

IMPROVEMENT OF FABA BEAN AND WHEAT VEGETATIVE GROWTH AND THEIR RHIZOSPHERE SOIL AGGREGATIONS BY SOME PGPR GROUP PRODUCING EXOPOLYSACCHARIDES

ABSTRACT

Some nitrogen fixing bacteria were found to be able to produce different types of Exopolysaccharides (EPS). In this study four nitrogen fixing bacterial strains were isolated and identified as: *Rhizobium* sp. *Azotobacter*.sp. *Bacillus* sp. *Azospirillum* sp. Eps were extracted from these isolates and were subjected to qualitative and quantitative determinations using Thin Layer Chromatography (TLC). The effect of bacterial inoculation as well as their extracted EPS on vegetative growth of wheat and faba bean cultivated in sandy soil was investigated. Aggregation size distribution, nitrogenase, dehydrogenase, nitrogen percentage, log number of bacteria and colonization patterns were also studied after 45 days of sowing. Data showed that shoots and roots dry weights were significant increased in all inoculated plants (either by bacteria or EPS) compared to the un-inoculated one. Nitrogenase activity and nitrogen percentage showed also similar trend. Data also revealed that inoculation with a combined mixture of *Azotobacter*.sp and *Bacillus* sp. together with their EPS increased the aggregate size distribution as expressed by Mean Wight Diameter.

Key words: Exopolysaccharides, Faba Bean, Wheat, PGPR, Rhizosphere, Soil aggregation.

INTRODUCTION

Soil structure is important in fertility and plant growth and may be affected by physical and biological factors. Organic residues applied to soils improve soil structure (Allison, 1973). In cropping the rhizosphere constituents are a principal source of organic materials in soil. The cultivated crops contribute to soil organic matter directly through the root material itself and its decomposition and indirectly through root exudation which stimulates microbial activity and biomass. Gouzou *et al.*, (1993) demonstrated that inoculation of wheat with *Bacillus polymyxa* enhanced the "rhizosphere effect" by increasing the amount of adhering soil to improve soil structure.

Reid and Goss (1981) found that increasing aggregate stability of soils was associated with root growth of rye-grass and alfa alfa could be attributed to polysaccharides produced in the rhizosphere.

The production of exopolysaccharides by bacterial populations in the rhizosphere has been demonstrated to contribute to water and nutrient uptake by plant roots through modification of the physical properties of rhizosphere soil (Yahia *et al.*, 2005). Colonization and efficient association of *Azospirillum* and cereal crops could potentially provide an environment for useful BNF, similar to rhizosphere-legume symbiosis (Michiels *et al.*, 1984). This study was performed to evaluate the effects of bacteria and their exopolysaccharides inoculation on growth and soil aggregation. Colonization patterns of certain diazotrophs associated with wheat and faba bean roots, were also studied

MATERIALS AND METHODS

Bacterial strains:

Bacterial strains of *Rhizobium* sp, *Azotobacter* sp, *Bacillus* sp and *Azospirillum* sp were supplied by SWERI, ARC, Giza, Egypt,

Table (1): Source of strains isolated from rhizosphere of different plants

Isolated strains	Plant	Source of strains
<i>Rhizobium</i> sp	Faba bean (<i>Vicia faba</i>)	Toshky
<i>Azotobacter</i> sp	Wheat (<i>Triticum aestivum</i>)	El-Bosiely
<i>Bacillus</i> sp	Maize (<i>Zea mays</i>)	Ismailia
<i>Azospirillum</i> sp	Maize (<i>Zea mays</i>)	Ismailia

These strains were grown on modified yeast extract mannitol (Bellogin *et al.*, 1984), N₂ modified Ashby's medium (Abdel-Malek and Ishac, 1968), Watanabe medium supplemented with different carbon sources and modified malate medium (Hebber *et al.*, 1992).

Extraction of exopolysaccharides

The exopolysaccharides were extracted from the treated bacterial strains by the method described by (Hebber *et al.*, 1992).

Bacterial strains were grown separately on nitrogen deficient selective media, at 28°C for 5-7 days, then the biomass was harvested by centrifugation at 15000 for 15 min. The obtained pellets were re-suspended in a bottle (250 ml capacity) containing 5ml of 0.85% KCl sterilized solution and 10ml of selective medium. Bottle was incubated on orbital shaker (150 rpm) for 3-5 days at 28°C pellets were dehydrated in alcohol and dried at 35°C for extracting hydro soluble slime .

Chemical analysis of EPS

Exopolysaccharides were hydrolyzed by heating at 100°C with 1N HCL for 7h. The acid hydrolyzate was reported under vacuum to dryness. Analysis of hydrolyzed bacterial exopolysaccharides was carried out using thin-layer chromatography (TLC) on cellulose plate F-254 (Merck). The solvent system used was ethyl acetate: pyridine: water: acetic acid: propionic acid (50:30:10:5:5) (v/v), (Bellogin *et al.*, 1984). The plates were developed by aniline hydrogen phthalate (Partridge, 1949).

Seed sterilization

Seeds of wheat (*Triticum aestivum* cv. Sakha 69) and faba bean (*Vicia faba* cv. Giza 717) were soaked in a saturated calcium hypochlorite solution for 2hr under agitation, washed thoroughly with sterile distilled water, then immersed in 10% hydrogen peroxide for 20 min.

Sterilized sandy soil (sand, 91.55%, 1.20% silt, 7.25% clay, pH value of 7.39 in 1:5 soil/water suspension) was used throughout the current work.

Wheat and faba bean seeds inoculation

Sterilized seeds were coated with 4×10^7 bacterial cells per seed either individually or mixed. The seeds were also coated with exopolysaccharides (35g/5 seeds) extracted from each strain, either individually or in mixture and coated with a mixture of bacteria and exopolysaccharides. The control treatment included seeds coated with peat. In the faba bean, all the treatments were inoculated by *Rhizobium leguminosarium*. Coated seeds (5 seeds per pot) were sown 10mm below the soil surface in plastic cylindrical pots (2 kg/pot) of air dried soil. Wheat and faba bean plants were grown for 45 days. Three seedlings for each pot were left.

The water content of the soil in each pot was adjusted ,at 60% WHC with sterile water, which was added by spraying on the soil surface. The roots together with the adhering soil were taken from the pots 45 days after sowing. They were carefully separated from the sandy soil. The size distribution of aggregates was determined by dry sieving according to (Rouiller *et al.*, 1972). A 100g (dry mass) of air were gently passed through a set of sieves of different sizes, e.g 5mm, 2mm, 1mm, 0.5mm and 0.25 mm. The fractions of aggregates of mean diameters of 10mm, 3.5 mm, 1.5 mm, 0.75 mm and 0.125 mm were weighed and their proportions to the whole sample of aggregates were calculated. The results were expressed as Mean Weight Diameter (MWD).

- Dehydrogenase enzyme activity (DHA) in rhizosphere soil (wheat and faba bean) was determined according to **Thalman, (1967)**.
- Nitrogenase activity (N₂-ase) in rhizosphere soil wheat and faba bean) was determined according to **Hardy *et al.*, (1973)**.
- Total viable aerobic bacteria in rhizosphere soil (log number) were counted to the method described by **Vincent (1970)**.
- Nitrogen percentage in shoots (wheat and faba bean) was determined by micro-Kjeldahel method as described by **Black *et al.*, (1965)**.
- **Colonization patterns:** colonization was characterized as the ability of bacterial cells to attach to the plant roots. The roots of wheat or faba bean plants were treated with 2, 3, 5 try-phenyl tetrazolium chloride (2ml solution of TTC /sample) for 2hr, then examined by microscope.

RESULTS AND DISCUSSION

Data in Table (2) showed that the four bacterial agents produce variable quantities of exopolysaccharides, depending on the carbon sources supplemented in the culture media. *Azotobacter.sp* produced higher quantities of EPS being 1.6g/L. EPS produced by *Bacillus sp*, *Rhizobium sp* and *Azospirillum sp* were 1.18, 0.77 and 0.63 g/L respectively. The modified Ashby's medium enhanced the EPS yield comparing with Watanabe C-rich medium. Similar results were reported by **Hebbar *et al.*, (1992)**; **Chenu (1995)**, who revealed that N-Limitation enhanced EPS production in a liquid culture of bacteria. The obtained results also, revealed that the extracted EPS of *Rhizobium sp* composed of mannose (R_f 0.22) ,lactose (R_f 0.28) and arabinose (R_f0.45)Table, 2 and Figure, 2). These results did not agree with **Yahia *et al.*, (2005)** who found that the exopolysaccharides produced from *Rhizobium sullae* was composed of glucose, galactose and mannuronic acid. On the other hand, EPS

obtained from *Bacillus sp* had R_f values 0.15, 0.26, 0.44 and 0.89, the second and the third were identified as lactose and arabinose whereas the other compound could not be identified. These results are agree with **Kang and Cottrell (1979)**, who reported that *B. polymyxa* produced monosaccharide such as fructose, glucose, and/or arabinose but don't agree with **Hebbar et al., (1992)**. Also TLC analysis of EPS produced by *Azotobacter.sp* revealed the presence of glucose (R_f 0.40) and ribose (R_f 0.58) .While, exopolysaccharides obtained from *Azospirillum sp* contained xylose (R_f 0.52) and unknown sugar (R_f 0.81). These results are on line with **Choma et al., (1984)** who found that the exopolysaccharides from *Azospirillum lipoferum* were composed of mannose, xylose, rhamnose, galactose and glucose.

Table (2): Yield of exopolysaccharides extracted from some PGPR group using thin layer chromatography (TLC) methods.

Bacterial strains	EPS g/L	. TLC * R_f values				
<i>Azotobacter sp</i>	1.60	0.40 glucose	0.58 ribose	0.79 un known	**N.F	N.F
<i>Azospirillum sp</i>	0.63	0.52 xylose	0.81un-known	N.F	N.F	N.F
<i>Bacillus sp</i>	1.18	0.15 un known	0.26 lactose	0.44 arabinose	0.89 un known	N.F
<i>Rhizobium sp</i>	0.77	0.22 mannose	0.28 lactose	0.45 arabinose	0.65un known	0.83

N.F.: Not found

Data in Table (3) showed the effect of inoculation with PGPR group and exopolysaccharides on total bacterial count and dehydrogenase activity in rhizosphere soil cultivated with wheat and faba bean plants. Generally results of the inoculation with PGPR group with EPS were higher than the control in both wheat and faba bean plants. The highest bacterial count (CFU 7.97) of wheat rhizosphere was observed in case of inoculation with *Azotobacter.sp* +EPS comparing with other treatments and control. In faba bean, inoculations with *Rhizobium sp* +EPS give the highest count being 8.42 cfu comparing with control. On the other hand, inoculation with *Azotobacter.sp*, *Bacillus*, *Azospirillum* and *Rhizobium* alone or EPS along giving the lowest count. These results are in harmony with **Amellal et al., (1999)** who found that inoculation of wheat with the EPS-produced from *P. agglomerans* led to spreading of bacteria all over the root system. Also, results showed that inoculation with PGPR plus with EPS led to a significantly increase of dehydrogenase enzyme activity in wheat and faba bean plants. **Mehanni (1995)** **Verma et al., (2001)** and found that inoculation of wheat with *Azospirillum* and *Azotobacter* increased the dehydrogenase activity.

Data in Table (4) showed that inoculation of wheat and faba bean with PGPR and exopolysaccharides were significant increase of dry weights and total length of roots. A combination of *Rhizobium sp* and *Azotobacter.sp* and their EPS increased dry weight and total length of roots compared to control. A general stimulation of root development and the formation of large numbers of short lateral roots led to promotion of plant growth. These results are in harmony with **Yahia, et al., (2005)** who cleared that inoculation of wheat with KYGT 2007 caused significant promotion

of plant growth. Interaction of the EPS with wheat germ agglutinin leads to increased numbers of wheat seedling root hair deformations Skivortsov *et al.*, (1995).

Table (3) : Bacterial total counts in (log number) and dehydrogenase activity as affected by bacterial inoculant and exopolysaccharides in wheat and faba bean plants.

Bacterial strains	Log No.		DHA $\mu\text{g TPF/g}$ soil /day	
	Wheat	Faba bean	Wheat	Fababean
Control	6.56	6.25	6.77	5.63
<i>Rhizobium sp</i> cell	7.18	7.76	16.29	13.09
<i>Rhizobium sp</i> EPS	6.82	7.82	14.41	12.54
<i>Rhizobium sp</i> cell+ EPS	7.93	8.42	27.84	10.76
<i>Azotobacter sp</i> cell	6.83	7.86	8.19	9.95
<i>Azotobacter sp</i> EPS	6.76	8.01	16.32	12.61
<i>Azotobacter sp</i> cell+ EPS	7.97	8.34	21.06	14.65
<i>Bacillus sp</i> cell	6.94	7.75	15.31	21.22
<i>Bacillus sp</i> EPS	6.67	7.77	9.78	21.48
<i>Bacillus sp</i> cell+ EPS	7.75	8.22	11.46	26.44
<i>Azospirillum sp</i> cell	6.83	7.659	26.59	16.18
<i>Azospirillum sp</i> cell	6.88	7.68	8.32	11.16
<i>Azospirillum</i> cell+ EPS	7.76	7.97	15.95	16.34
Mixed bacteria	6.78	7.68	44.94	28.18
Mixed. EPS	6.79	7.51	9.37	24.14
Mixed bacteria+mixed EPS	7.08	7.52	11.36	15.52
L.S.D. (5%)			6.720	3.303

Mixed bacterial strains and mixture of EPS gave a lower total length compared with other treatment in both wheat and faba bean plants. This may be due to the quantities and qualitative of EP

S. The same trend was observed in dry weight of shoots and plant height of wheat and faba bean plants. The increase in dry weight of vegetative growth which could be considered as a criterion for the photosynthetic efficiency of the plant was reflect on the increase of yield These results are in agree with those obtained by Verma *et al.*, (2001) who found that *Azospirillum sp*, *Azotobacter sp* and *Bacillus sp* had the ability to fix nitrogen and produce plant growth promoting substances which help more absorption of nutrients by root plants.

Table (4): Growth parameters of wheat and faba bean plants as affected by bacterial inoculation and exopolysaccharides (EPS) in the sterilized soil after 45 days from sowing.

Bacterial strains	Wheat				Faba bean			
	Roots		shoots		Roots		shoots	
	Dry wt. (g)	Total length (cm)	Dry wt. (g)	Plant height (cm)	Dry wt. (g)	Total length (cm)	Dry wt. (g)	Plant height (cm)
Control	0.07	7.78	0.04	9.47	0.27	6.64	0.19	12.48
<i>Rhizobium sp</i> cell	0.61	12.14	0.15	14.78	0.38	13.67	0.92	18.27
<i>Rhizobium sp</i> EPS	0.26	11.18	0.12	12.74	0.37	12.78	0.96	16.91
<i>Rhizobium sp</i> cell+ EPS	2.07	16.61	0.43	22.91	0.39	13.67	1.00	20.01
<i>Azotobacter sp</i> cell	0.58	12.14	0.22	17.08	0.46	18.21	1.06	22.93
<i>Azotobacter sp</i> EPS	0.28	10.68	0.15	15.74	0.45	16.44	1.02	22.21
<i>Azotobacter sp</i> cell+Eps	1.78	13.10	0.34	19.41	0.51	18.48	1.33	24.45
<i>Bacillus sp</i> cell	0.86	9.48	0.15	16.81	0.40	13.95	1.01	20.08
<i>Bacillus sp</i> EPS	0.76	10.64	0.11	11.78	0.42	14.47	1.03	18.01
<i>Bacillus sp</i> cell+ EPS	1.19	10.91	0.22	16.34	0.43	14.68	1.05	19.22
<i>Azospirillum sp</i> cell	0.40	8.81	0.12	14.84	0.35	11.81	0.59	15.30
<i>Azospirillum sp</i> cell	0.44	9.18	0.11	11.68	0.31	9.81	0.41	13.91
<i>Azospirillum</i> cell+ EPS	0.72	9.24	0.15	16.91	0.35	12.15	0.64	16.64
Mixed bacteria	0.81	9.01	0.13	17.01	0.38	17.14	0.92	21.39
Mixed EPS	0.08	8.21	0.06	9.47	0.39	12.34	0.60	14.94
Mixed bacteria+ mixed EPS	0.08	8.10	0.07	10.58	0.31	11.34	0.24	12.88
L.S.D. (5%)	0.330	1.085	0.002	1.248	0.0017	1.156	0.055	1.137

Data in Table (5) indicated that inoculation with *Azotobacter sp* +EPS and *Bacillus sp* +EPS combined with *Rhizobium sp* significantly increased of dry weight of root nodules being 101,100 mg/plant compared to the other treatments, while the inoculation with mixed bacteria and mixed EPS decreased in the dry weight of nodules (81 mg/plant). These results not agree with Abo El-Soud *et al.*, 2003) who found that triple inoculation treatments (*Rhizobium*+ *Azotobacter*+*Bacillus*) gave significant increases in nodule dry weight.

Table (5): dry weight (mg/plant) of faba bean nodules as affected by inoculation and exopolysaccharides.

Bacterial strains	D. wt. (mg/plant)
Control	80
<i>Rhizobium sp</i> cell	90
<i>Rhizobium sp</i> EPS	92
<i>Rhizobium sp</i> cell+ EPS	92
<i>Azotobacter sp</i> cell	94
<i>Azotobacter sp</i> EPS	90
<i>Azotobacter sp</i> cell+ EPS	100
<i>Bacillus sp</i> cell	92
<i>Bacillus sp</i> EPS	92
<i>Bacillus sp</i> cell+ EPS	101
<i>Azospirillum sp</i> cell	92
<i>Azospirillum sp</i> cell	91
<i>Azospirillum</i> cell+ EPS	96
Mixed bacteria	94
Mixed EPS	82
Mixed bacteria+ mixed EPS	81
L.S.D. (5%)	19.60

Data in Table (6) showed that inoculation with PGPR either individually or with EPS significantly increased N₂-ase activity and nitrogen content in wheat and faba bean shoots. Inoculation of faba bean with *Rhizobium sp* plus EPS induced significant increase in N₂-ase activity comparing with the other treatments in wheat, while in faba bean, inoculation with *Azotobacter sp* +EPS gave the highest value, but inoculation with mixed bacteria and EPS gave the lowest value of N₂-ase activity and nitrogen content in shoots. These results are in harmony with Rodelas *et al.*, (1999) who found that inoculating faba bean with *Rhizobium*+ *Azospirillum* +*Azotobacter* under gontobiotic conditions significantly increased the total N content compared with plants inoculated with *Rhizobium* alone.

Data presented in Tables (7&8) showed the values of dry sieving aggregates (D.S.A %) under wheat and faba bean plants inoculated with either PGPR or extracted EPS. The aggregate values having diameters (0.5-0.25) were higher than any other aggregate fractions. The effect of inoculation could be arranged in the following order, *Azotobacter.sp* +EPS>*Bacillus sp*+EPS >*Azospirillum sp*+EPS. Such increases in total aggregate were possibly due to the root exudate and increase of the exopolysaccharides.

Table(6): Nitrogenase activity (μ mole C_2H_4 /dry weight soil) and nitrogen percentage in wheat and faba bean shoots as affected by inoculation and exopolysaccharinely in sterilized soil.

Bacterial strains	Nitrogenase activity		Nitrogen percentage	
	Wheat	Fababean	Wheat	Fababean
Control	0.19	0.34	0.50	0.41
<i>Rhizobium sp</i> cell	0.73	1.05	2.50	2.64
<i>Rhizobium sp</i> EPS	0.42	0.82	0.64	1.23
<i>Rhizobium sp</i> cell+ EPS	2.57	1.01	2.41	4.32
<i>Azotobacter sp</i> cell	1.24	1.32	4.09	2.72
<i>Azotobacter sp</i> EPS	0.81	0.86	3.60	2.36
<i>Azotobacter sp</i> cell+ EPS	1.38	1.51	3.60	4.73
<i>Bacillus sp</i> cell	0.96	1.25	1.95	1.39
<i>Bacillus sp</i> EPS	0.74	0.68	0.60	4.01
<i>Bacillus sp</i> cell+Eps	1.29	0.47	4.01	2.75
<i>Azopirillum sp</i> cell	0.40	1.15	1.93	1.37
<i>Azopirillum sp</i> cell	0.36	0.62	0.83	1.90
<i>Azopirillum</i> cell+ EPS	1.09	0.42	3.63	2.73
Mixed bacteria	0.70	1.41	1.51	1.38
Mixed. EPS	0.32	0.61	0.41	1.35
Mixed bacteria+ mixed EPS	0.30	0.37	0.45	0.61
L.S.D. (5%)	0.174	0.123	0.078	0.066

The inoculation caused a decrease in the sizes of <0.25 mm. **Yahia Kaci et al., (2005)** demonstrated that EPS producing bacteria present in sandy soils play an important role in the rhizosphere through their contribution to soil aggregation. In conclusion, inoculation with PGPR plus EPS caused a slight increase in total aggregates.

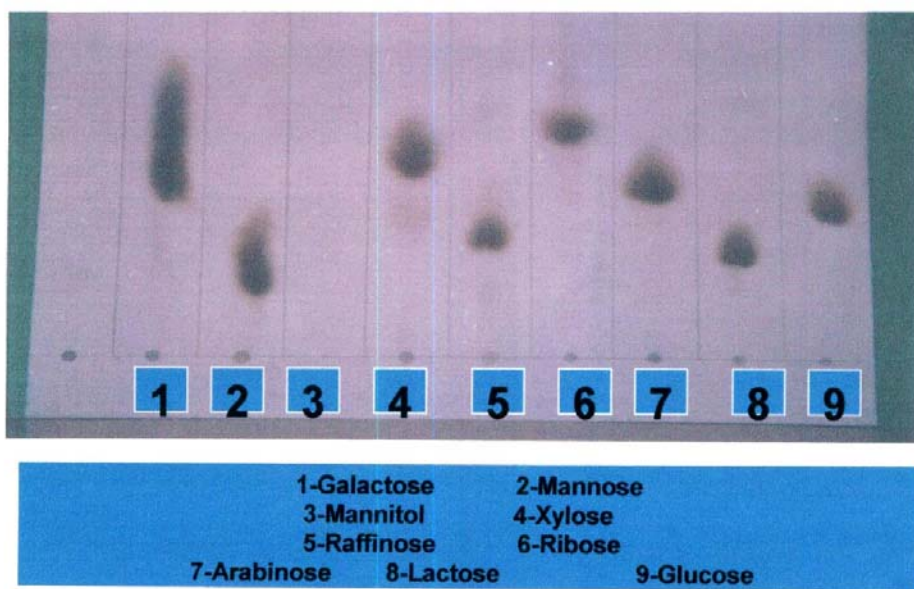
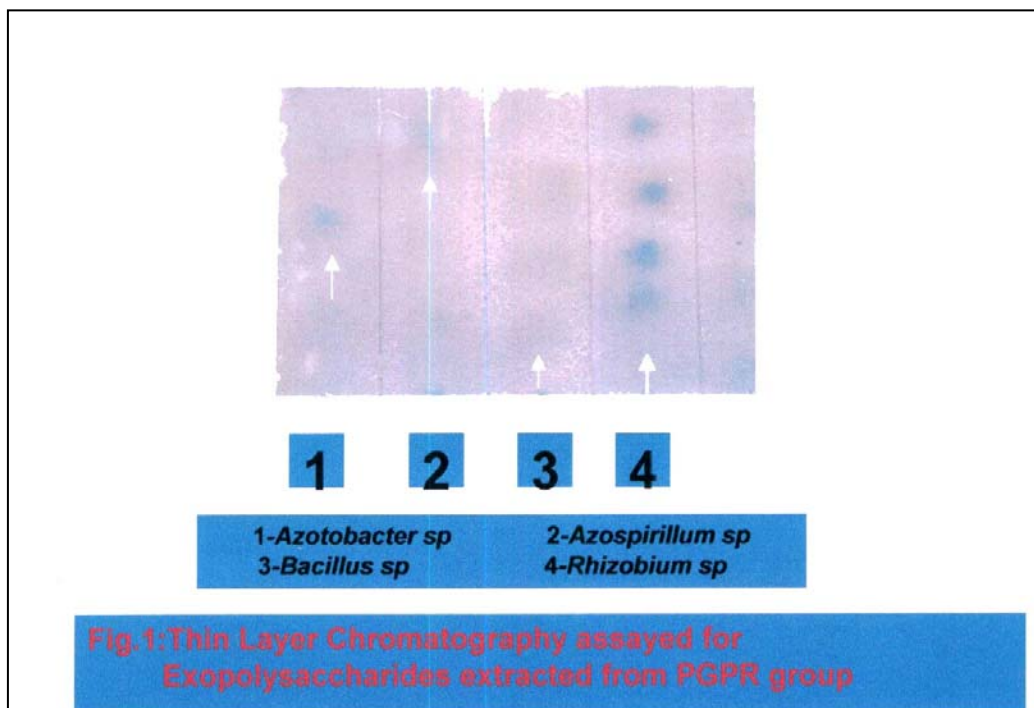
Table (7): Total aggregates and aggregates size distribution of the sterilized soil of wheat (Sakha 69) per gram as affected by the bacterial inoculation and exopolysaccharides.

Bacterial strains	Aggregate size Diameters (mm)				Total M.W.D.
	1-0.5	0.5-0.25	0.25-0.125	0.125-0.63	
Control	0.009	0.12	0.02	0.002	0.151
<i>Rhizobium sp</i> cell	0.13	0.16	0.03	0.002	0.322
<i>Rhizobium sp</i> EPS	0.09	0.13	0.02	0.001	0.241
<i>Rhizobium sp</i> cell+ EPS	0.11	0.14	0.03	0.001	0.281
<i>Azotobacter sp</i> cell	0.09	0.17	0.03	0.001	0.291
<i>Azotobacter sp</i> EPS	0.106	0.14	0.03	0.001	0.277
<i>Azotobacter sp</i> cell+ EPS	0.117	0.19	0.04	0.016	0.363
<i>Bacillus sp</i> cell	0.08	0.16	0.04	0.002	0.282
<i>Bacillus sp</i> EPS	0.102	0.14	0.03	0.001	0.273
<i>Bacillus sp</i> cell+ EPS	0.11	0.18	0.04	0.012	0.342
<i>Azospirillum sp</i> cell	0.07	0.16	0.03	0.001	0.261
<i>Azospirillum sp</i> cell	0.06	0.12	0.02	0.001	0.201
<i>Azospirillum</i> cell+ EPS	0.117	0.17	0.03	0.002	0.317
Mixed bacteria	0.094	0.17	0.04	0.002	0.306
Mixed. EPS	0.091	0.13	0.02	0.001	0.242
Mixed bacteria+ mixed EPS	0.091	0.12	0.02	0.001	0.232

Colonization patterns of the diazotrophs associated with wheat and faba bean. Colonization was characterized as the ability of some bacterial cells to attach the root. To investigate the colonization patterns of PGPR group on wheat (Fig.3 (A,B,C,D and E) and faba bean roots (Fig. 4 (A,B,C,D and E) showed bacteria stained by 1ml 2, 3, 5 triphenyltetrazolium chloride (TTC), to investigate the presence of colonization of root by immersing roots in (TTC) for 1 to 2hr. Plant tissue were visible in a red color due to reduction of TTC to TPF by bacteria, while uninoculated wheat and faba bean roots were colorless.

Table (8): Total aggregates and aggregates size distribution of the sterilized soil of faba bean (Giza717) per gram as affected by the bacterial inoculation and exopoly-saccharides

Bacterial strains	Aggregate size Diameters (mm)				Total M.W.D
	1-0.5	0.5-0.25	0.25-0.125	0.125-0.63	
Control	0.11	0.16	0.02	0.0001	0.290
<i>Rhizobium sp</i> cell	0.14	0.18	0.02	0.001	0.341
<i>Rhizobium sp</i> EPS	0.13	0.18	0.02	0.001	0.331
<i>Rhizobium sp</i> cell+ EPS	0.15	0.20	0.02	0.001	0.371
<i>Azotobacter sp</i> cell	0.14	0.20	0.02	0.001	0.361
<i>Azotobacter sp</i> EPS	0.13	0.18	0.02	0.001	0.341
<i>Azotobacter sp</i> cell+ EPS	0.17	0.24	0.04	0.002	0.452
<i>Bacillus sp</i> cell	0.13	0.21	0.03	0.001	0.371
<i>Bacillus sp</i> EPS	0.14	0.17	0.02	0.001	0.331
<i>Bacillus sp</i> cell+ EPS	0.16	0.22	0.03	0.002	0.412
<i>Azospirillum sp</i> cell	0.13	0.20	0.02	0.001	0.351
<i>Azospirillum sp</i> cell	0.14	0.16	0.02	0.001	0.321
<i>Azospirillum</i> cell+ EPS	0.15	0.22	0.03	0.001	0.401
Mixed bacteria	0.15	0.22	0.03	0.001	0.401
Mixed. EPS	0.11	0.16	0.03	0.001	0.301
Mixed bacteria+ mixed EPS	0.11	0.16	0.02	0.001	0.291



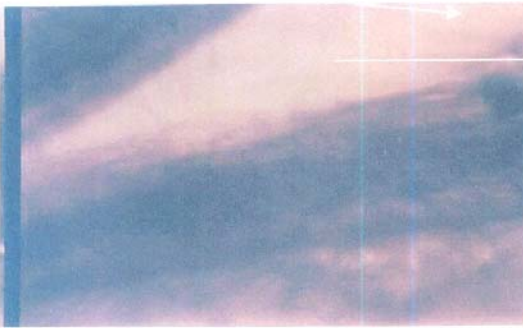


Fig.3A-Wheat roots without inoculation

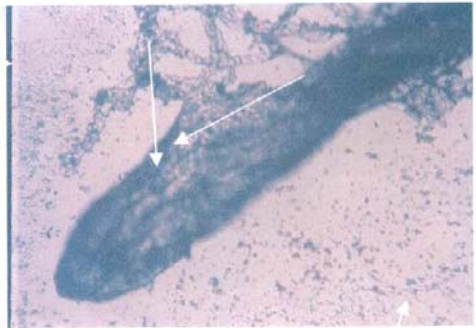


Fig.3-BWheat roots inoculated with Azotobacter



Fig.3D-Wheat roots inoculated with *B. polymyxa*



Fig.3C-Wheat roots inoculated with Azotobacter and its polysaccharides



Fig.3E-Wheat roots inoculated with *B. polymyxa* and its polysaccharides



Fig.4A-Faba bean roots without inoculation



Fig.4C-Faba bean roots inoculated with *Rhizobium* and its polysaccharides



Fig.4 B-Faba bean roots inoculated with *Rhizobium*



Fig.4E-Faba bean roots inoculated with *Azotobacter* and its polysaccharides

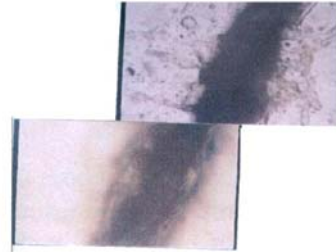


Fig.4D-Faba bean roots inoculated with *Azotobacter*

**Fig.3 Colonization patterns of wheat (A, B, C and D)
Fig.4 Colonization patterns of faba bean (A, B, C and D)**

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الملخص العربي

تحسين النمو الخضري لمحصولي الفول البلدي و القمح و التجمعات الارضية في منطقة الجذور باستخدام بعض سلالات من البكتيريا المنشطة للنمو والمنتجة للسكريات العديدة
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في هذا البحث تم استخدام 4 أنواع من العزلات البكتيرية المعزولة من توشكى الاسماعيلية والبوصيلي وتم تعريفها كالاتى:

Rhizobium sp, Azotobacter sp., Bacillus sp, Azospirillum sp.

وتم استخلاص السكر من هذه السلالات باستخدام الطرد المركزي وتم تعريف انواع السكر المستخلص وذلك بتفريدها على ألواح مغطاة بالسليولوز (Thin layer chromatograph, TLC). تم استخدام هذا السكر المستخلص بالاضافة إلى الأربع انواع من البكتريا كلقاح حيوى فى تلقيح نباتات القمح والفول البلدى فى تربة رملية معقمة وتركت لمدة 45 يوم بعد ذلك تم قياس نشاط انزيم الدهيدروجينيز والنيتروجينيز وعدد البكتريا فى منطقة الريزو سفير و محتوى النيتروجين فى النبات ووزن وطول الجذور والسيقان، حساب التجمعات الرملية الحقيقية، وتم اختبار قدرة السلالات البكتيرية على الالتصاق على جذور بادرات القمح والفول البلدى وذلك بصبغها باضافة 5مل ترى فينيل تترازوليم كلوريد وتم الفحص بواسطة الميكروسكوب الضوئى.وأظهرت النتائج أن هناك زيادة معنوية باستخدام مستخلص السكر والعزلات المذكورة على النشاط الانزيمى للنبات وكذلك أطوال السيقان والبذور وعدد البكتريا فى منطقة الريزوسفير، وأوضحت عملية الالتصاق على جذور النباتات وجود أعداد أكبر من هذه البكتريا عن هذه النباتات الغير ملقحة.