*J. Agric. Sci. Mansoura Univ., 32 (10): 8263 - 8271, 2007*

# GENETIC IMPROVEMENT OF HIGHLY GELLAN GUM PRODUCING STRAINS OF *Sphingomonas paucimobilis* via PROTOPLAST FUSION

**Abd-El-Aal, S. kh.[1] and M. S. Asker[2]**

**[1]Microbial Genetic Dept., National Research Center, Cairo, EGYPT**

**[2]Microbial Biotechnology Dept., National Research Center, Cairo, Egypt**

## ABSTRACT

**T**hree local strains; *Sphingomonas paucimobilis Kb1( S.p.Kb1), Sphingomonas paucimobilis CAAS11*, *(S.p.CAAS11*) and *Sphingomonas paucimobilis .CAAS6 S.p (CAAS6)* were used throughout this investigation to improve their potentialities as gellan gum producers. Three of protoplast fusion experiments were done between the three *Sphingomonas paucimobilis* local strains; *S.p.Kb1* (P1), *CAAS6* (P2) and *S.p.CAAS11* (P3) at all possible combinations and the fusants were obtained after 1 hr incubation period. Four fusants were obtained after one hour producing gellan gum higher than their parents. The obtained fusant *Sp2::Sp3- F4* from the third protoplast fusion experiment P2:: P3 is the best in their gum efficiency rate (two times and half than their parents), followed by the two obtained fusants; *Sp1::Sp2- F1* and *Sp1::Sp3-F2*, from the other two experiments; P1 :: P2 and P1 :: P3, respectively have the same gum efficiency rate, two times than their parents. They have higher viscosity values than that of their parents. All the obtained four fusants have promising economic value and can be used from further genetic improvement.

***keywords;*** *Sphingomonas paucimobilis*, Protoplast fusion, Polysaccharides, Gellan gum, Viscosity and genetic improvement

## INTRODUCTION

Many microorganisms produce extracellular polysaccharides. The main reason is unknown, but two possibilities seem likely. Polysaccharides may serve as a protective barrier against adverse environmental conditions or may facilitate colonization or attachment to surfaces (Costerton *et al.,* 1981). *Sphingomonas paucimobilis* strains are of agriculture and industrial importance due to their production of useful polysaccharide, gellan gum (Murooka *et al.*, 1987). It is composed of a repeating linear tetrasaccharide with d-glucose (Glc), d-glucuronic acid (GlcA) and l-rhamnose residues in a 2:1:1 ratio with glycerate and acetate substitutions (Videira *et al.,* 2001).

Gellan gum is an extracellular polysaccharide obtained from an aerobic culture of *Pseudomonas elodea* (Sutherland 2002). Gellan gum is 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. (Nitrchke *et al.,*2001). Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Sphingomonas paucimobilis* using D-glucose or corn syrup as the major carbon source (Kennedy and Bradshaw, 1984).

The main objective of this study was to improve gellan gum production strain(s) via protoplast fusion technique to induce new genetic recombination.

Protoplast was prepared from three parents different in their gellan gum production and also differs in their antibiotic marker in all possible combinations.

## MATERIALS AND METHODS

### I-MATERIALS

**1.1- Bacterial strains:**

Three *Sphingomonas paucimobilis* local identified strains are used in this study and listed in Table (1).

**Table (1): The bacterial strains and its properties.**

| Strains | Viscosity (CPS) | Polysacc-haride (g/l) | Code |
|---|---|---|---|
| *Sphingomonas paucimobilis Kb1* | 3210 | 5.0 | P1 |
| *Sphingomonas paucimobilis CAAS6* | 3500 | 4.6 | P2 |
| *Sphingomonas paucimobilis CAAS11* | 3770 | 4.1 | P3 |

**1.2- Media**

**1.2.1-** Maintenance and propagation medium was used for gellan production by all tested *Sphingomonas paucimobilis* cultures_(Videira *et. al.,* 2001 and Martins *et al.,* 1996).

**1.3- Buffers and Reagents**

**1.3.1-**TBE buffer and TAE B buffer; 10X were prepared and used for plasmid isolation according to Maniatis *et al.(* 1982 ).

**1.3.2-** Gel- Loading Mix; 0.25 % Bromophenol blue and 30% glycerol.

**1.3.3-** Phenol saturated with 1.0 M Tris-HCl, pH 8.0.

**1.3.4-** The Agarose – gel ; 0.7 % in TAE buffer.

**1.3.5-** Protoplast buffer was prepared and used according to El-Gaali Eisa *et al*. (1995).

### II-METHODS

**1.1 -** Protoplast fusion of *Sphingomonas paucimobilis* strains was carried out according to El-Gaali Eisa *et al*. (1995).

**1.2 -** 4-Isolation of *Sphingomonas paucimobilis* antibiotic resistant strains was done according to Weng *et al*. (1996)

**1.3 -**Gellan gum determination method was used as described by Martins and SA Coreeia (1994) .

**1.4 -**Viscosity determination method was done according to that described by Abd El-Aal (2000).

## RESULTS AND DISCUSSION

The aim of this investigation is to improve the production of gellan gum from *Sphingomonas paucimobilis* (local strain) using protoplast fusion technique.

Some attempts to construct superior *Sphingomonas paucimobilis* strains protoplast fusion was done. Enzymatic removal of the cell wall, in the

presence of an osmotic stabilizer such as sucrose, generates protoplasts in which large areas of the cytoplasmic membrane are exposed. The formation and regeneration of protoplasts is a key feature of an allied procedure known as protoplast fusion, where the addition of PEG to a mixture of protoplasts of two different strains results in the formation of a fused cell that contains the complete genome of both parents (at least transiently). Protoplast fusion has been more widely used for eukaryotic cells (Jeremy, 1998)

**1- Genetic markers:**

Isolation of *Sphingomonas paucimobilis* genetically stable markered strains (antibiotic sensitivity or resistant) were necessary to facilitate tracing and manipulating of desired strains through genetic experiments.

Six of the more frequently antibiotics applied in genetic studies with known mode of action; kanamycin (Km), chloramphincol (Cm), rifampicin (Rf), tetracycline, (Tc) streptomycin (Sm), neomycin (Nm) and ampicilline (Am) at concentrations; 50μg, 30μg, 150μg, 150μg, 200μg, 100μg and 150μg, respectively, were used with three strains; *Sphingomonas paucimobilis Kb1, Sphingomonas paucimobilis CAAS6* and *Sphingomonas paucimobilis CAAS11* (P1,P2 and P3 respectively) in order to obtain parental strains genetically marking (Moazed and Noller, 1987).

All the three identified strains; P1, P2 and P3 were sensitive to three antibiotics; Sm, Nm and Rf, but they showed different resistant patterns versus the four antibiotics; Am, Cm, Km and Tc. Strain (P2) was sensitive to the antibiotic Ac and resistant to the antibiotics; Km, Cm and Tc, while the strains; P1and P3 were resistant to Am and sensitive to Cm. In addition P1 was sensitive to Cm and Tc , while it was resistant to Am and Km. In the contrary, strain P3 was resistant to Ac and Tc while it was sensitive to Km and Cm (Table 2).These results provides genetic markers required for selection of fusants.

**Table (2): genetic marking of *Sphingomonas paucimobilis* using different antibiotics.**

| code | Strains | Am | | | | Km | | | | Cm | | | | Tc | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Am | Am Km | *Am* ***Cm*** | *Am* ***Tc*** | Km | Kn Am | Kn Cm | Kn Tc | Cm | Cm Am | Cm Km | Cm Tc | Tc | Tc Am | Tc Km | Tc Cm |
| P1 | *Sphingomonas paucimobilis Kb1* | + | + | + | + | + | + | - | - | - | + | - | - | - | + | - | - |
| P2 | *Sphingomonas paucimobilis CAAS6* | - | + | + | + | + | + | + | - | + | - | - | + | + | - | - | - |
| P3 | *Sphingomonas paucimobilis CAAS11* | + | + | + | + | - | - | - | + | - | - | - | + | + | + | + | + |

**2- Protoplast fusion:**

**2.1- Protoplast induction and regeneration efficiency.**

Strains; P1,P2 and P3 which characterized as distinctive strains for gellan gum production were chosen for genetic improvement attempts via protoplast fusion. The primary step was induction of protoplasts parental strain by enzymatic treatments to remove the cell wall (El-Gaali Eisa *et al.,* 1995).

In attempts to construct *Sphingomonas paucimobilis* strains protoplast fusion were done. Lysozyme was added to the treated *Sphingomonas*

*paucimobilis* cells at final concentration of 1.5-% mg/ml and incubated at 37°C for 3 hr. at 40rpm. Protoplast induction was followed periodically by microscopic examination. The results obtained revealed that almost all enzymatic treated cells were converted into protoplasts .

In order to determine the efficiency of protoplast induction and number of regenerated protoplasts for each parental strain two equal samples of pretreated cells, protoplast suspension, were used. The first sample was spreaded onto YM medium after osmotic shock and the number of intact cells was counted (Table 3). The second sample was added to the top layer medium then overplayed on the regeneration medium and grown cells were also counted. From these results the number of regenerated protoplasts and efficiency of protoplast induction for each parental strain were calculated (Table 3).

**Table (3): Regeneration efficiency of induced protoplast of parental *Sphingomonas paucimobilis* strains.**

| Strain | Regenerated and intact cells /ml | Intact cells /ml | Protoplasts /ml | Regeneration efficiency % |
|---|---|---|---|---|
| P1 | 910 | 510 | 440 | 48.4 % |
| P2 | 780 | 370 | 500 | 64.1 % |
| P3 | 490 | 180 | 270 | 55.1 % |

Data in Table (3) revealed that the protoplast induction for the parental strains P1, P2 and P3 were; 48.4 %, 64.1 % and 55.1 %, respectively. Strain P2 showed higher regeneration efficiency of protoplast induction followed by P3 and P1. On the contrary, Strain P1 showed higher efficiency of gellan production followed by P2 and P3. These results were in agreement with those observed by El-Gaali Eisa *et al*. (1995). Such results are consistent with those obtained by Pigac *et al*. (1981) whom got frequencies of protoplasting range from 56 to 75% depending upon the genome of mutants originized from a slant of *S.rimosus.*

**2.2- Intra- specific protoplast fusion**

Three attempts to obtain *Sphingomonas paucimobilis* fusants were carried out using protoplast fusion technique between induced protoplasts of the parental strains as follow; (P1 :: P2), (P1 :: P3) and (P2 :: P3) for one hour incubation period.

**2.2.1- Protoplast fusion (P1:: P2)**

This attempt was carried out between the two parental strains; P1 and P2 which have distinctive characters illustrated before in Tables (2 and 3). Table(4) represents the characters of the parental strains and their one genetically stable fusant obtained after one hour of incubation period and designated *Sp1::Sp2- F1* (F1). This fusant showed higher efficiency of gellan production than their parents; 11.9 g/l. On the other hand, data in table (4) revealed that the gellan gum production ratio for the obtained fusant was 208% (about two folds) comparing with the mean of gellan gum production of the inserted parental strains (5.7 g/l). The results also showed that fusant, had higher efficiency of gellan production than their parental strains. The

results were in agreements with the previous results obtained by Papoutsopoulou *et al* (1994).

**Table (4): Characteristics of the parental strains; P1 & P2 and their fusants obtained after one hour incubation period**.

| Code | Strains | Antibiotic | Polysaccha-ride g/L |
|---|---|---|---|
| P1 | *Sphingomonas paucimobilis Kb1* | $Am^r$ $Km^r$ $Cm^s$ $Tc^s$ | 5 |
| P2 | *Sphingomonas paucimobilis CAAS6* | $Am^s$ $Km^r$ $Cm^r$ $Tc^r$ | 6.4 |
| F 1 | *Sp1::Sp2- F1* | $Am^r$ $Km^r$ $Cm^r$ $Tc^r$ | 11.9 |

**2.2.2 Protoplast fusion (P1:: P3)**

In this attempt two parents; P1 and P3 were used and their characters were illustrated in Tables (2 and 3). Table (5) proved the success of fusant formation between the parents; P1 and P3. Four genetically stable fusant were obtained after one hour of incubation period and designated *Sp1::Sp3-F2* (F2). All obtained fusant showed higher efficiency of gellan production than their parents (10.8 g/L). Also, data in table (5) showed that gellan production ratio for these fusant was 194% (about two folds) comparison with the mean of gellan gum production of the inserted parental strains (5.55g/l). These results were in agreements with the previous results obtained by Thorne *et al.* (1988)

**Table (5) Characteristics of the parental strains; P1 and P3 and their fusants after one hour incubation period**.

| **Code** | **Strains** | **Antibiotic** | **Polysaccha-ride g/L** |
|---|---|---|---|
| P1 | *Sphingomonas paucimobilis Kb1* | $Am^r$ $Km^r$ $Cm^s$ $Tc^s$ | 5 |
| P3 | *Sphingomonas paucimobilis CAAS11* | $Am^r$ $Km^s$ $Cm^s$ $Tc^r$ | 6.1 |
| F 2 | *Sp1::Sp3- F2* | $Am^r$ $Km^r$ $Cm^r$ $Tc^r$ | 10.8 |

**2.2.3- Protoplast fusion (P2:: P3)**

Two parental strains; P2 and P3 were used in this trial and their two fusants successfully obtained after one hour of incubation are present in Table (6). These fusants designated; *Sp2::Sp3-F3* (F3), and *Sp2::Sp3-F4* (F4), showed higher efficiencies of gellan production 9.4 and 15.6 g/l, respectively than both parents. In addition, data in Table (6) showed that the gellan production ratios for these fusants were; 150 % (one time and half) for F3 and 252% (about two times and half) for F4 comparing with the mean of gellan gum production of the inserted parental strains (6.25 g/l). The fusant F4 has highest efficiency of gellan gum production (compare with F3) about 234% and 256% of that of P2 and P3 strains, respectively while the another fusant have gellan gum efficiency were 147% and 154% of the same parents. These results were in agreements with that reported by Aralova *et al.* (1993).

**Table (6): Characteristics of parental strains; P2 & P3 and their fusants after one hour incubation period**.

| Code | Strains | Antibiotic | Polysaccha-ride g/L |
|---|---|---|---|
| P2 | *Sphingomonas paucimobilis CAAS6* | $Am^s$ $Km^r$ $Cm^r$ $Tc^r$ | 6.4 |
| P3 | *Sphingomonas paucimobilis CAAS11* | $Am^r$ $Km^s$ $Cm^s$ $Tc^r$ | 6.1 |
| F3 | *Sp2::Sp3- F3* | $Am^r$ $Km^r$ $Cm^r$ $Tc^r$ | 9.4 |
| F4 | *Sp2::Sp3- F4* | $Am^r$ $Km^r$ $Cm^r$ $Tc^r$ | 15.6 |

**3 - Viscosity determination**

The obtained four fusants from three protoplast experiments were determined their viscosity in comparison with their parental strains; P1, P2 and P3. The differences in the viscosity readings (measuring with c p) are high as presented in table (7). Positive relationship was showed between the gellan gum production (measuring as polysaccharide g/L) and its viscosity readings for the three parents and there four fusants.

Also, table (7) showed that all the four fusants had higher viscosity values than that of their parents and there are clear differences in viscosity values between them. The maximum viscosity value was observed for fusant F4 (1420 cP) which has high gellan gum production (14.3 g/l), while the minimum viscosity value was 880 (cP) for fusant F3, which has gellan gum production (9.4 g/l).

**Table (7): Viscosity readings and Polysaccharide of the three *Sphingomonas paucimobilis* parents and their four fusants.**

| Strains | Viscosity values (cP) | Polysaccharide g/L |
|---|---|---|
| P1 | 315 | 5 |
| P2 | 470 | 6.4 |
| P3 | 390 | 6.1 |
| F1 | 1250 | 11.9 |
| F2 | 1190 | 10.8 |
| F3 | 880 | 9.4 |
| F4 | 1420 | 15.6 |

Generally, the obtained fusant *Sp2::Sp3- F4* from the third protoplast fusion experiment P2:: P3 (Table 6) is the best in their gum efficiency rate (two times and half than their parents) viscosity readings (1420 cP), followed by the two obtained fusants; *Sp1::Sp2- F1* and *Sp1::Sp3- F2*, from the other two experiments; P1 :: P2 and P1 :: P3, respectively nearly have the same gum efficiency rate, two times than their parents . However, the low yield of the obtained fusant *Sp2::Sp3- F3* could be caused by the disruption of some fused protoplasts during washing with PEG and mainly by insufficient embedding of protoplast in medium which is the most important requirement for successful regeneration of the cell wall (Jeans *et al.*, 1976).

The variation in ability of gum production by the different fusants can be due to the number copies of gene(s) introduced, probably the introduction of different genes from the gene pools and location in which genes had been

integrated in the same chromosome (Peters *et al* ,1989 and Papagianni *et al,* 2001). A problem that may impede the functionality of some strains constructed using DNA from diverse origins is a lack of gene expression. Although many foreign genes are expressed in frequently used hosts, a number of exceptions are known (Li – YouZhi. *et al.* 1999). Theoretically, the lake or failure of any of the steps from transcription of DNA onward can pose a barrier to proper gene expression. The possibility for failures of transcription, RNA processing, translation and the processing of preproteins may be due to the product of an rDNA gene (Abd-El-Halim *et. al.* 2001).

The data of polysaccharide and viscosity values listed in Tables (4,5,6 and 7), it could be recommended to use fusants F4, F2 and F1 for gellan gum production. The efficiency of gellan production and stability of these fusants can be increased through genetic improvement experiments either integration of several gene copies in the chromosome and/or select the best location for the introduced genes for the best production. These results were in agreements with that reported by Widjaja *et al* . (1999) and Nitschke *et al*. ( 2001).

There is no correlation between the number of plasmids in all fusants obtained and their gum production. This result may be considered as an identifying character for fusants obtained. These results were in agreements with the previous results obtained by Ferreira *et al* (1995).

In conclusion, the success of protoplast fusion in *Sphingomonas paucimobilis* was proved to develop new genetically stable strains with high efficiency of gellan gum production. These results were recommended that the the genetic improvement must be do on the high efficiency fusants to increase its efficiency or to obtain a new strains harboring desirable economical traits concerning gum induction .

## REFERENCES

Abd-El-Aal, S.KH. (2000) Xanthan gum *Xanthomonas campestris* producer on permeate through genomic *Kluyvromyces lactis* transformation. Proccedings of the Tenth Microbiology Conference,Cairo Egypt, 449-459.

Abd-El-Halim, M.M.; Abd-El-Salam, M.S.; Ibrahim, S.A. Abd-El-Aal, S. KH. and Atallah, A.G. (2001) Genetic improvement of *B. japonicum* to tolerate abiotic stresses via protoplast fusion. J. Agric. Sci. Mansoura Univ., 26 (9): 5427-5436.

Aralova, G.D.; Panchev,I.N.; Kratchanov,C.G. and Kirchev,N.A.(1993). Optimization of the xanthan biosynthesis using *Xanthomonas campestris*. Pv vesic0atoria 36. Food hydrocalloids., 7:299-306

Costerton, J.W.; Irvin, R.T. and Cheng K.J. (1981). The role of bacterial surface structures in pathogenesis. Crit. Rev. Microbial, 8: 303-338.

El-Gaali Eisa; Kazuhiko, M. and Mori, N. (1995) Enhanced nitrogen fixation capability of Soybean Rhizobia by inter–and intra–specific cell fusion. Japanese J. of Crop Sci., 64: 273-280.

Ferreira,H;Barrientos,F.J.A.;Baldini, R.L. and Rosato,Y.B. (1995) Electrotransformation of 3 pathovars of *Xanthomonas campestris* . Appl .Microbiol .Biotechnol . , 43, 4: 651-655

.Jeans, A.R.; Rogovin, S.P.; Cadmus, M.C., Silman, R.W. and Knutson, A.C. (1976) Polysaccharide (Xanthan) of *xanthomonas campestris* NRRL B-1459 producers for culture maintenance and polysaccharide production purification and analysis ARS-NC-51 peoria, II Agricultural Research Service US Department of Agriculture.

Jeremy,W.D. (1998) Molecular Genetics of Bacteria. Jhon Wily & Sons, LTD. Third ed. New York. Pp. 167-168.

Kennedy, J.F. and Bradshaw, I.J. (1984) Production, properties and application of xanthan. Prog Ind Microbiol., 19: 319-371.

Li – YouZhi ; Tang – JiLiang ; Feng – JianXun ; Cha – DongXing ; Ma – QinSheng; Li YZ ; Tang – JL ; Feng – JX; Cha – DX; //ma – QS (1999) Sequencing analysis of " 1.9" kb EcoRI DNA fragment related to biosynthesis of xanthan gum. Journal of Guangxi -Agricultural and Biological Sci., 18 (1):6-9.

Maniatis, T.; Fritsch, E. F. and Sambrock, J. (1982 ) Molecular Cloning, A Laboratory Manual , Cold Spring Harbor , New York.

Martins. L. O.; Fialho, A. M.; Roodrigus, P. L. and Sa Coreeia, I. (1996). Gellan gum production and activity of biosynthetic enzyme in *Sphingomonas paucimobolis mucoid and nonmucoid variants. Biotechnol. Appl. B iochem, 24:47.*

Martins, L. O. and Sa Correia, I. (1994). Temperature profiles of gellan gum synthesis and activity of biosynthetic enzymes. Biotechnol. Appl. Biochem., 20:385

Moazed, D. and Noller, H. F. (1987) Interaction of antibiotics with functional sites in 16s ribosomal RNA. Nature, 327: 389-394.

Murooka, Y.; Iwamoto, H.; Hamanoto, A. and Yamauchi, T. (1987). Efficient transformation of phytopathogenic of *Xanthomonas* species. J. Bacteriol., 169: 4406-4409.

Nitrchke, M.; Rodrigues, V. and Schinato, L. F. (2001) Formulation of whey-based media for xanthan gum production by *X. campestris* C7L isolate. Ciencia-e-Technologia-de-Alimentos., 21 (1):82-85.

Papabianni – M ; Psomas – Sx ; Batsilas – L ; Paras – SV ; Kyriakidis – DA; Liakopoulou–Kyriakides-M (2001) Xanthan production by *Xanthomonas campestris* in batch cultures..proccess- Biochemistry. 37 (1):73-80.

Papoutsopoulou, S.V; Ekateriniadou, L. V. and Kyriaidis, D.A.(1994) Genetic construction of *Xanthomonas campestris* and xanthan gum production from whey. Biotechnol-Lett., 16 (12) :1235-1240.

Peters,H-U; Bagyana-E; Hesselink-P-G – M ; Schumpe –A; Deckwer – W-D ( 1989) Modelling the production of microbial polysaccharide xanthan. DECHEMA - Biotechnol . Conf., 669-673.

Piagc, J.; D.Hranueli; T. Smokvina and M. Alacevic (1981). Optimal cultural and physiological conditions for handling *Streptomyces rimosus* protoplast. Appl. Environ. Microbiol. 44 : 1178-1186.

Sutherland, I.W. (2002). Microbial exopolysaccharides control of synthesis and acylation, p. 1-34. In R.C.W. Berkeley, G.W. Gooday and D.C. Ellwood (eds), Microbial Polysaccharides and Polysaccharides. Academic Press. Inc. (London). LTD., London.

Thorne, L; Tansey, L. and Pollock, T.J. (1988) Direct for xanthan gum synthesis by *Xanthomonas campestris* Industerial Microbiology, 3: 321-328.

Videira, P.; Fialho, A.; Geremia, R. A.; Breton, C. and SA-Correia, I. (2001). Biochemical characterization of the β-1,4 glucuronosyltransferase Gelk in the gellan gum-producing strain *Sphongomonas paucimobilis* A.T.C.C. 31461.Biochem. J. ,358: 457-464.

Weng Shu-Fen ;Nien-Tsung Lin; Yu-Fen Fan; Juey-Wen Lin and Yi-Hsiung Tseng(1996) Characterization of the 1.8-Kb plasmid pXV64 from *Xanthomonas campestris* pv.*vesicatoria*. Bot. .Bull.Acad .Sci.,37:93-98.

Widjaja, R ; Suwanto – A; Tjahjono – B(1999) Genome size and macrorestriction map of *Xanthomonas campestris* pv.glycines YR32 chromosome. FEMS-Microbiology- Letters. 175 (1) : 59-68.

## التحسين الوراثى لسلالات من بكتريا سفنجوموناس باتشيموبيلس لانتاج صمغ الجيلان بكميات عالية باستخدام الدمج البروتوبلاستى

**سمير خلف عبد العال(1) و محسن عسكر(2)**

**(1) قسم الوراثة الميكروبية – المركز القومى للبحوث- الدقى – القاهرة- مصر**

**(2) قسم التكنولوجية الحيوية الميكروبية – المركز القومى للبحوث- الدقى – القاهرة- مصر**

استخدم فى هذا البحث ثلاث من السلالات المحلية من من بكتريا سفنجوموناس باتشيموبيلس هم *Sphingomonas paucimobilis Kb1, Sphingomonas paucimobilis CAAS11* and *Sphingomonas paucimobilis CAAS6 S.p* بغرض تحسين كفائتهم من حيث انتاجية صمغ الجيلان . تم تقدير انتاجيهم لصمغ الجيلان وكانت 5 و 4,6 و 4.1 جم لكل ليتر و درجة اللزوجة وايضا تم عمل المعلمات الوراثية بطريقة المضدات الحيوية . تم عمل ثلاث تجارب الدمج البروتوبلاستى بين تلك الثلاث سلالات بطريقة التبادل بينهم وكانت حصيلة الثلاث تجارب اربعة مندمجات وكانوا جميعهم اعلى انتاجية للصمغ من الاباء الناتجة منهم . اعطى المندمج *Sp2::Sp3- F4* والناتج من تجربة الدمج البروتوبلاستى الثالثة اعلى انتاجية من بين الاربعة مندمجات (15.6 جم لكل ليتر) يليه فى الترتيب كل من المندمجات *Sp2::Sp3- F1 بانتاجية* (11,9 جم لكل ليتر ) و المندمج *Sp2::Sp3- F2 بانتاجية* (10,8 جم لكل ليتر ) و المندمج *Sp2::Sp3- F3 بانتاجية* (9,4 جم لكل ليتر ) وكذلك تم تقدير درجة اللزوجة لهم . اوجدت النتائج ان هناك علاقة طردية بين كمية الصمغ المنتج ودرجة اللزوجة مما يؤكد ارتفاع نوعية الصمغ بالاضافة لزيادة الكمية وذلك مقارنة بالاباء الناتجة منهم. ومن ناحية اخرى اثبتت النتائج ان الاربع مندمجات الناتجة ذات قيمة اقتصادية عالية وينصح باخالهم فى برامج تحسين وراثى اخرى.