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STUDIES ON BIOEMULSIFIER PRODUCTION BY *ACINETOBACTER CALCOACETICUS* C42 ISOLATED FROM RHIZOSPHERE OF CORN

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ABSTRACT

The aim of this study is production, characterization and partial purification of bioemulsifier produced by *Acinetobacter calcoaceticus* C42 isolated from rhizosphere of *Zea mays* and analysis of C42 to be used as a biofertilizer. The effect of physical parameters such as temperature, pH, and agitation on the production of bioemulsifier by *Ac. calcoaceticus* C42 was studied. Influences of various parameters such as salts, carbon, nitrogen source, oils and hydrocarbons on bioemulsifier production were also tested. The bioemulsifier was partially purified by acetone precipitation followed by dialysis. The possibility of the use of *Ac. calcoaceticus* C42 as a biofertilizer was checked in *Zea mays*, *Phaseolus lunatus* and *Lycospersicon esculentus* and was compared with commercially available biofertilizers of *Azospirillum* and *Azotobacter*. Maximum bioemulsifier was produced in Acinetobacter Minimal Medium (supplemented with 2 gm% glucose and ammonium chloride each) at pH 7.0, 30°C for 72 h. The bioemulsifier was composed majorly of 95% lipids. *Acinetobacter calcoaceticus* C42 isolated from rhizosphere of corn produced lipopeptide bioemulsifier and can be used as a biofertilizer. Use of *Acinetobacter calcoaceticus* C42 producing bioemulsifier as a biofertilizer can augment corn productivity and participate to relieve economical pressures of farmers. This report focuses on novel application of bioemulsifier producing *Acinetobacter calcoaceticus* C42 as plant growth promoting bacteria for corn *in vitro*.

INTRODUCTION

Microorganisms adhere to surfaces by producing certain metabolites. These metabolites called as surfactants tend to increase the area of attachment thereby strengthening their association. Surfactants are amphipathic in nature, possessing hydrophilic (polar) heads and hydrophobic (non-polar) tails[I]. It is this very dual nature which brings about changes at the boundary between the two surfaces thus promoting the interaction. A category of surfactants called as emulsifiers bring about the dispersion of two immiscible liquids[II, III].

These surface active compounds may be produced during any phase of microbial growth and may be secreted outside the cell or bound to the cell membrane[IV]. The stability at the interface of the two immiscible layers is due to their arrangement, wherein the polar end of the bioemulsifier is attached to water and its non-polar end is attached to oil[V,VI,VII]. In presence of bioemulsifiers water insoluble hydrophobic materials such as hydrocarbons may act as substrates for the growth of microorganisms. The various advantages of bioemulsifiers encompass its specificity, less toxicity, biodegradability, effectiveness at extreme conditions of pH, temperature, salinity and its easy synthesis from cheaper renewable feed stock[VIII,IX,X].

Water insoluble substrate can facilitate the growth of microorganisms by virtue of the production of bioemulsifiers which brings about the emulsification of the substrates[XI,XII,XIII]. In addition, bioemulsifers may assist nutrient transport, microbe-host interaction or act as biocides[XIV,XV]. Production of extracellular emulsifiers has been observed to occur majorly among *Acinetobacter* genera[XI]. Emulsan is the most studied polymeric bioemulsifier produced by *Ac. calcoaceticus* RAG-1 ATCC 31012[XVI, XVII, XVIII, XIX, XX, XXI, XXII, XXIII, XXIV].

Corn is the third most important cereal crop in the world and is referred to as miracle crop or queen of cereals due to its high productivity potential[XXV]. Corn is gaining more popularity due to its significant utility in food & feed industry, pharmaceutical and brewing industry. In addition, low cost of cultivation, easy adaptability to various climatic conditions, increasing productivity, minor fluctuations in prices and high potential for export demand makes corn an economic asset[XXVI].

Biofertilizers are microbial inoculants or group of microorganisms, which are able to fix atmospheric nitrogen or solubilize phosphorus, decompose organic material or oxidize sulfur in the soil[XXVII, XXVIII].

This report studies a novel application of *Acinetobacter calcoaceticus* C42 as a PGPR (plant growth promoting rhizobacteria) on corn *in vitro*. There are no reports till date either on bioemulsifier production from *Acinetobacter* species isolated from the rhizosphere of corn and more importantly its application as PGPR.

MATERIAL AND METHODS

The culture isolated from rhizosphere of corn from Mahatma Phule Agriculture College, Pune was obtained in a previous study. It was identified by API32GN system (BioMerieux, France) and 16S rDNA sequencing as *Acinetobacter calcoaceticus* and was named *Acinetobacter calcoaceticus* C42.

Screening of *Acinetobacter calcoaceticus* C42 for bioemulsifier production by emulsification assay

Ac. calcoaceticus C42 was grown in Luria broth up to 72 h. Cells were harvested by centrifugation at 10,000 rpm for 15 min at 30°C. Three ml of cell free culture supernatant was mixed with 0.5 ml test oil, vortexed vigorously for two min and incubated at 30°C for one hour for phase separation. Lower aqueous phase was removed carefully and absorbance was recorded at 400 nm (UV-2700 Double beam spectrophotometer, Chemito, Germany). Blank was prepared similarly with sterile medium. An absorbance of 0.010 units at 400 nm multiplied by dilution factor, if any, was considered as one unit of emulsification activity per ml (EU/ml)[XXIX].

Screening of *Ac. calcoaceticus* C42 for lipase production

For lipase activity Luria Bertani agar supplemented with an olive oil emulsion plates were made and spot inoculated with fresh culture of *Ac. calcoaceticus* C42 and incubated at 30°C for 24 h. After incubation, the plates were observed for clear zone of hydrolysis around the colony[XXX].

Bioemulsifier production *Ac. calcoaceticus* C42

Ac. calcoaceticus C42 was inoculated in 250 ml Acinetobacter Minimal Medium (AMM)[XXXI], Luria broth[XXXII], BNP[XXII] and Bushnell Haas Minimal Medium[XXXIII] each and incubated at 30°C at 250 rpm. Emulsification assay was performed as described earlier. Growth was monitored at 660 nm[XXIX].

Effect of physico-chemical factors on bioemulsifier production by C42

Optimization was done by a single factor approach using the emulsification assay described earlier. The effect of pH such as 5.0, 6.0, 7.0, 8.0 and 9.0 on bioemulsifier production and activity was studied. AMM was adjusted to pH of 7.0 and inoculated with *Ac. calcoaceticus* C42, incubated at 30°C at 150 rpm. Emulsification assay was determined at intervals of 24 h [XXIX] .To

determine the effect of temperature on bioemulsifier production, AMM was inoculated with *Ac. calcoaceticus* C42 and incubated at different temperatures such as 30°C, 40°C and 50°C. After every 24 h emulsification assay was carried out. Effect of agitation at100, 150 and 200 rpm on bioemulsifier production was studied similarly. Optimum temperature of 37°C obtained was used for all further experiments unless otherwise stated.

AMM was supplemented with salts NaCl, $CaCl_2$ and $MgSO_4$ separately with 0.5, 1, 1.5 and 2 g% to check the effects of salts on bioemulsifier production. Bioemulsifier production was checked by emulsification assay at regular intervals of 24 h. Effect of varying concentrations (0.5, 1, 1.5 and 2 g%) of nitrogen sources such as ammonium chloride and sodium nitrate and carbon sources such as glucose, mannitol and sucrose on bioemulsifier production was studied separately using the emulsification assay. Amino acids supplemented in the production media were tyrosine, phenylalanine, tryptophan, arginine, histidine, glycine, proline at concentrations similar to that of original glutamic acid component (0.25 g%) of the AMM broth[XXIX] .

Effect of different oils and hydrocarbons as substrates for bioemulsification

Edible oils such as groundnut oil (Chakan depot, Pune, India), sunflower oil (Gemini, India), sesame oil (Chakan depot, Pune, India), mustard oil (Chakan depot, Pune, India), and hydrocarbons such as, toluene, kerosene, xylene (Sisco Research Laboratories, India), petrol oil (Indian oil, India), 'Castrol', 'Xtra premium', petrol (Bharat Petroleum, India) were used. The emulsification assay was performed as described earlier[XXIX].

Effect of inducer oil on bioemulsifier production by C42

Ac. calcoaceticus C42 was grown in AMM supplemented with varying concentrations of sesame oil such as 0.5, 1, 1.5 and 2% v/v. Control experiments were run in AMM without oil. Emulsification assay was performed after 24 h upto a period of 72 h.

Preparation of *Ac. calcoaceticus* C42 bioinoculum

Ac. calcoaceticus C42 bioinoculum was prepared by using lignite as a carrier. Lignite was sterilized at 121°C for one hour. After autoclaving, lignite was cooled for three hours. *Ac. calcoaceticus* C42 was inoculated in sterile 250 ml AMM broth which was the optimized medium and incubated at 30°C, 150 rpm for 72 h. After 72 h the culture (O.D. 0.42) was aseptically mixed with lignite. Calcium carbonate was added to adjust pH of lignite. *Zea mays* seeds were coated aseptically with the bioinoculum and used for the pot experiment[XXXIV].

Effect of *Ac. calcoaceticus* C42 bioinoculum on growth of *Zea mays*

Growth promotion was analysed by measuring the height of corn, root length, width and length of leaves of *Zea mays*. Results were statistically analysed in terms of mean, standard deviation. Total chlorophyll,protein,carbohydrate, nitrogen content of *Zea mays* leaves were analysed[XXXIV].

Effect of C42 bioinoculum on growth of different crops

Seeds of *Phaseolus lunatus* (Lima bean) and *Lycospersicon esculentus* (Tomato) were coated aseptically with C42 bioinoculum and sowed in sterile soil. Control experiments were performed with seeds not coated with the C42 bioinoculumn. The growth of the plants was observed for three months to determine the effect of the bioinoculum on these plants.

Comparison of C42 bioinoculum with other biofertilizers

The C42 bioinoculum was compared with other biofertilizers presently being used for *Zea mays* production. *Azospirillum* & *Azotobacter*, were procured from Mahatma Phule Krishi Vidyapeeth, Pune, India. Results were analysed and compared with *Zea mays* inoculated with C42.

Partial purification of bioemulsifier from *Ac. calcoaceticus* C42

Partial purification of bioemulsifier was carried out using one liter of 72 h old broth at 30°C. Broth was centrifuged at 10,000 *g* for 20 min at room temperature. After centrifugation three volumes of chilled acetone was added in cell free supernatant and kept at 4°C for 15 h. The mixture was centrifuged at 8000 *g* for 30 min at 10°C to obtain a white precipitate. The white precipitate was then dissolved in three ml sterile distilled water and dialysed extensively against sterile distilled water at 10°C for 48 h (Cellulose seamless tubing, retaining most proteins of molecular weight 12,000 or more, Sigma Aldrich, Steinheim, Germany). Distilled water was changed after every 12 h. The dialysate was then frozen at 4°C and lyophilized. The lyophilized powder was stored at room temperature in air tight glass vials[XXIX].

Chemical analysis of partially purified bioemulsifier

The partially purified bioemulsifier was chemically analyzed. Protein content was determined by Folin-Lowry[XXXV] with bovine serum albumin as standard; Carbohydrate content[XXXVI] and reducing sugar was estimated using dinitro-salicylic acid method[XXXVII] with glucose as standard. Extraction and quantification of lipid and fatty acids was performed[XXXVIII].

Stability of bioemulsifier produced by *Ac. calcoaceticus* C42

Emulsification stability was checked by incubating the emulsion for seven days at 28°C. The emulsification assay was performed to observe any decrease in the emulsification activity[XXIX].

Cleaning property of bioemulsifier

Twenty milligrams of partially purified powder of bioemulsifier was dissolved in four ml of distilled water. In clean glass tubes seven ml glycerol, groundnut oil, mustard oil, sesame oil and sunflower oil were added separately. The tubes were inverted and excess oils were removed such that portion of oil was coated on the walls of the tubes. After this, one ml of bioemulsifier solution was added drop by drop in each tube by holding the tube in the horizontal position and the inner surface of each glass was observed carefully for cleansing property[I].

RESULTS

Screening of *Acinetobacter calcoaceticus* C42 for bioemulsifier production by emulsification Assay

Maximum bioemulsification activity was observed with the vegetable oils than the hydrocarbons. It was observed that bioemulsifier production was directly proportional to the cell density.

Screening of *Ac. calcoaceticus* C42 for lipase production

Ac. calcoaceticus C42 produced lipase as indicated by the development of a clear zone around the colony on Luria Bertani agar supplemented with olive oil emulsion plate.

Bioemulsifier production by C42

Bioemulsifier production was observed in AMM and LB broth with maximum production obtained in AMM (Fig. I & II). No activity was obtained in *Ac. calcoaceticus* C42 inoculated Bushnell Haas Minimal medium.

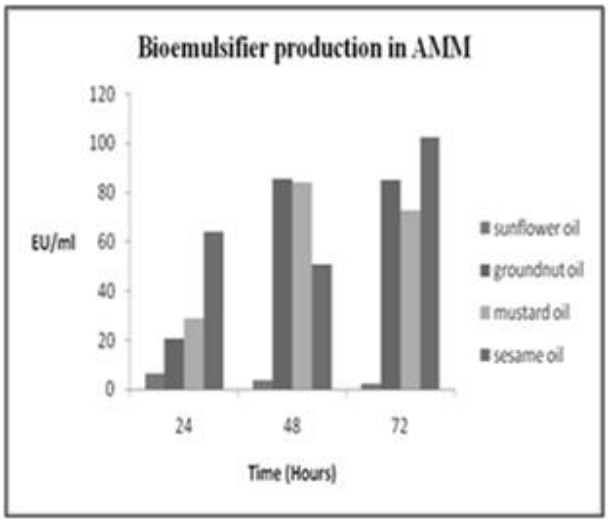

Fig. I: Effect of growth in AMM media on bioemulsifier production

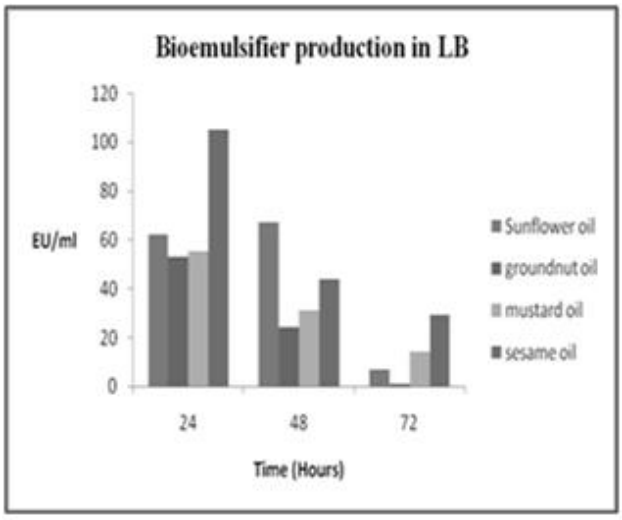

Fig. II: Effect of growth in LB media on bioemulsifier production

Effect of physico-chemical factors on bioemulsifier production by C42

Maximum bioemulsifier production was observed at pH 7.0 (250 EU/ml). No bioemulsifier was produced at pH 8.0 & 9.0 (98 EU/ml) and C42 was unable to grow at pH 5.0. Maximum bioemulsifier was produced at 30°C as compared to 40°C & 50°C (Fig. III). Agitation at 200 rpm was found to be most suitable for the bioemulsifier production (Fig. VI). Magnesium sulphate (0.5 g%) enhanced bioemulsifier production; in contrast calcium chloride was inhibitory for the bioemulsifier production (Fig. V). Ammonium chloride (2 g%) was a better salt than sodium nitrate. Glucose (2 g%) promoted the bioemulsifier production while mannitol and sucrose decreased the activity of the bioemulsifier. Amino acids glycine, tyrosine and histidine enhanced bioemulsifier production with most significant increase observed with tryptophan (100 EU/ml).

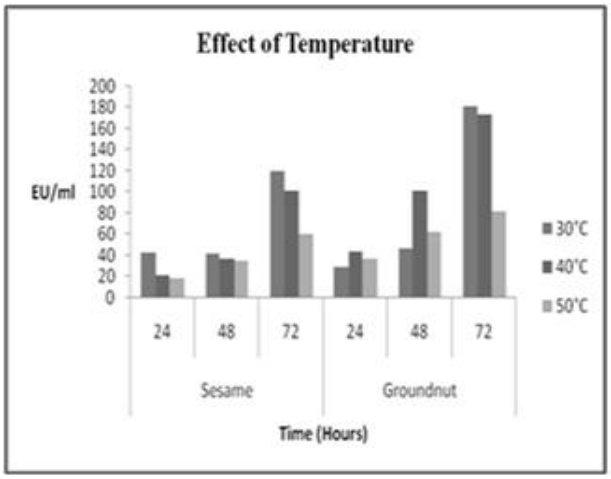

Fig. III: Effect of temperature on bioemulsifier production

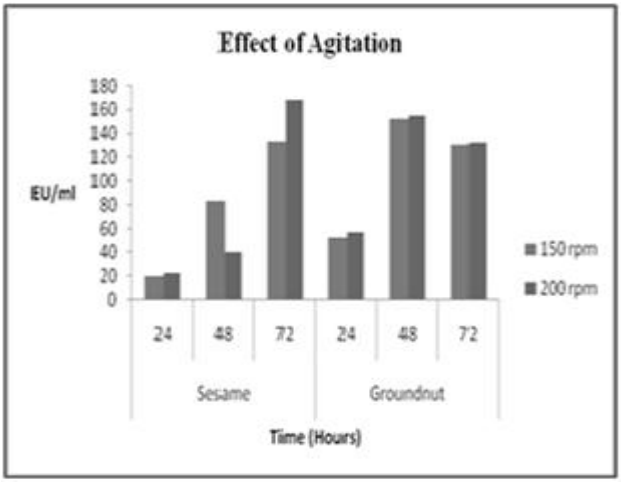

Fig. IV: Effect of agitation on bioemulsifier production

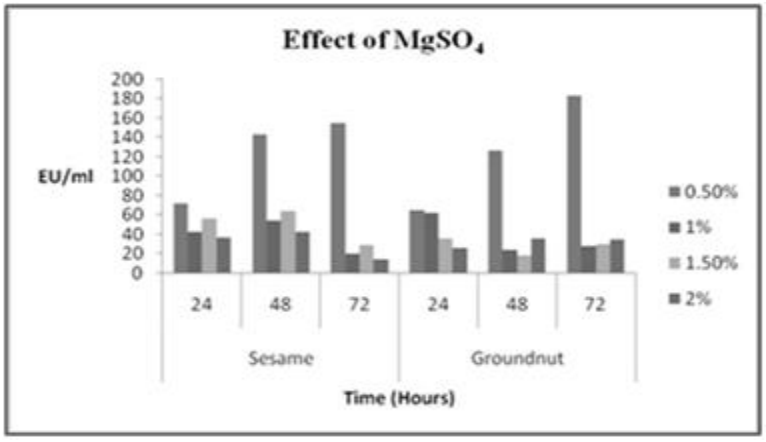

Fig. V: Effect of different $MgSO_4$ concentrations on bioemulsifier

Effect of different oils and hydrocarbons as substrates on bioemulsifier production

Sesame oil was used for further studies as it resulted in maximum production of bioemulsifier (120 EU/ml).Xylene was a preferable substrate as compared to other hydrocarbons(Fig.VI&VII).

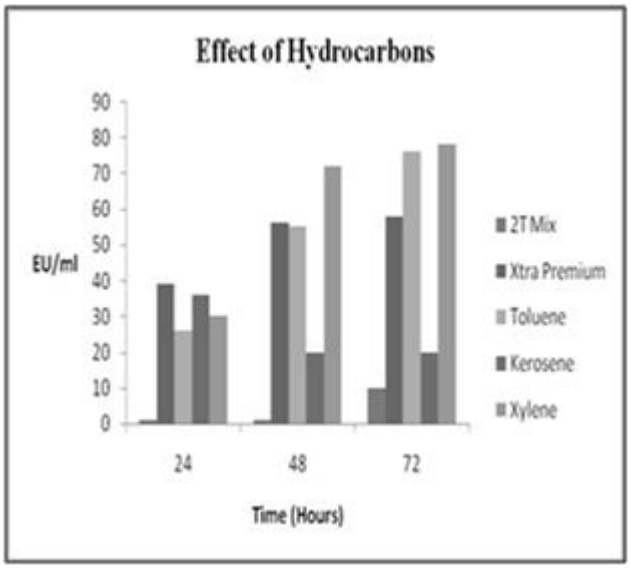

Fig. VI: Effect of different hydrocarbons on bioemulsifier

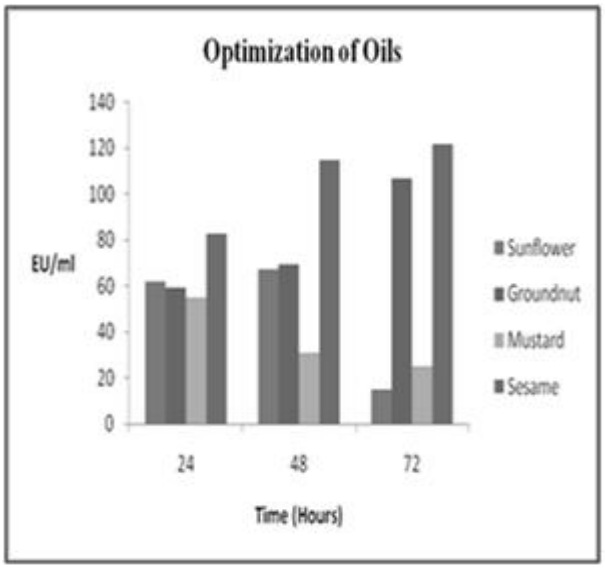

Fig. VII: Effect of different oils on bioemulsifier production by C42

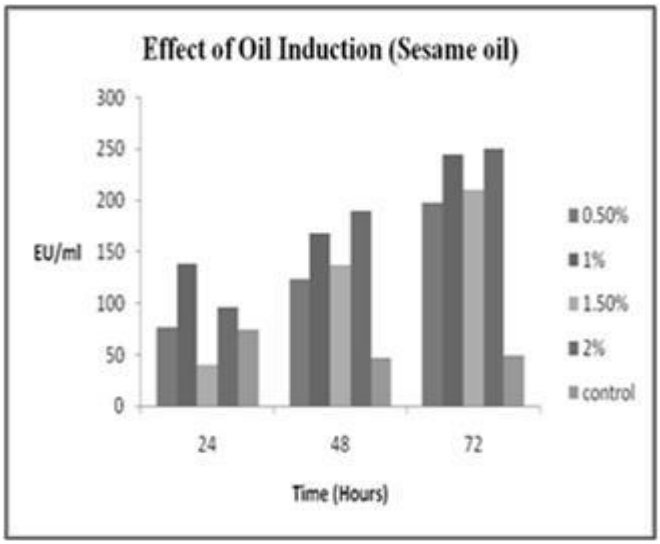

Fig. VIII: Effect of sesame oil induction on bioemulsifier production by C42 strain.

Effect of inducer oil on bioemulsifier production by C42

Bioemulsifier production by C42 was found to be inducible. Sesame oil 2% v/v induced production of bioemulsifier (250 EU/ml). (Fig. VIII).

Figure IX: Effect of C42 on *Zea mays*

Effect of C42 bioinoculum on growth of *Zea mays*

Significant differences were observed in the physical characteristics of the control and test *Zea mays* (Fig. IX). Biochemical analysis in terms of protein, chlorophyll, carbohydrate and nitrogen content of leaves of the test and control plants showed significant difference. The corn seeds were aseptically coated with the C42 bioinoculum and its effect on the growth of the plant was studied by measuring the height of the corn plant, width and length of the corn leaves and the length of the corn leaves in centimeter. Statistical methods such as mean and standard deviation were calculated to significant difference between the control and the test corn plant.(Table I & II)

Table I: Comparison of physical parameters of corn plants treated with and without C42 bioinoculum.

Name of Treatment* given to corn seeds	**Number of Samples**	**Mean (cm)**	**Standard Deviation**
1) Height of corn plant (cm)			
Control	5	42.60	0.423
Test	5	**53.72**	0.356
2) Width of corn plant leaves (cm)			
Control	5	1.77	0.033
Test	5	**2.18**	0.254
3) Length of corn plant leaves (cm)			
Control	5	30.72	0.370
Test	5	**53.54**	2.894
4) **Length of corn roots (cm)**			
Control	5	19.86	0.270
Test	5	**22.42**	0.526

Control: corn seed not treated with C42 bioinoculum, Test: corn seed treated with C42 bioinoculum, *: coating of corn seeds with or without C42 bioinoculum

Table II: Biochemical analysis of corn seeds with and without C42 treatments

Treatments given to corn seeds†	Total chlorophyll (a+b)* in mg/g	Protein content (mg/ml)	Carbohydrate content (mg/ml)	Nitrogen content (%)
Control	9.83	1.40	2.00	1.07
Test	**15.23**	**2.10**	**2.80**	**2.46**

*: chlorophyll a + chlorophyll b

†: Coating of corn seeds with or without *Ac. calcoaceticus* C42

Control: corn seed not treated with C42 bioinoculum

Test: corn seed treated with C42 bioinoculum

Effect of C42 bioinoculum on growth of different crops

The C42 biofertilizer promoted growth of both *Phaseolus lunatus* and *Lycospersicon esculentus* plants as compared to their respective controls. The height of the plants increased significantly. Their roots were found to be longer than those of the control plants. Equal number of seeds of corn was aseptically coated with C42 bioinoculum, standard *Azotobacter* and *Azospirillum* biofertilizer and sowed in sterile soil. The effect of each biofertilizer was studied by detecting the physical parameters and calculating their mean and standard deviation for five samples. The experiment is performed in triplicates. (Table III)

Table III: Comparison of physical parameters between different biofertilizer treatment

Name of Treatment* given to corn seeds	Sample nos.	Mean (cm)	Standard Deviation
1) Height of corn plant (cm)			
Ac. calcoaceticus C42	5	**37.68**	0.2397
Azospirillum	5	34.94	0.2408
Azotobacter	5	33.92	0.1483
2) Width of corn plant leaves (cm)			
Ac. calcoaceticus C42	5	**2.18**	0.254
Azospirillum	5	1.52	0.228
Azotobacter	5	1.48	0.311
3) Length of corn plant leaves (cm)			
Ac. calcoaceticus C42	5	**53.54**	2.894
Azospirillum	5	34.88	3.061
Azotobacter	5	38.86	6.692
4) Length of corn plant roots (cm)			
Ac. calcoaceticus C42	5	**22.42**	0.526
Azospirillum	5	20.26	0.288
Azotobacter	5	21.62	0.500

Control: corn seed not treated with C42 bioinoculum, Test: corn seed treated with C42 bioinoculum, *: coating of corn seeds with or without C42 bioinoculum

Comparison of C42 bioinoculum with other biofertilizers

The protein, carbohydrate and chlorophyll content of corn plant treated with C42 biofertilizer was greater as compared to the corn plant treated with *Azotobacter* biofertilizer. The values are mean of 5 samples of each treatment. Equal number of seeds of corn was aseptically coated with C42 bioinoculum, standard *Azotobacter* and *Azospirillum* biofertilizer and sowed in sterile soil. The effect of each biofertilizer was studied by detecting the chlorophyll content, protein content, total carbohydrate content as described by Sadashivam and Manickam and nitrogen content was analysed at Mahatma Phule Krishi Vidyapeeth, Agriculture College, Pune. (Table IV)

Table IV: Comparison of biochemical analysis between different biofertilizer treatments.

Treatment given to corn seeds†	**Total chlorophyll (a+b)* in mg g^{-1}**	**Protein content (mg ml^{-1})**	**Carbohydrate content (mg ml^{-1})**	**Nitrogen content (%)**
Ac. calcoaceticus C42	15.23	0.77	0.50	2.46
Azospirillum	19.46	0.77	0.80	2.75
Azotobacter	12.74	0.13	0.18	4.73

*: chlorophyll a + chlorophyll b

†: Coating of corn seeds with *Ac. calcoaceticus* C42, *Azospirillum* & *Azotobacter*

Control: corn seed not treated with C42 bioinoculum, Test: corn seed treated with C42 bioinoculum

Partial purification of bioemulsifier from *Ac. calcoaceticus* C42

Partially purified bioemulsifier obtained by acetone precipitation yielded a white powder. Bioemulsifier was made up of lipid (95.22%), protein (0.62%) and carbohydrate (1.74%).

The partially purified bioemulsifier was obtained in chilled acetone and dialysed with sterile distilled water and lyophilized for further analysis of its chemical nature (Table V).

Table V: Characteristics of partially purified bioemulsifier from *Ac. calcoaceticus* C42.

Colour	White
Yield	19.2 mg l^{-1}
Nature	White dry powder, non-hygroscopic
Chemical composition	Protein, polysaccharide, reducing sugar and lipid. Protein: 0.62% Polysaccharide :1.74% Reducing sugar: 2.3% Lipids and fatty acids: 95.22%
Solubility	Weakly soluble in cold water
Stability	Room temperature.
Storage at	Room temperature in tight glass vials.

Stability of partially purified bioemulsifier

Ninety percent of emulsification activity was retained for seven days after which the activity decreased.

Cleaning property of bioemulsifier

Bioemulsifier exhibited good cleaning property against glycerol, groundnut oil and sesame oil.

DISCUSSION

This is an innovative study which attributes bioemulsifier producing *Acinetobacter calcoaceticus* C42, isolated from rhizosphere of corn, with plant growth promoting ability.

Ac. calcoaceticus C42 isolated from rhizosphere of corn produced maximum bioemulsifier (250 EU/ml) after 72 h of growth which coincided with the growth phase of C42. Sesame oil was found to be the best substrate for emulsification. This pattern of production is similar to that of emulsan from *Ac. calcoaceticus* RAG-1 where the bioemulsifier was produced only on induction by hydrocarbons or oils[XXI]. Vegetable oils were emulsified to a greater extent than hydrocarbons. For *Ac. calcoaceticus* C42, AMM encouraged high cell density. Cell mass is directly proportional to the bioemulsifier production, hence used for further studies[XXXIX].

Optimum pH has an important effect on bioemulsifier production and activity[XL]. The optimum pH for both activity and bioemulsifier production by *Ac. calcoaceticus* C42 was found to be 7. In case of alasan produced by *Ac. radioresistens* KA 53, both bioemulsifier production and activity was maximum at acidic pH. *Candida glabrata* UCP 100 showed activity over a wide range of pH of 2-12 [V], whereas *Ac. junii* SC 14 showed significant activity at slightly alkaline pH[XXIX]. It was found that the pH of the soil is in the range of 7.0- 8.2, thus it is obvious that rhizosphere strains showed higher activity at corresponding pH[XLI, XLII].

Temperature 30°C was found to be optimum for bioemulsifier production. This is similar to *Arthrobacter*- RAG-1 which showed maximum production of emulsan at 30°C [XVI, XVII]. It was found that production of bioemulsifier increases with increase in agitation. An agitation of 200 rpm was found to be optimum for the bioemulsifier production and activity by *Ac. calcoaceticus* C42. It was reported that 150 rpm for *Ac. calcoaceticus* A2 and 250 rpm for *Ac. calcoaceticus* BD4 provided the most suitable conditions for bioemulsifier production[XLIII].

Increase in bioemulsifier production, produced by *Ps. flourescens* was due to magnesium sulphate[XLIV]. Bioemulsifier production by *Ac. calcoaceticus* C42 was also enhanced when magnesium sulphate (0.5%) was supplemented in the medium.

Root exudates of corn plant such as amino acids and sugars may affect the production and activity of bioemulsifier produced by *Ac. calcoaceticus* C42. In case of *Ac. calcoaceticus* C42, only glucose (2%) increased activity, of the bioemulsifier as it is readily metabolized. Effect of different carbon sources on bioemulsifier produced by *Pseudomonas* species and *Acinetobacter* sp strain H13-A & H01-N was reported[XLV, XLVI]. Amino acid L-tryptophan stimulated the bioemulsifier production by *Ac. calcoaceticus* C42. It was reported that *Ac. baumannii* A25 produced high yields of bioemulsifier when grown in presence of amino acids such as L-ornithine, L- tyrosine, L-arginine, L- phenylalanine[XLII]. In case of C42 we found that ammonium chloride was consumed as nitrogen source suggesting that C42 can utilize nitrogen when it is in the ammonium form. Nitrate was reported to be the best nitrogen source for biosurfactant production by *Pseudomonas* 44T1 and *Rhodococcus* strain ST-5[XLVII, XLVIII].

Stability of bioemulsifier is a very important characteristic as it helps in increasing shelf life of the product and for easier storage[XXIX]. Stability of bioemulsifier in terms of its activity was checked with sesame oil and ninety percent of activity was found to be retained. The bioemulsifier of *Ac. calcoaceticus* C42 is weakly soluble in water. The partially purified bioemulsifier contained protein (0.62%), reducing sugars (2.3%), carbohydrates (1.74%) and lipid & fatty acids (95.22%). Surface active compounds with maximum lipid and fatty acid content were reported in *Corynebacterium lepus*[XLIX].

Their surface active compound mostly consists of 25% polar lipids and 10% saponifiable saturated fatty acids and corynomycolic acids[XLIX]. *Ac. calcoaceticus* BD4 was reported to produce RAG-1 emulsan which constituted 12% of fatty acid[XL]. It contains maximum percentage of lipids and hence it is weakly soluble in water. The bioemulsifier produced by *Ac. baumannii* A25 was reported to be an effective cleaning agent[XLII]. Similarly the bioemulsifier produced by *Ac. calcoaceticus* C42 exhibited good cleaning property; hence it can be used for cleaning purposes in detergent industries.

Acinetobacter calcoaceticus, till date is not reported as a plant pathogen. There have been preliminary reports of presence of *Acinetobacter* in wheat and maize rhizosphere. The production of IAA and phosphate solubilization was only reported from maize rhizosphere[L, LI] and production of IAA by *Acinetobacter* species isolated from the wheat rhizosphere[LII]. However there have been no reports of possible role of bioemulsifier producing *Acinetobacter* as a PGPR.

Bioemulsifier producing *Acinetobacter* species as a PGPR for corn is not yet been reported. There have been various reports of PGPR used for corn production including *Azospirillum lipoferum* DSM1691, *Azospirillium. brasilense* DSM1690, *Ps. putida* R-168, *Ps. fluorescens* R-93[XXV, LIII], *Azotobacter chroococcum*, *B. lentus*[LIV] and *Rhodotorula glutinis*[LV]. *Acinetobacter calcoaceticus* C42 bioinoculum was prepared using lignite as the carrier since it has high water holding capacity and high organic content which enhances the growth of microorganisms[LVI].

The effect of C42 bioinoculum on corn was checked by observing the physiological changes in the plant. The height of the C42 treated plant (53.72 cm) was greater than the control. The increase in leaf length (53.54 cm) in *Ac. calcoaceticus* C42 treated corn plant could be attributed to an increase in the length of the petiole which in turn is capable of assimilating maximum sunlight through the lamina[XLII]. The increase in the leaf width (2.18 cm) of the test corn plant is related to the primary productivity which exhibits maximum efficiency in carbon-di-oxide and glucose production[XLII]. Increase in the root length of the test plant (22.42 cm) was also observed to be greater than the plant treated without C42 bioinoculum (19.86cm). The protein (2.10 mg ml^{-1}), carbohydrate (2.80 mg ml^{-1}), chlorophyll (15.23 mg g^{-1}) and nitrogen (2.46%) content was significantly greater due to the enhanced physiology of the plant clearly suggesting the positive effect of *Ac. calcoaceticus* C42 on the growth of corn plant.

Since *Azospirillum* has been studied to have a positive effect on the growth of corn[LVII, XXV] it was selected as a standard for comparative study. The plant height, leaf length, leaf width and root length of the C42 treated corn plants were observed to be greater than that of the plants supplemented with *Azospirillum* and *Azotobacter*. This demonstrates the potential of C42 producing bioemulsifier as a biofertilizer. Similarly chlorophyll content of the *Azospirillum* treated plant was the highest (19.46 mg g^{-1}) and C42 treated plant with 15.23 mg $g^{-1}$of chlorophyll which was comparatively less than the *Azospirillum* treated plants.

The protein (2.10 mg ml^{-1}) and carbohydrate (2.8 mg ml^{-1}) content of C42 treated plant was greater than that of the other two treatments. The decrease (0.80 mg ml^{-1}) in sugar content of the plants treated with *Azospirillum*, in spite of high chlorophyll content, may attribute to the redistribution of sugar from the vegetative part to the reproductive part of the plant[LII]. The nitrogen content of the corn leaf was measured after 45 days of sowing. There is no significant difference in nitrogen content of C42 treated plants (2.46%), *Azospirillum* treated plants (2.75%).

The present investigation reveals that under *in vitro* conditions seed treated with *Ac. calcoaceticus* C42 as plant growth promoting bacteria improved seed germination as compared to the control. This may be due to increase in availability of water insoluble substrates due to bioemulsifier production by our strain C42.

Ac. calcoaceticus C42 is not able to fix nitrogen, siderophores antibiotics, phytohormones or solubilize phosphate. Hence the positive effect of this bioinoculum can be suggested to be due to the production of bioemulsifier.

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