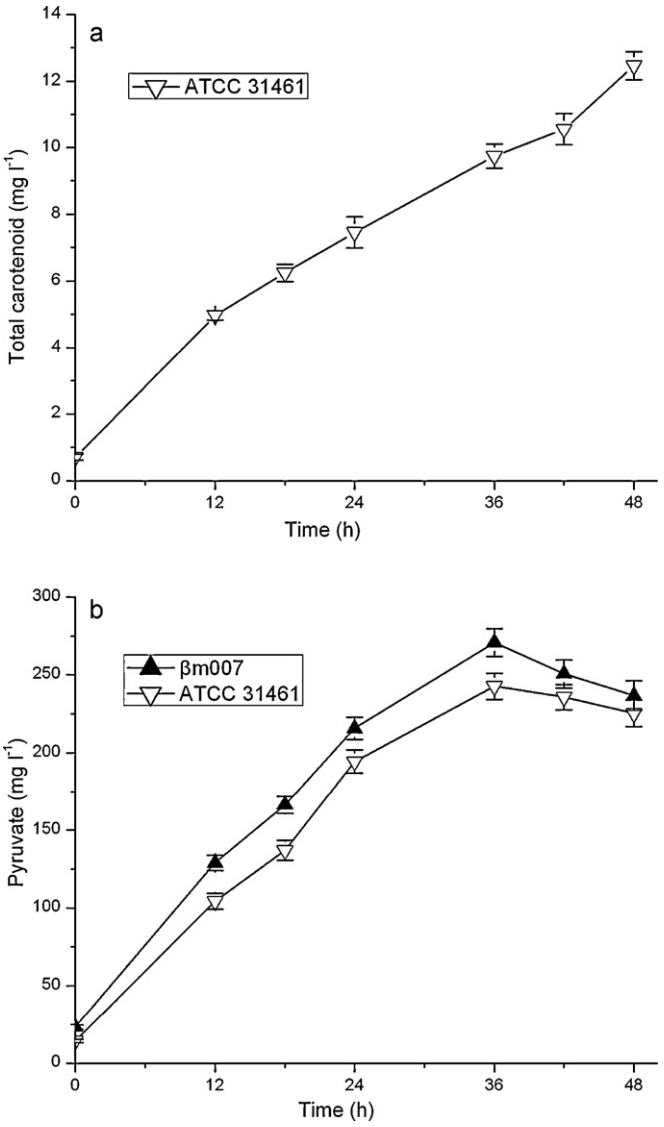

Fig. 1. (a) Carotenoids content of ATCC 31461 and (b) pyruvate content of the βm007 and ATCC 31461 broths in a 6.7 l bioreactors for 48 h. There was no pigment found in the broth of βm007. Values are the averages of three parallel samples (error bars indicate standard deviations).

3.3. Carotenoids and pyruvate content

To determine the reasons behind the different acyl content of the two strains, a comparison of the carotenoids and the key intermediate metabolite pyruvate content produced by both strains was made (Fig. 1). The broth of strain βm007 was visually white with no carotenoids detected throughout the fermentation pro-

Table 3
Chemical composition[a] and molecular weight analysis of gellan produced by βmw007 and ATCC 31461.

Gellan sample	Glucuronic acid	Glucose	Rhamsan	Glycerate	Acetate	Molecular weight[b]
βmw007	1 ± 0.04	1.86 ± 0.07	1.29 ± 0.05	0.54 ± 0.02	0.38 ± 0.01	1.42
ATCC 31461	1 ± 0.03	1.82 ± 0.05	1.28 ± 0.06	0.46 ± 0.02	0.23 ± 0.01	1.00

[a] Data is shown in the molar ratio according to glucuronic acid and presents the average value and standard deviation of three individual experiments.
[b] The gellan sample of ATCC 31461 was averaged to provide the control relative molecular weight, which was assigned a value of 1.0.

cess, whereas the carotenoid content for ATCC 31461 continued to increase during cultivation. The carotenoid content of ATCC after 48 h cultivation was 12.47 mg l^{-1} (Fig. 1a). The time courses of the pyruvate content in the broths of both strains were compared in Fig. 1b. In the initial phase of fermentation, the pyruvate content of both strains increased dramatically and reached their peaks at 36 h, followed by small decreases until 48 h due to the decrease in metabolic rate. βm007 had noticeably higher pyruvate content compared with ATCC 31461 at each period of analysis.

3.4. Analysis of substrate consumption, gellan production, and viscosity in batch cultivation

βm007 was found to achieve higher gellan levels and viscosity than ATCC 31461 throughout the fermentation process (Fig. 2a and b). After 48 h of cultivation, the gellan level accumulated by the mutant strain βm007 was up to 16.33 g l^{-1}, 13% higher than the level produced by the parent strain (Fig. 2a). Such an increase was even more apparent in terms of the viscosity of the fermentation broth. After 24 h of incubation, the broth viscosity of βm007 was 10,039 cP, about twofold higher than that of ATCC 31461 at 48 h, whereas gellan production was only 10.07 g l^{-1}, much lower than that of ATCC 31461 at 48 h. Viscosity elaboration by βm007 (30,310 cP) was more than fivefold that observed for ATCC 31461 following 48 h of growth (Fig. 2b). Furthermore, the sucrose consumption rate was clearly higher for βm007 than for ATCC 31461 over the same period of fermentation, especially during the initial 24 h (Fig. 2c).

3.5. Stability of the fermentation broths

The gellan purification is a time-consuming process. Thus, maintaining the stability of gellan during recovery is of great importance. Fig. 3 shows a comparison of the percentage changes in viscosity of the fermentation broths for βm007 and ATCC 31461 during storage at room temperature. The viscosity of the broth of βm007 was stable for the initial 24 h (Fig. 3a), and a slight decrease was observed over a period of 100 d (Fig. 3b). Most of the viscosity (85% of the initial value) of the broth from this strain was retained. In comparison, the broth viscosity of ATCC 31461 was significantly reduced over the first 24 h (Fig. 3a), and it retained only 5% of the original value after 100 d (Fig. 3b).

3.6. Sequence analysis of acetyl transferase gene and its flanking sequences

The sequence analysis indicated that the acetyl transferase gene and its flanking sequences were the same for the wild-type strain ATCC 31461 and the mutant strain βm007, which is identical to the acetyl transferase gene sequences reported by Harding, Patel, and McQuown (2002).

4. Discussion

When a clarified gellan product is desired, the key step in the recovery of gellan is the removal of the pigments (major impurities) tightly bound to gellan in the broth. Two full volumes of isopropanol were used to eliminate the pigment impurities, raising the total cost by 20% (personal communication). The economic effects of such a requirement are a challenge to overcome in gellan production. In this study, a mutant strain deficient in carotenoid showed potential for a more economic downstream purification process. In this study, a new method using less solvent was developed to recover gellan from the colorless fermentation broth of βmw007; it is useless for the fermentation broth of ATCC 31461 due to the presence of pigments. The proposed method appears highly efficient and may

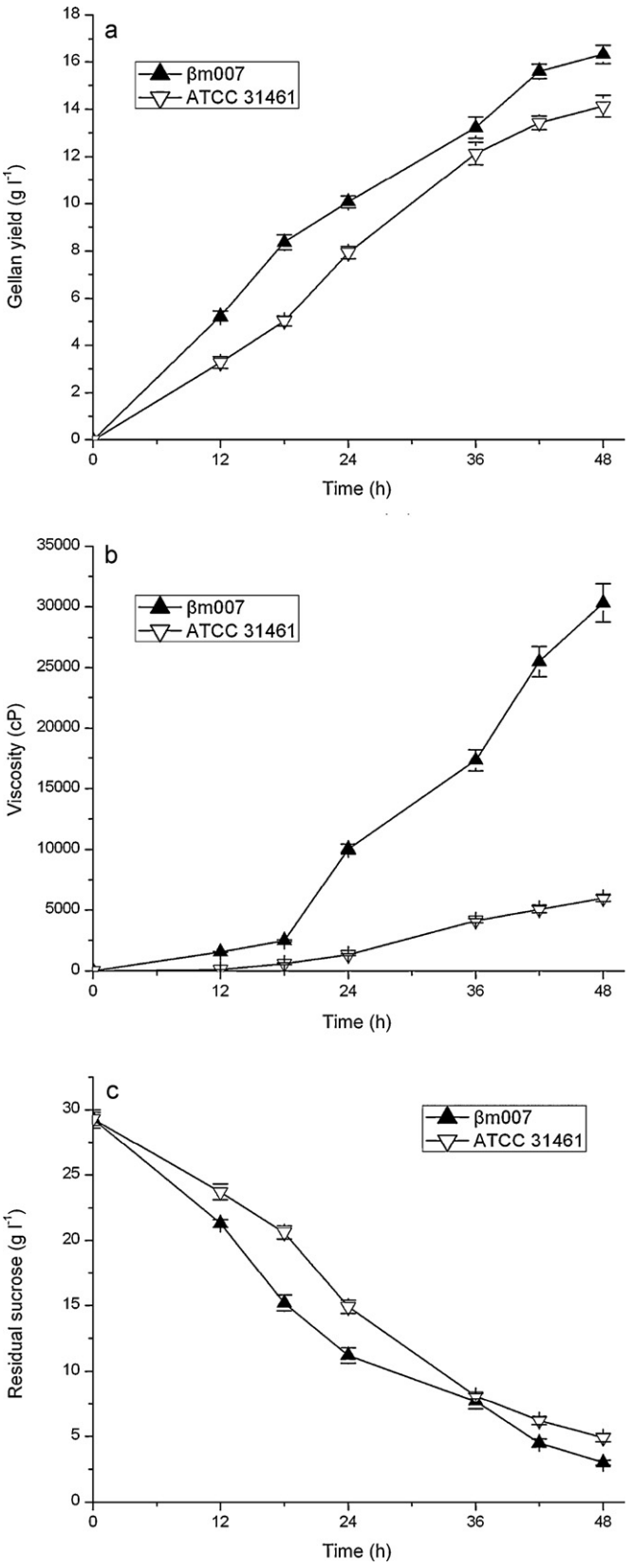

Fig. 2. Fermentation profile of βm007 and ATCC 31461 in a 6.7-l bioreactor for 48h. Cells were grown under the same fermentation conditions (agitation 300 rpm; air supply 1 vvm). (a) Gellan production, (b) broth viscosity, (c) residual sucrose concentration. Values are the averages of three parallel samples (error bars indicate standard deviations).

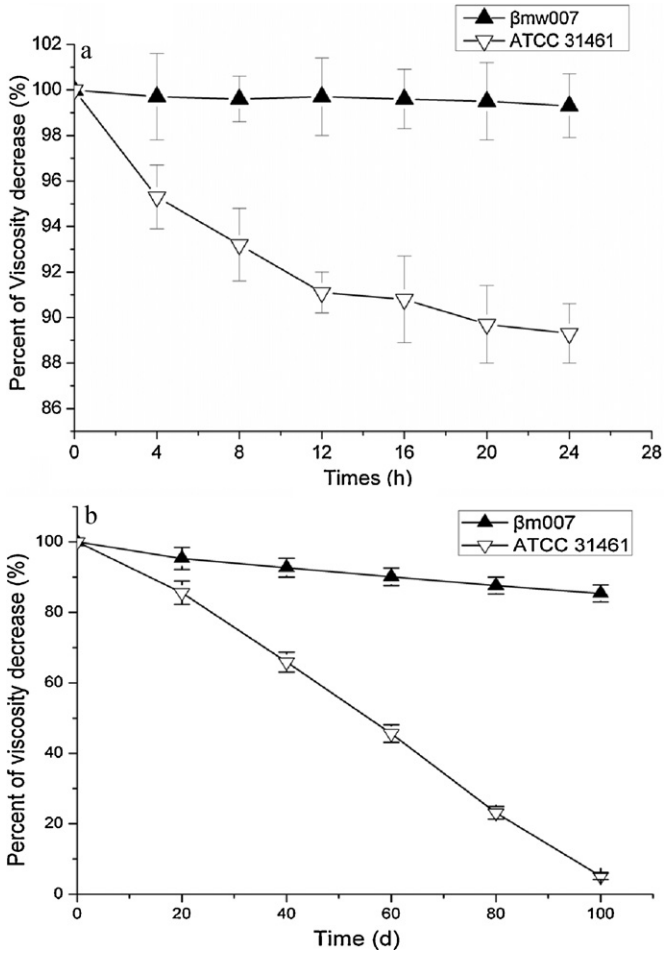

Fig. 3. (a) Short-term and (b) long-term stabilities of the fermentation broths of βm007 and ATCC 31461. About 200 ml of each fermentation broth were stored in sterile 500-ml Erlenmeyer flasks at room temperature without agitation, after which the broth viscosity was measured. Values are the averages of three parallel samples (error bars indicate standard deviations).

be able to reduce costs by over 20%. This is promising for large-scale manufacturing needs.

We examined the fermentation of gellan by both strains. The clearly higher sucrose consumption rate of βmw007 may be attributed to its faster rate of gellan production, as is evident in Fig. 2a. Sucrose consumption rates for both strains decreased when the broth viscosity increased because gellan accumulation significantly reduced the mass transfer rate for oxygen and other nutrients. Given its relatively higher sucrose consumption rate, βmw007 showed a final gellan concentration that was 13% higher than its parent strain, and its space-time yield after 48 h of cultivation was 0.34 g l^{-1} per hour compared with 0.30 g l^{-1} per hour for ATCC 31461. The yield increase observed for βmw007 was lower than those previously reported by West (2002) and Lobas et al. (1992). However, the conversion of all kinds of carbon sources to gellan by βmw007 was 62%, which was relatively high and was only slightly lower compared with the closely related species *Xanthomonas campestris*, which converts nearly 60–80% of the sugar in its culture medium to commercially important xanthan. In comparison, the conversion of all kinds of carbon sources to gellan for ATCC 31461, including the results reported here, is only about 50% or less (Vartak, Lin, Cleary, Fagan, & Saier, 1995). The reason for the increase in conversion by the strain βmw007 isolated in the current study was much higher than that of gellan production is still unclear.

The results by West suggested that the increase in viscosity is directly related to the increase in gellan production after 48 h (West, 2002). However, in this study, the broth viscosity of βmw007 significantly increased and was much higher than that of ATCC 31461 compared with the increase in gellan production. The broth viscosity increased more than five times the original value. In contrast, the amount of gellan increased by only about 13%. In fact, many factors may influence the viscosity of the broth. Aside from the type and concentration of added cation species (Horinaka, Honda, & Takigawa, 2009; Huang, Singh, Tang, & Swanson, 2004; Kani, Horinaka, & Maeda, 2005; Nickerson, Paulson, & Speers, 2003), which were equal in both fermentation broths since they were cultured in the same media, the nature of gellan, i.e., the chemical composition (especially the glycerate and acetate content, as well as the relative molecular weight of gellan) is another main influencing factor. Jay et al. (1998) and Morris, Gothard, Hember, Manning, and Robinson (1996) have proven that glycerate and acetate played significant roles in the rheology of the broth. Our results were in agreement with these conclusions. Chemical analysis of the two gellan samples indicated that they had exactly the same primary carbohydrate structure, but their acetate and glycerate content were different (Table 3). The gellan produced by βmw007 had higher levels of acylation, especially in terms of acetate substituents (1.7-fold). The acylation appears to be associated with the promotion of the aggregated form of gellan, thereby enhancing the viscosity. Moreover, differences in the molecular weights between the gellan produced by the two different strains may have also contributed to the enhancement of broth viscosity, which is in accordance with the results of Giavasis et al. (2006) and Dreveton, Monot, Lecourtier, Ballerini, and Choplin (1996), who noted that the viscosity of the broth seemed to be a function of the molecular weight of gellan.

As shown in Fig. 4, the synthesis of gellan, especially the acylation substituent, shares a common initial pathway with carotenoid formation. Carotenoids are synthesized via an initial condensation reaction between pyruvate and glyceraldehyde-3-phosphate (G3P) (Das et al., 2007; Rodriguez-Concepcion & Boronat, 2002), which are also required as precursors for the acylation substituent of gellan (Giavasis, Harvey, & McNeil, 2000). The higher acylation levels of gellan from βmw007 may be associated with higher pyruvate content during the fermentation process. βmw007 was a mutant strain in which at least one of the genes that encode the relevant proteins involved in the synthesis of colorless carotenoid precursors was mutated, thereby blocking the synthesis of yellow carotenoid pigments (about 13 mg l^{-1} in ATCC 31461). This blocking resulted in the increase of intermetabolite pyruvate by about 20 mg l^{-1} (Fig. 1b). From Table 3, we can calculated that the increased content of acylation took up 1.2% of the total amount of gellan (about 16 g l^{-1}), that is to say the content of acylation rose by about 19 mg l^{-1}, which is close to the increase in pyruvate (Fig. 1b). The disruption of carotenoid production may be partially involved in the increase in pyruvate, and may eventually lead to redirection of pyruvate flux from carotenoids to gellan. In a randomly mutagenized strain, many factors could also be modified by mutation. For example, the genes encoding for the enzymes responsible for acylation may be modified. In this study, the acetyl transferase gene sequences and its flanking sequences obtained from βm007 and ATCC 31461 indicated that the expression of the gellan responsible for acylation was not influenced by the randomly mutagenized βm007. Other genetic alterations that may also contribute to changes in metabolism require further investigation.

Highlighting the higher acylation substitution and viscosity of gellan produced by βmw007 is necessary as these are advantages for some of its applications (Yuan, Morrison, & Clark, 2005). Glycerate and acetate play significant roles in controlling the polymer association and gelation of gellan. The acyl groups present in gel-

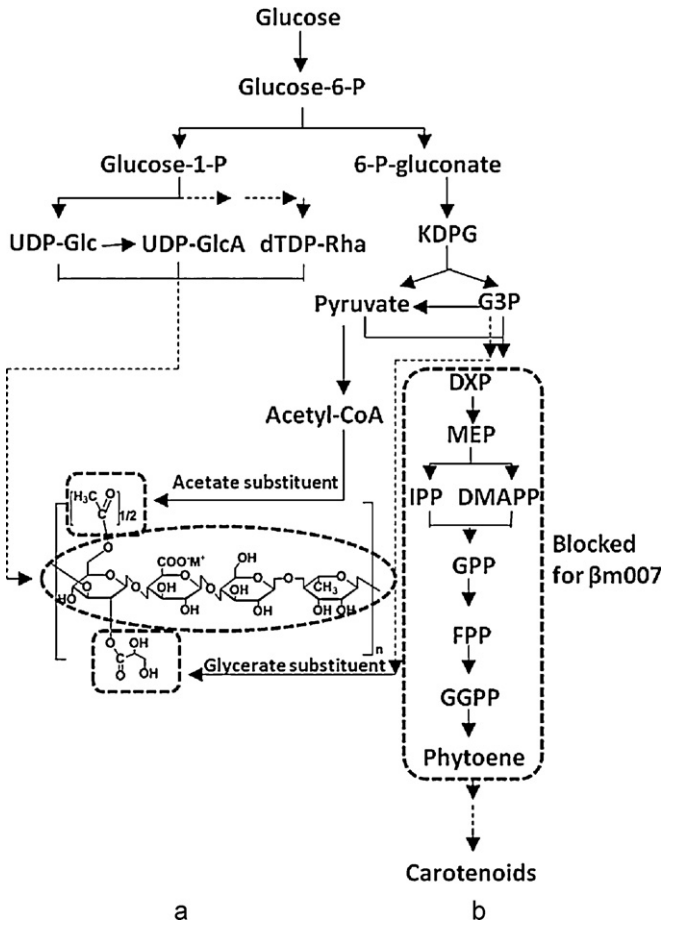

Fig. 4. Proposed biosynthetic pathway for (a) gellan and (b) carotenoids. The conformation within the oval in (a) represents the primary backbone of the gellan, whereas that within the box shows the acetate and glycerate substituents, respectively. The box in (b) shows that at least one metabolite may be blocked in the mutant strain βmw007. *Abbreviations*: UDP-Glc, UDP-D-Glucose; UDP-GlcA, UDP-D-glucuronic acid; dTDP-Rha, dTDP-L-rhamnose; KDPG, 2-keto-3-deoxy-6-phospho-gluconate; G3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

lan cause massive changes in the thermal stability of the double helix and in its ability to form cation-mediated aggregates (Morris, Gothard, Hember, Manning, & Robinson, 1996). Higher acylation substituents enhanced colloidal stability in beverages, allowing for the greatly enhanced suspension performance of gellan. Gellan has a high set point (greater than 75–80 °C) with thermoreversible behavior and low calcium sensitivity (Yuan, Morrison, & Clark, 2005). Thus, it is beneficial for use in yogurt drink products with fruit pulp suspensions and in retort milk beverages.

Another important characteristic of the gellan produced by βmw007 that reflects polymer quality is its stability over prolonged storage (Fig. 3). Such stability is likely due to the resistance of βmw007-produced gellan against the gellan lyase produced by the strain over the period of fermentation. Previous research had proven that a gene, designated as *gel*R, found in the biosynthetic gene cluster of gellan encoding a gellan lyase was involved in the hydrolysis of gellan and liberatee cells from the capsule under conditions of nutrient limitation (Harding, Patel, & Coleman, 2004). In addition, it has also been found that enzymes prefer to degrade low acyl gellan due to extracellular eliminase-types of enzymes (lyases), which cleave to the sequence – β-D-glucosyl(1,4)-β-glucuronosyl – in the tetrasaccharide repeat unit of the gellan substrate, and exhibit negligible activity against highly acylated gellan (Kennedy & Sutherland, 1994; Mikolajczak, Thorne, Pollock, & Armentrout, 1994; Sutherland, 1995; Sutherland & Kennedy, 1996). As shown in Table 3 and Fig. 3, the βmw007-derived gellan, with a higher total acyl content, showed remarkably stable storage compared with that of the parent strain, probably due to its stronger resistance to the depolymerizing activity of gellan lyase. Resistance to gellan lyase may also be associated with differences in the molecular weights of the two gellan samples (Das et al., 2007). In industrial processes, degradation by the gellan lyase produced by the strain itself decreases the viscosity of the gellan in the broth. The decrease in viscosity is mainly observed in the early stages of recovery because transferring large amounts (i.e., 30 m^3) of the fermentation broth from the fermentor to the post-treatment tank is time consuming. Moreover, the broth cannot all be treated timely or at the same time. This pretreating process of industrial practice are, to some extent, similar to the process our stability tests (Fig. 3). When the broth was mixed with $CaCl_2$ and isopropanol, the gellan precipitated from the broth and it was able to resist lyase degradation. Thus, the gellan produced by βmw007 may be of better quality by virtue of its extremely stable broth.

5. Conclusions

A non-pigmented mutant strain, βmw007, was derived from *S. paucimobilis* ATCC 31461 by ethyl methanesulfonate mutagenesis and ultraviolet irradiation. A new recovery method based on this mutant strain was established, decreasing the consumption of isopropanol during the recovery process and, in turn, reducing the cost of recovery by more than 20%. βmw007 was observed to accumulate 13% more gellan compared with its parent strain. The high acyl substitution rate and molecular weight of the gellan produced by βmw007 are advantages for some of its applications. Tests indicated that the broth produced by the mutant strain is more stable than that by the parent strain. Therefore, the mutant strain is a more favorable gellan-producing strain.

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