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Induction of Highly Gellan Gum Productive *Sphingomonas paucimobilis* Strain(s) via Conjugation Experiments

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Abstract: Via conjugation technique, three attempts were done to construct new *Sphingomonas* strains with high production of gellan using three *Sphingomonas* strains; one wild type *Sphingomonas paucimobilis ATCC 31461* (P0) and two local strains; *Sphingomonas paucimobilis CAAS6* (P1) and *Sphingomonas paucimobilis CAAS6* (P2).Three tansconjugante were obtained, one for each attempt. These transconjugants were *Sphingomonas paucimobilis* NRC-C01, C02 and C03 with gellan production; 15.9, 10.5 and 11.9 g/l, respectively. SDS-PAGE protein fingerprinting techniques, Gel documentation system and image analysis Gel works 1D advanced software which used in this study in order to emphasize how much the difference between each of the three parents and their transconjugants.

Key words: *Sphingomonas paucimobilis,* conjugation, transconjugants, SDS-Protein patterns.

INTRODUCTION

Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea (Sphongomonas paucimobilis),* purified by recovery with isopropyl alcohol, dried, and milled. Gellan gum is a microbial polysaccharide of great commercial interest, especially in food industry which was used as a thickening, stabilizer of the gelled dessert; jam, jelly, pudding, confectionery, sugarcoating of confectionery and other applications; pharmaceutical industries (capsules), perfumes, cosmetics, etc.[9] Gellan has been widely employed as a gelling agent in plant biotechnology under the trade name of gelrite or phytogel; it can also be used in place of agar in bacterial culture media[13]. Also, it may be used to make electrophoresis gels in a range of polymer concentrations and buffer compositions. Once the gellan gum particles are in solution, the divalent cation is added. The divalent cation most commonly used to cast the gels is calcium; however, magnesium may also be used. The gels are characterized as mechanically strong yet brittle, which means that the gels will crack when not supported. Gellan electrophoresis gels as low as 0.03% may be constructed. These low concentration gels are preferable, because when converted back to solution, there are lower concentrations of gellan gum present in the resulting DNA solutions[1]. Gellan gum gels are prepared by cooling hot solutions in the presence of appropriate cations. These gels show a large melting/setting syneresis similar to agar gels. Although gellan gum has not yet been approved for food use, it shows potential for use in a number of foods including milk gels and acidified milk products. The range of molecular weight can be separated by gellan gum electrophoresis between (1.0 kbp to 48.5 kbp) by using 0.1% gellan gum formed with 5 mmol/L $CaCl_2$. Liu *et al.*[7]. Recent reviews showed the importance of gene mechanisms in the molecular biology studies of bacterial strains in constructing new or improved strains via recombinant DNA technology[14]. Conjugation is one of these mechanisms and it is major mode of gene transfer among prokaryotes, e.g., bacteria as a tool of gene transfer in different environment[11]. Conjugation can be used to transferred plasmids from *Lactococcus loctis* into *Xanthomonas campestris* to obtain transconjugants can produce high quantity of xanthan gum and proteinase on medium containing whey. After several generations (about 20) 67% of the colonies tested were resistant to chloroamphenical[3,10].

In this investigation, conjugation technique was used to construct new *Sphingomonas* transconjugants strains with high production of gellan gum.

MATERIALS AND METHODS

Three *Sphingomonas* strains with high production of gellan were used in this work; one wild type *Sphingomonas paucimobilis ATCC 31461* (P0) and two local strains; *Sphingomonas paucimobilis CAAS6* (P1) and *Sphingomonas paucimobilis CAAS6* (P2). For isolation, preservation and standard inoculums preparation S medium was used[15]. Via conjugation technique described by Ekateriniadou *et al.*[3]and Liu *et*

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al.[6] three attempts were done to produce new *Sphingomonas* strains with high production of gellan using the above three *Sphingomonas* strains. These strains have distinctive antibiotic markers which beneficial in transconjugants selection.

In order to select transconjugants between each two parents, the cell suspension of these two parents were gently mixed and plating them on solid medium nutrient agar. After 18 hours of incubation at 30°C, 100ml, sample was plating on antibiotic selective medium. Cells that resisted of antibiotics were selected *i.e*. conjugate cells. The above method was repeated three times for each attempt. Total protein extraction and banding patterns SDS-polyacrylamid gelelectrophoresis for *Sphingomonas paucimobilis* will be performed according to Sheri *et. al.*[12] and Davis *et al.*[2].

Gel documentation system, image analysis Gel works 1D advanced software, was used for more accurate analysis and comparison between the local strains via biochemical genetic analysis. This method is recommended to determine the relationship within and between of species[4].

RESULTS AND DISCUSSIONS

Conjugation Experiments: Results in Table (1) proved the success of transconjugants formation for the three attempts between the three *Sphingomonas paucimobilis* strains. One tansconjugante was result for each attempt. The first tansconjugante *Sphingomonas paucimobilis* NRC-C01 was obtained between the two parents *Sphingomonas paucimobilis ATCC 31461* and *Sphingomonas paucimobilis CAAS6*. The second tansconjugante *Sphingomonas paucimobilis* NRC-C02 was obtained between the two parents *Sphingomonas paucimobilis ATCC 31461* and *Sphingomonas paucimobilis CAAS11*.The last tansconjugante *Sphingomonas paucimobilis* NRC-C03 was obtained between the two parents *Sphingomonas paucimobilis CAAS6* and *Sphingomonas paucimobilis CAAS11*. These transconjugants have polysaccharide values; 15.9, 10.5 and 11.9 g/l for *Sphingomonas paucimobilis* NRC-C01, NRC-C02 and NRC-C03, respectively.

Data in Table (1) show that the tansconjugante *Sphingomonas paucimobilis* NRC-C01 had maximum efficiency of gellan production followed by tansconjugante *Sphingomonas paucimobilis* NRC-C03 and C02. Tansconjugante *Sphingomonas paucimobilis* NRC-C01 had maximum efficiency of gellan production it have about two fold and half than the higher efficiency of gellan production parent *Sphingomonas paucimobilis ATCC 31461* and about four times than its lower efficiency of gellan production parent *Sphingomonas paucimobilis CAAS6*. on the other hand it have gellan production efficiency about three times of gellan production efficiency average than its two parents, approximately.

The other two transconjugants; *Sphingomonas paucimobilis* NRC-C02 and C03 showed efficiency of gellan production about two fold and half than their parents average, approximately. These results are in agreement with Ekateriniadou *et al.*[3] they constructed new strains (transconjugants) of *Xanthomonas campestrus* (gram negative bacteria) between by conjugation technique with *Lactococcus lactis* capable of producing xanthan gum from whey in large quantities and Lloyd and Low[8] obtained transconjugants between *Escherichia coli* and *Salmonella*. Also, in 2000, Liu and others construct new strains (transconjugants) between two strains of *Thiobacillus ferrooxidans* using this technique.

SDS-PAGE Protein Fingerprinting:

Protein Banding Pattern: Total protein extraction and banding patterns SDS-polyacrylamid gel electrophoresis for five treatments resulting from using three *Sphingomonas paucimobilis* strains and their transconjugants are illustrated in Fig.(3). There are observable differences in the protein banding pattern for all the parents and their transconjugants. Some minor differences in banding patterns between the parents from one side and between the transconjugants from other side which resulting from the three conjugation attempts. The three parents and their transconjugants on the gel were; *Sphingomonas paucimobilis ATCC 31461* (lane 1), *Sphingomonas paucimobilis* CAAS6 (lane 2), Sphingomonas paucimobilis CAAS11 (lane 3), Sphingomonas paucimobilis NRC- C01 (lane 4), Sphingomonas paucimobilis NRC- C02 (lane 5) and Sphingomonas paucimobilis NRC- C03 (lane 6).

Data from Fig (3) also revealed that the total bands number for the parents and there transconjugants ranged from 7 bands for the tansconjugante *Sphingomonas paucimobilis* NRC- C03 (lane 6), to 19 bands for tansconjugante *Sphingomonas paucimobilis* NRC- C01 (lane 3). The higher total number of bands for the three parents and there conjugates were 19 bands for the tansconjugante *Sphingomonas paucimobilis CAAS11* followed by the parent *Sphingomonas paucimobilis CAAS11* (18 bands), the two parents *Sphingomonas paucimobilis CAAS11& Sphingomonas paucimobilis* ATCC 31461 (15 bands of each) and in the end 12 bands for tansconjugante *Sphingomonas paucimobilis CAAS6*. The molecular weight ranged from 14, 4 to 94 KDs. There are common bands found in all strains. The results also revealed that the two strains *Sphingomonas paucimobilis ATCC 31461* and *Sphingomonas*

Table 2: Characteristics of three *Sphingomonas paucimobilis* strains; one wild type and two local strains in three recombinations and their transconjugants.

Parents				Transconjugants		
Strain			Recombination	Strain	Polysaccaharide g/l	
Sphingomonas *paucimobilis* ATCC 31461*á	PO	6.4	PO × P1	Sphingomonas *paucimobilis* NRC- C01á	15.9	Nm^r Amp^r Cm^r Sm^r
Sphingomonas *paucimobilis* CAAS6**á	P1	4.6	PO×P2	Sphingomonas *paucimobilis* NRC- C02á	10.5	Nm^r Amp^r Cm^r
Sphingomonas *paucimobilis* CAAS11**á	P2	4.1	P1×P2	Sphingomonas *paucimobilis* NRC- C03á	11.9	Nm^r Amp^r Cm^r Sm^r

** Local strains ; * Wild type strainá

Fig 3: The SDS-PAGE total protein pattern for the three parents their transconjugants; Sphingomonas paucimobilis ATCC 3146 (lane1) Sphingomonas paucimobilis CAAS6 (lane2), Sphingomonas paucimobilis CAAS11 (lane3), Sphingomonas paucimobilis NRC- C01 (lane4), Sphingomonas paucimobilis NRC- C01 (lane5) and Sphingomonas paucimobilis NRC-C01 (lane6). Lane (lane M) the marker with molecular weigh (12 to 97 KDs).

paucimobilis NRC- C03had one monomorphic and six polymorphic bands. Each of *Sphingomonas paucimobilis CAAS6* & *Sphingomonas paucimobilis* NRC-C02 had three monomorphic and 12 & 18

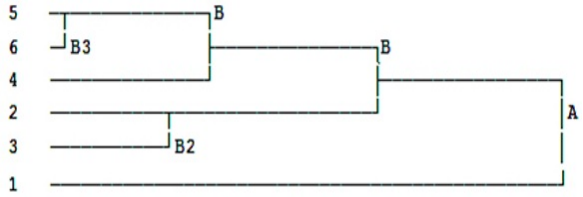

Fig. 4: Dendogram tree for the three parents their conjugates; *Sphingomonas paucimobilis* ATCC 3146 (1) *Sphingomonas paucimobilis* CAAS6 (2), *Sphingomonas paucimobilis* CAAS11 (3), *Sphingomonas paucimobilis* NRC- C01 (4), *Sphingomonas paucimobilis* NRC- C01 (5) and *Sphingomonas paucimobilis* NRC- C01 (6).

polymorphic bands, respectively and the parent *Sphingomonas paucimobilis CAAS11had two* monomorphic and 16 polymorphic bands. The wild type strain *Sphingomonas paucimobilis ATCC 31461* have not any unique or specific band.

Statistical Analysis for SDS-PAGE data: Package SPSS system. Significant different were determine at $p<0.05$**.** Data from SDS-PAGE were pooled and transferred into 1 and o, their were interred into the input of the program as shown in the dendogram below (Fig. 4). The statistical analysis data were carried out with the statistical software according to the method which described by Iruela[5]. The dendogram generated by (Gel works 1D) analysis confirmed the above pattern of diversity using SDS-PAGE. The total three parents their conjugates were classified for the dendogram into two main pool clusters; A and B. The first cluster (A) includes one the wild type strain *Sphingomonas paucimobilis ATCC 31461*, while the other cluster (B) include the rest strains with different

distance between them. This strains so far from the other clusters with genetic with completely100% dissimilarity (or 0.0% similarity) than the other strains. The two strains; *Sphingomonas paucimobilis* NRC- C02 and *Sphingomonas paucimobilis* NRC- C03 have a one group cluster (B3) with very close distance between them (96 % similarity). This result is in parallel with the data in Figure (4) which showed that the two conjugates strains produced equal approximately efficiency of gellan gum. The conjugant *Sphingomonas paucimobilis* NRC- C03 have one group (cluster B1) near the last cluster (B3) but it is little distance from them (68% similarity). The two parents strains; *Sphingomonas paucimobilis CAAS6 and Sphingomonas paucimobilis CAAS11* have a one group (cluster B2) with close distance between them (about 78% similarity) and far the last cluster (B1) it is have distance from them (38% similarity) and have a common ancestor the clusters B 1.

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