Cloning of gellan gum biosynthetic genes gelC, gelD and gelE for enhanced production of gellan gum by *Sphingomonas paucimobilis* ATCC 31461

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Abstract

Gellan gum is a water soluble, high molecular weight anionic exopolysaccharide with rheological characteristics. Sphingomonas paucimobilis ATCC 31461 is the bacterium used for the industrial production of gellan gum. The biopolymer has gained wide applications in food, biomedical and pharmaceutical industries and has been used as a replacement for gelatin and agar. The genes gelC, gelD and gelE are involved in the polymerization and export of the gellan. In this study, gellan gum genes gelC, gelD and gelE were cloned into pBBR122 vector and the recombinant plasmids containing target gene-pBBR122 was transformed into competent Sphingomonas cells by electroporation method. The expressed proteins were purified by affinity chromatography using Ni-NTA resin and the molecular weight of the recombinant proteins were estimated by SDS-PAGE.

The biomass and gellan gum production by both wild and recombinant strains harbouring gelC, gelD and gelE were carried out in the laboratory fermentor. The recombinant genes gelC, gelD and gelE had a molecular mass of 110KDa, 74KDa and 60KDa respectively. The results indicated that gellan gum production was higher in case of recombinant strains compared to the wild type. Recombinant strain containing multiple copies of gelD showed the maximum biomass and gellan gum yield compared to other two recombinant strains. The construction of a recombinant strain for the gellan gum production could lead to a considerable increase in its production yield and thus enabling a reduction in the cost of gellan.

Keywords: Gellan gum, electroporation, pBBR122, Ni-NTA, SDS-PAGE.

Introduction

Microbial biopolymers are long chain molecules that are composed of sugar residues. They are high-molecular-weight biodegradable polymers synthesized by a broad range of bacteria. The most well known and industrially used biopolymers are xanthan from *Xanthomonas campestris*, gellan from *Pseudomonas* and dextran secreted by the *Leuconostoc* species[1]. Gellan gum has been the subject of extensive research recently due to its functional properties. Structure, composition and viscosity of polysaccharides is influenced by various factor such as the culture medium composition, carbon and nitrogen sources, precursor molecules, minerals, salts, trace elements, type of strain and fermentation parameters such as pH, temperature, dissolved oxygen and agitation.

Gellan gum is of great interest due to its rheological characteristics. It is a water soluble exopolysaccharide produced by the non-pathogenic bacterium *Sphingomonas paucimobilis.* Gellan gum is a multifunctional gelling agent presently produced by C. P. Kelco in Japan and the USA[2]. In its natal form, gellan is a linear anionic exopolysaccharide based on a tetrasaccharide repeat unit comprised of two molecules of D-glucose, one of L-rhamnose and one of D-glucuronic acid. The composition of repeating unit of gellan polysaccharide is 60 % glucose, 20 % rhamnose and 20 % of glucuronic acid[3].

The gellan gum is classified into few types which includes deactivated, clarified and native gums and is marketed by various trade names like Kelcogel, Gelrite, Phytagel and Gel-Gro. Kelcogel is extensively used in food processing industry as a gelling agent whereas Gelrite, Phytagel and Gel-Gro are used as solidifying agent[4].

The bacterial strain *Sphingomonas paucimobilis* ATCC 31461 is used for the industrial production of gellan gum[5]. Gellan gum producing microorganism *Sphingomonas paucimobilis* was originally isolated from the surface of a plant *Elodea*. The genus *Sphingomonas* is a group of gram-negative, rod-shaped, aerobic, chemoheterotrophic bacteria comprising of glycosphingolipids in their cell envelopes and they generally produce yellow-pigmented colonies[6].

The gel cluster of *Sphingomonas paucimobilis* comprises of 18 genes (gel Q,I,K,L,J,F,D,C, E,M,N,B, rmlA,B,C,D atrD,B) associated with the production of dTDP-L-Rha, glycosyltransferases and proteins required for polymerization and export of gellan. Other genes pgmG, ugpG and ugdG gellan genes do not map in the same locus and are not present in the cluster of genes involved in gellan biosynthesis[7]. Gellan gum biosynthesis pathway comprises of three different segments namely intracellular synthesis of sugar activated precursors, assemblage of the tetrasaccharide adhered to a membrane anchored C55-isoprenyl pyrophosphate carrier, the polymerization of the repeat units and transport of the polysaccharide[8].

This present work focuses on the genes gelC, gelD and gelE of the *Sphingomonas paucimobilis* ATCC 31461 which are involved in the polymerization and export of the gellan gum[8].

In this present study, we report the cloning of the gellan gum genes namely gelC, gelD and gelE into the broad-host-range vector pBBR122 and over expression in *S.paucimobilis* ATCC 31461.The expression host containing the cloned gene were induced for protein expression and the resultant protein was purified by affinity chromatography using Ni-NTA resin. Protein expression levels in *S.paucimobilis* carrying the gellan gum genes were estimated by SDS-PAGE. In addition, the biomass and gellan gum yield of recombinant strains and wild type strain was also studied.

Material and Methods

Bacterial Strains, Vectors, Regents and growth conditions: *Sphingomonas paucimobilis* ATCC 31461 was obtained from LGC, Promochem, Bangalore. YPG (Yeast Peptone Glucose, Sigma, USA) and LB medium (Luria Bertani Broth, Sigma, USA) were used for culturing bacteria. *S.paucimobilis* was grown at 30°C at an aerobic environment in a YPG medium comprising 3g of yeast extract, 5g of peptone, 30g of glucose and 5g of NaCl per liter of distilled water, adjusted to pH 6.5. *E. Coli* was grown at 37°C in LB broth and was supplemented by antibiotics kanamycin (50µg/ml) and chloramphenicol (33.3µg/ml) (Sigma, USA).

The pBBR122 vector was procured from MoBiTec. *E.coli* DH5α and the enzymes were purchased from Promega (USA). DNA isolation, PCR purification, Gel extraction and plasmid isolation kits were purchased from Thermo Fisher Scientific (USA). Recombinant protein was purified by utilizing Ni-NTA column (Novagen, USA). All other chemicals were obtained from Merck (Germany).

Isolation of Genomic DNA: After overnight incubation of *S.paucimobilis* at 30°C in YPG, cells were centrifuged at 5000rpm for 5min to pellet the cells. Chromosomal DNA of *Sphingomonas paucimobilis* ATCC 31461 was isolated using the Promega DNA isolation kit and was run on 0.8% agarose gel for confirmation.

Polymerase Chain Reaction: The primers for genes gelC, gelD and gelE were designed using the Primer Premier Software. The forward primer with a NcoI restriction site and reverse primer with EcoRI restriction site were used for gelD and gelE. The forward primer containing HindIII and reverse primer containing PvuI restriction site were designed for gelC. The gene gelC (1346bp), gelD (902bp) and gelE (707bp) were augmented by PCR using the following primer sequences (Table 1). The polymerase chain reactions for genes gelC, gelD and gelE were carried out using a thermal cycler (GeneAtlas). PCR mixture was performed in a total volume of 50µl containing, 1µl of template DNA (ng), 1µl of each primer (10µM), 5µl of *Pfu* buffer with $MgSO_4$(10x), 2µl of deoxynucleotide triphosphate (10mM), 2µl of *Pfu* DNA polymerase (2.5 U/µl) (100 U) and 38µl of nuclease-free water.

Reaction conditions for the amplification of the PCR mixture is as follows: initial denaturation at 95°C for two minutes, 30 cycle of denaturation at 95°C for 30 sec, annealing at corresponding temperature for 45 sec and extension at 72°C for three minute. It was followed by a final extension at 72°C for ten minutes. The amplified PCR products were examined by 0.8% agarose gel and purified using Gene Jet PCR purification kit.

Cloning of the gelC, gelD and gelE gene: The purified gelD, gelE and pBBR122 vector were subjected to double digestion with restriction enzymes NcoI and EcoRI whereas the purified gelC were double digested with HindIII and PvuI. After double digestion, Gel extraction was done using thermo scientific GeneJET Gel extraction kit. The eluted DNA and vector was run on 0.8% agarose for confirmation of its purity. In order to generate the recombinant plasmid, gel eluted inserts (gelC, gelD and gelE) and vector were ligated using the enzyme T4 DNA ligase at 16^0C overnight.

E.coli DH5α cells were made competent by calcium chloride method and heat shock technique was carried out for the transformation of ligated samples (pBBR122-gelC, pBBR122-gelD and pBBR122-gelE) into the competent *E. coli* DH5α cells. The cells were then plated on LB medium containing antibiotic (33.3µg/ml) chloramphenicol for gelD and gelE and kanamycin (50µg/ml) for gelC respectively for colony screening .The plasmid DNA was isolated from the transformants according to the protocol of the plasmid DNA isolation kit and was run on 0.8% agarose gel for confirmation.

Table 1
The Primers used in this study

Primer names	Primer Sequence (5′–3′)
gelC-F	ACCCAAGCTTATGGTGAGTATTACCCAATTCT
gelC-R	ACCCCGATCGTCAGTTTACTGAAGCTCCGT
gelD-F	ACCCGAATTCATGAACGAAAACCGCCGGT
gelD-R	ACCCCCATGGTCAGAACAACCGTTCGCCGA
gelE-F	ACCCGAATTCATGGACGCGATGACCAGCG
gelE-R	ACCCCCATGGCTGATCCAAGTCAGTAGCCG

Confirmation of Clones: The presence of target gene was confirmed using three techniques:

Double digestion with restriction enzymes: The plasmid DNA was subjected to restriction digestion using restriction enzymes. After digestion of the plasmid DNA, sample was electrophoresed on 0.8% agarose gel.

Colony PCR: Colonies were picked from the antibiotic plate and screened by colony PCR in order to check for the presence of gene insert. Colony PCR was carried out using specific forward and reverse primers. The PCR products attained were examined using agarose gel electrophoresis.

Sequencing by Sanger di-deoxy method[9]: The plasmid DNA was sequenced by sanger method. The sequencing was carried out in order to validate the identity of the inserts. The nucleotide sequences for gelC, gelD and gelE were deposited at Gen-Bank.

Expression of gelC, gelD and gelE genes in *Sphingomonas paucimobilis* ATCC 31461: The recombinant plasmids (pBBR122-gelC, pBBR122-gelD and pBBR122-gel E) were transformed into *Sphingomonas paucimobilis* ATCC31461 by electroporation technique. *Sphingomonas paucimobilis* was streaked on YPG agar plate and grown overnight at 30°C. A single colony was picked from streaked YPG plate and grown in 10ml YPG medium at 30°C overnight with shaking at 200rpm. The 5ml of overnight culture was transferred to 500ml YPG medium and incubated at 30°C with shaking at 200rpm until the OD600 reached 0.6. Cell growth was examined by measurement of optical density at 600nm using a Genesys 10S UV-Visible spectrophotometer.

This culture was then incubated in ice for 15min and the cells were collected by centrifugation at 5000rpm for 20min at 4°C and the pellet was resuspended in 50ml sterile ice cold 50% glycerol. Centrifugation was repeated twice in order to collect the cells and the pellet was resuspended in 50ml sterile ice cold 50% glycerol.

After competent cell preparation 50µl aliquots of electrocompetent cells were stored at -80°C for subsequent procedure. Electroporation was done in a Bio-Rad MicroPulser apparatus using 1mm cuvettes containing 25µl of electrocompetent cells and 1µl of DNA. The cells and DNA were electroporated at 20°C with optimum time constant of 5ms at 1.8 kV. After electroporation, cells were instantly resuspended in 1ml of YPG broth, transferred to a falcon tube and incubated at 30°C with shaking at 200rpm for 1hr. Cells were then plated on YPG agar plates supplemented with the appropriate antibiotic chloramphenicol for gelD and gelE and Kanamycin for gelC and incubated at 30°C overnight. Transformants containing antibiotic resistance were isolated subsequent to overnight incubation. The cells were inoculated into YPG broth containing antibiotic and grown overnight at 30°C for 48hrs with 200 rpm shaking. The cell suspension were collected and centrifuged at 12,000rpm for 20minute at 4°C to remove cell debris and insoluble proteins. The resulting supernatant was used for SDS-PAGE[10].

Purification of Recombinant Protein: The recombinant protein obtained from the supernatant was purified by affinity chromatography using Ni-nitrilotriacetate (Ni-NTA) agarose column. The Ni-NTA column was initially washed with deionized water. The crude protein solution was loaded onto column pre equilibrated with lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0). The column was then washed with wash buffer (50 mM NaH_2PO_4, 300mMNaCl, 30 mM imidazole, pH 8.0). The protein was eluted from the column using elution buffer (50 mM NaH_2PO_4, 300mMNaCl, pH 8.0) with a stepwise increase in imidazole concentration 100-250mM[11].The purification steps were carried out at 4°C and the purified recombinant protein obtained was stored at -20°C. The purified protein and protein concentration were analyzed by SDS–PAGE and Lowry's method respectively[12].

Ammonium sulfate Precipitation: The ammonium sulfate was slowly added to the culture supernatant containing crude wild protein in order to precipitate the protein at saturation level 0-90%, incubated at 4°C overnight and followed by centrifugation at 12,000rpm for 20min. The obtained pellet proteins were resuspended in Tris-phosphate buffer. The ammonium sulfate fraction was then analyzed on 12% SDS-PAGE.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis: SDS-PAGE was done on 12% separating and 5% stacking polyacrylamide gels. The protein sample containing SDS-PAGE loading buffer was heated for 3minutes at 100°C in a dry bath. 20µl of the sample was loaded onto wells of a Clear PAGE Precast Gels positioned in a Dual Cool Mini-Vertical PAGE system comprising 1x SDS-PAGE running buffer. The SDS PAGE system was connected to an electric source and run at 70V for 3hr. Protein band was visualized by staining with Coomassie brilliant blue and the molecular mass of the protein was determined using protein ladder. The gel was visualized on a gel documentation system (Mediccare-Gelstan, India)[13]. The protein concentration was determined by the Lowry method[14] by measuring the optical density at 660nm using bovine serum albumin as standard. Standard curve of absorbance was plotted as a function of protein concentration.

Production of gellan gum using recombinant *S. paucimobilis:* YPG medium was used for the growth and maintenance of recombinant *S.paucimobilis* ATCC 31461. The YPG agar slants containing bacterial cells were incubated at 30°C for 48h and the fully grown slants were stored at 4°C.The inoculum was developed by transferring a loopfull of recombinant *S.paucimobilis* cells from YPG slant culture into 50mL of the YPG broth in a 250ml Erlenmeyer

flask. The flasks were aerobically incubated for 24h at 30°C, 250rpm on a rotary shaker.

The production of gellan was carried out in a 6.0- L stirred-type fermenter. The 10% inoculum (24h old) were inoculated into fermentor containing 4.5L of the YPG production medium. The temperature of fermentor was maintained at 30°C using a cooling system. The pH of the medium was maintained at 6.5 using pH probe. The dissolved oxygen (DO) in the broth was measured using a polarographic type electrode and DO was maintained at 100% saturation by adjusting air flow rate and agitation rate. The fermenter was operated for 48hr and the production of biomass and gellan were studied[15].

Isolation of gellan gum: After fermentation, the broth was heated for 15 min in a boiling water bath and cooled. The pH was adjusted to 10.0 by 2M NaOH. Then the broth was again heated for 10min in a water bath, cooled and pH was lowered to 7.0 by the addition of 2M H_2SO_4. The pre-treated broth was centrifuged at 8000rpm for 30min at 4°C to separate the cell mass. In order to remove any adhering polymers, the cells were treated with dimethyl sulphoxide and centrifuged at 8000rpm for 30min at 4°C. The cell pellet was then dried to a constant weight by keeping in a hot air oven at 80°C for 6h. The dry weight of cell was determined gravimetrically and expressed in g/l.

The cell free supernatant was utilized for the gellan recovery. The supernatant was added with three volumes of isopropyl alcohol and held at 4°C overnight to precipitate the polymer. The gellan precipitate was recovered by centrifugation at 8000rpm for 30min at 4°C and dried in a hot air oven at 80°C for 12h. The produced gellan was then determined gravimetrically and expressed in g/l[16].

Results and Discussion

Isolation of Genomic DNA and PCR amplification: The genomic DNA of *S.Paucimobilis* was isolated using the genomic DNA isolation kit and was used as a template for the gene amplification. High molecular weight single band was observed on agarose gel electrophoresis. The primers comprising appropriate restriction enzyme sites were designed using primer premier software. The gellan gum biosynthetic genes gelC, gelD and gelE were amplified by PCR. The PCR products were run on agarose gel electrophoresis in order to confirm the size of the product. The expected size of gelC, gelD and gelE are 1346bp, 902bp and 707bp respectively. Fig.1 represents PCR amplified DNA of gelC, gelD and gelE. The amplified gene products were found to be within its expected size.

Cloning of gellan gum biosynthetic genes gelC, gelD and gelE: The purification of the PCR products (gelC, gelD and gelE) was performed using Gene Jet PCR purification kit and the purity was checked using agarose gel electrophoresis. The purified genes gelD, gelE and pBBR122 vector were double digested using the restriction enzymes NcoI and EcoRI in order to create sticky end for ligation. The gene gelC was double digested with HindIII and PvuI. Digested genes gelC, gelD, gelE and pBBR122 vector were run on agarose gel and the gel pieces were incised and gel extraction was performed. The result showed that bands were observed on the corresponding size of vector and insert. The ligation reaction depended upon concentration of insert and vector with the optimum being 2:1 for insert and vector respectively.

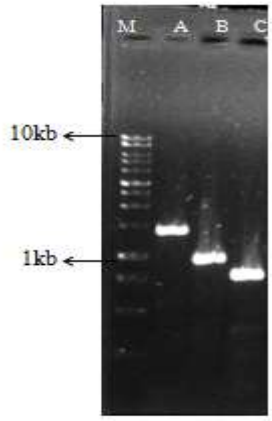

Figure 1: Agarose gel electrophoresis of amplified fragments of gelC, gelD and gelE: Lane M: 1kbp DNA Ladder, Lane A: gel C fragment showing band at 1346bp, Lane B: gelD fragment showing band at 902bp, Lane C: gelE fragment showing band at 707bp

The gel eluted pBBR122 vector and the genes gelC, gelD and gelE was ligated at 16°C overnight using T4 DNA ligase. The ligated samples were transformed into competent *E.coli* DH5α cells by calcium chloride transformation. *E. coli DH5α* colonies was transformed with ligated pBBR122 vector and inserts gelC, gelD and gelE were selected on the LB medium containing chloramphenicol for gelD and gelE and kanamycin for gelC. Several colonies appeared on antibiotic containing plates and single colonies were picked from the antibiotic plates, grown over night and plasmid DNA was isolated.

The isolated plasmids were examined on 0.8% agarose gel electrophoresis. The presence of desired DNA fragment in the cloning vector was confirmed by colony PCR, restriction digestion of plasmid DNA and sequencing. The isolated plasmids were double digested by restriction enzymes NcoI and EcoRI for gelD and gelE and HindIII, PvuI for gelC. The digested gene products were run on agarose gel electrophoresis and are illustrated in fig.2, fig.3 and fig.4. The results revealed that the presence of two bands that corresponds to the size of vector (5307bp) and genes gelC (1346bp), gelD (902bp) and gelE (707bp) validated the presence of clone. Colony PCR was carried out using forward and reverse primers in order to examine the presence of the gene insert. The transformation of *E.coli* with genes gelC, gelD and gelE ligated to the pBBR122 vector ensued many colonies of *E.Coli* comprising the

recombinant gelC-pBBR122, gelD -pBBR122 and gelE-pBBR122.One colony was selected from the antibiotic plate and colony PCR was performed. The products were loaded onto 0.8% agarose gel and are depicted in fig.5, fig.6 and fig.7.

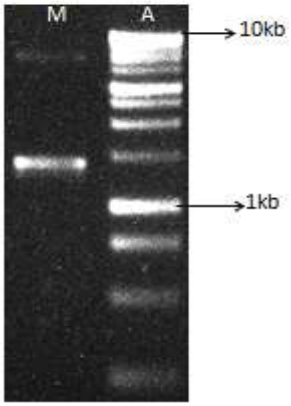

Figure 2: Verification of presence of gelC gene in the pBBR122 vector by restriction digestion: Lane M: Restriction digested clone with gelC and pBBR122 vector, Lane A: 1kbp DNA Ladder

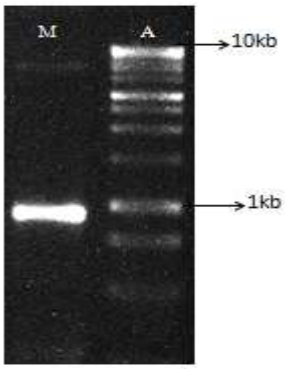

Figure 3: Verification of presence of gelD gene in the pBBR122 vector by restriction digestion: Lane M: Restriction digested clone with gelD and pBBR122 vector, Lane A: 1kbp DNA Ladder

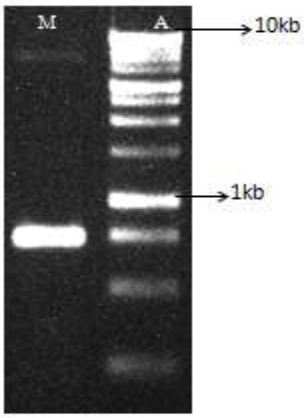

Figure 4: Verification of presence of gelE gene in the pBBR122 vector by restriction digestion: Lane M: Restriction digested clone with gelE and pBBR122 vector, Lane A: 1kbp DNA Ladder

The colony PCR of the selected colony resulted in amplification of the expected fragment size gelC (1346bp), gelD (902bp) and gelE (707bp) and thus confirming the insertion of the genes gelC, gelD and gelE into pBBR122 vector.

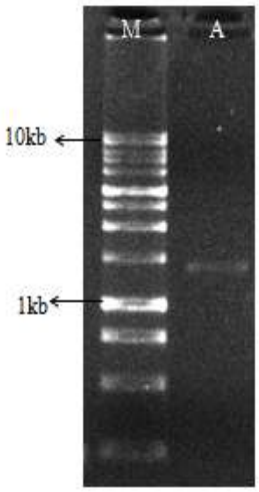

Figure 5: Colony PCR confirmation of gelC: Lane M: 1kbp DNA Ladder, Lane A: gelC fragment showing band at 1346bp

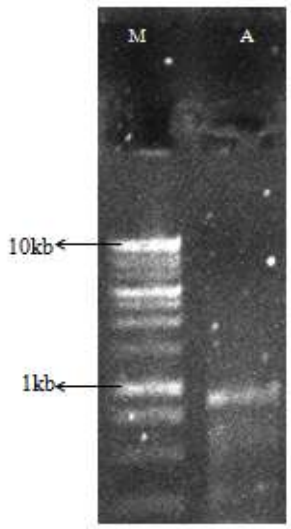

Figure 6: Colony PCR confirmation of gelD: Lane M: 1 kbp DNA Ladder, Lane A: gelD fragment showing band at 902 bp

The sequencing of the cloned gene was carried out by using specific primers based on the gene sequence. Sequence homology was analyzed using software basic local alignment search tool (BLAST) from the National Center for Biotechnology Information (NCBI).

The nucleotide sequences for gelC, gelD and gelE from *Sphingomonas paucimobilis* ATCC 31461 were submitted to Genbank with assigned accession number MK430035, MK430034 and MK430033 respectively.

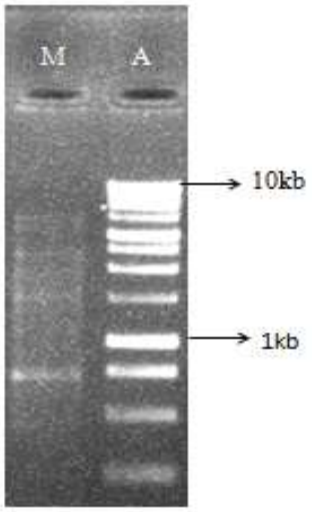

Figure 7: Colony PCR confirmation of gelE: Lane M: gelE fragment showing band at 707 bp, Lane A: 1kbp DNA Ladder

Expression and Purification of Recombinant Protein: The recombinant plasmids comprising the gene of interest were transformed into *Sphingomonas paucimobilis* ATCC 31461 by electroporation method. The expressed cells were grown on YPG agar plates supplemented by antibiotic. Single colonies were picked from the antibiotic plate and inoculated into YPG broth followed by incubation at 30°C overnight. The cell suspension was then centrifuged at 12,000rpm for 20minutes and the supernatant containing the recombinant proteins were collected.

This supernatant containing crude wild protein were subjected to ammonium sulfate precipitation and was followed by the separation of ammonium sulfate fraction using SDS –PAGE. The recombinant proteins were purified using Ni-NTA resin affinity chromatography due to the presence of His-tag containing expression vector pBBR122. The bound recombinant proteins were eluted from the Ni-NTA column using 250mM imidazole at 4°C. The purified recombinant gellan proteins were analyzed by SDS-polyacrylamide gel electrophoresis.

The supernatant of the control and recombinant strains were used for SDS-PAGE. SDS-PAGE analysis revealed the molecular mass of the recombinant proteins. The molecular weight of the recombinant gellan proteins gelC, gelD and gelE were found to be 110KDa, 74KDa and 60KDa respectively. The gels were stained with Coomassie blue and photographed as illustrated in fig.8, fig.9 and fig.10. The size of the band was approximated using molecular weight markers. The protein concentration was determined using Lowry method and the concentration of recombinant protein was found to be 0.46 mg/ml purified protein.

Comparison of Biomass and gellan gum productivity of wild type and recombinant strains: The recombinant S*phingomonas paucimobilis* carrying genes gelC, gelD and gelE and wild type strain were cultured in the fermenter. The result of the fermentation runs (Table 2) showed that the maximum biomass and gellan gum production was observed for recombinant strain gelD compared to other strains. The maximum biomass and gellan gum production yielded with the wild-type strain was 7.18±0.3g/l and 10.68±0.1g/l, and the recombinant gelD was 12.77±0.3g/l and 15.51±0.2g/l respectively. The above observation indicated that when comparing the wild type strain to the recombinant one, the gellan gum yield was double for recombinant strains.

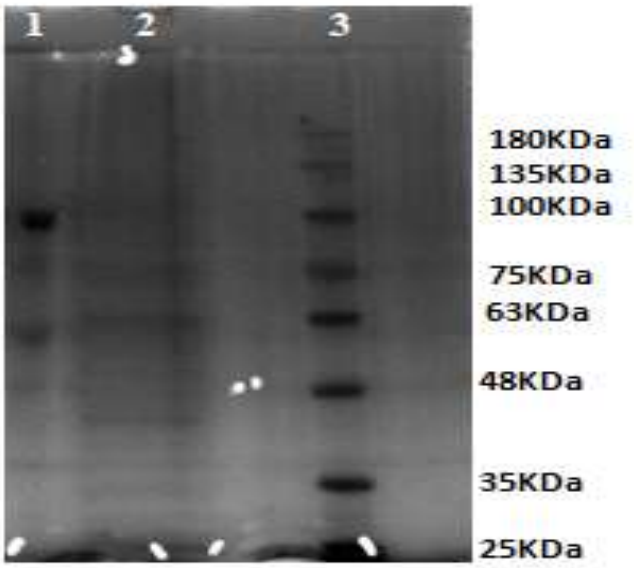

Figure 8: SDS-PAGE analysis of the recombinant gelC gene expressed in *Sphingomonas paucimobilis* ATCC 31461: Lane 1: Purified recombinant gelC protein, Lane 2: Crude extract, Lane 3: Protein marker

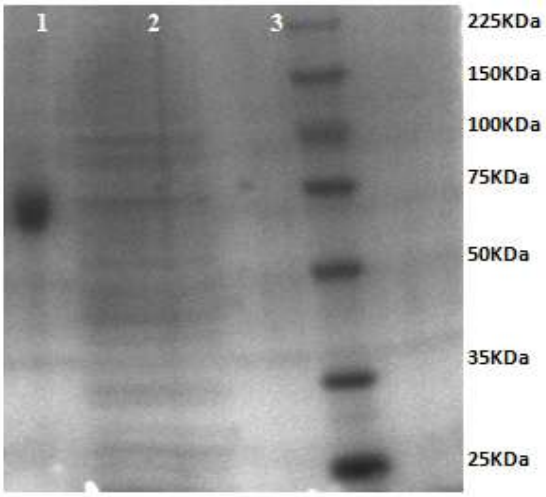

Figure 9: SDS-PAGE analysis of the recombinant gelD gene expressed in *Sphingomonas paucimobilis* ATCC 31461: Lane 1: Purified recombinant gelD protein, Lane 2: Crude extract, Lane 3: Protein marker

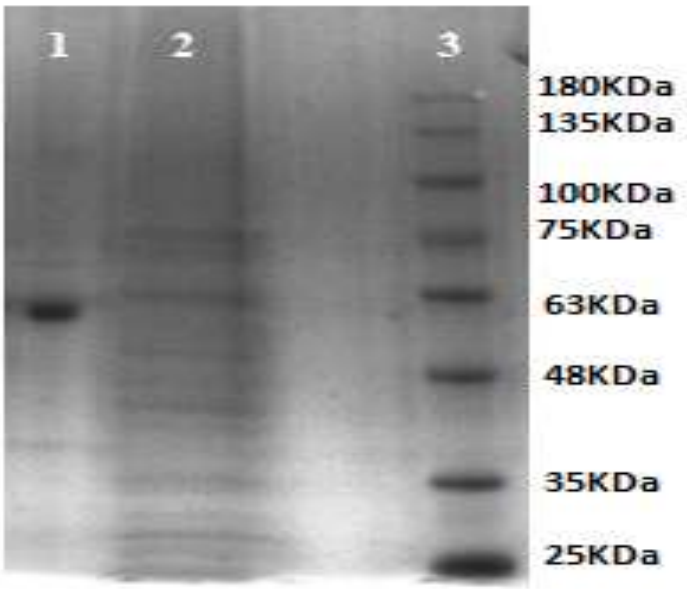

Figure 10: SDS-PAGE analysis of the recombinant gelE gene expressed in *Sphingomonas paucimobilis* ATCC 31461: Lane 1: Purified recombinant gelE protein, Lane 2: Crude Extract, Lane 3: Protein marker

Table 2
Biomass and gellan gum yield for recombinant strain carrying gelC, gelD and gelE

Recombinant Strain	Biomass yield (g/l)	Gellan gum yield (g/l)
gelC	8.84±0.1	12.93±0.1
gelD	12.77 ±0.3	15.51 ±0.2
gelE	10.58±0.2	13.42±0.3

Conclusion

Gellan gum is a bacterial polysaccharide which has wide applications in the food and pharmaceutical industries. The properties of gellan gum mainly gelling, textural and rheological characteristics makes gellan gum one of the industrially beneficial exopolysaccharides. Gellan genes gelC, gelD and gelE from *Sphingomonas paucimobilis* ATCC 31461 were amplified and cloned into pBBR122 vector. The cloned gellan genes were over expressed in expression host *Sphingomonas paucimobilis* ATCC 31461 using the electroporation technique. The recombinant protein was purified by Ni-NTA affinity chromatography. The purified protein migrated as a single band on SDS-PAGE and the analysis revealed the molecular mass of the recombinant proteins.

Concentration of purified protein was estimated by Lowry method. The recombinant strain carrying gelD showed higher gellan gum production (15.51g/l) compared to the wild type as well as other recombinant strains gelC and gelE. The present study indicated that multiple expression of gelD in *Sphingomonas paucimobilis* increased the production of gellan gum significantly.

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