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Traits Biologically Interacting *Azospirillum lipoferum* Strain R_{23}

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Abstract: The effect of *Azospirillum. lipoferum* (strain R_{23}) inoculation and / or wheat straw (0.5 and 10 t ha^{-1}) amendment on the growth and N_2 fixation of two wheat cultivars (Giza 167 and local cultivar) was determined in pot experiments using the difference method (DM). *Azospirillum* inoculation resulted in accumulation of fixed nitrogen and N% from atmosphere was 36.7 and 12.9 for wheat Giza 167 and the local wheat cultivar, respectively. Wheat straw amendment reduced the fixed N of local cultivar to 9.7 and 11.2% at ca 5 and 10 t ha^{-1} and recorded 23.9 and 20.1% for wheat cultivar Giza 167. Rational nitrogen fertilization (180 kg N ha^{-1}) recorded the lowest N concentration from air and recorded 7.1 and 11.2% for wheat local and Giza 167 cultivars, respectively. The highest levels of increased growth parameters were obtained by N-fertilization in both inoculated and uninoculated plants for both cultivars because of readily forms of N in the mineral fertilizers. Wheat straw addition had no significant effects in both cultivars used in the present study and such outcome attributed to the nutrient and carbon quality of the wheat straw residues.

Key words: *Triticum aestivum* · *Azospirillum lipoferum* · Wheat straw · Al Jabal Al Akhdar · Libya

INTRODUCTION

Nitrogen (N), an element vital for animal and plant growth, constitutes 78% of the earth's atmosphere. In spite of its abundance, it is one of the most limiting factors for crop growth and nitrogen fertilizers represent one of the major costs in crop production. Biological dinitrogen fixation (N_2-NH_3) is the single most important alternative for the increasing high costs of N fertilizer input into cropping systems without substantial loss in yield. Moreover, the dramatic rise in the costs of ammonia fertilizers and the steady increasing world demands for fertilizers provides the importance for the exploitation of biological N_2 fixation. The amount of nitrogen added to the soil by biological N_2 fixation could improve the efficiency of crop production by reducing the need for N fertilizer [1].

Associative nitrogen fixation is defined as, nitrogen fixation by free-living diazotrophs under the direct influence of the host plant. In an associative nitrogen-fixing system, plants supply the diazotrophs with organic substrates (exudates, secretions, lysates and sloughed cells), in turn the microorganisms fix atmospheric N_2 that is directly or indirectly transferred to the plant. It has been suggested that free-living bacteria fix N_2 only while they are actively growing, which could mean that they require organic substrates not only to generate energy but also to synthesize new cells. Low efficiency in a symbiotic diazotrophs is a product of two important constraints not confronted by symbiotic bacteria. Firstly, asymbiotic bacteria only fix N_2 while actively growing, in contrast to symbiotic bacteria which are non-dividing and thus a great deal of carbon is required for growth. Secondly, the aerobes invest a significant amount of carbon in mechanisms to reduce the impact of oxygen.

Following the discovery of the C_2H_2 reduction method for determinations of nitrogenase activity, associative N_2 fixers including *Azospirillum* spp. were demonstrated in many grasses and cereals [2]. The first species of *Azospirillum* was isolated by Beijerinck [3] from most of the tested soil samples (N-poor sandy soils) in the Netherlands and originally named *Spirillum lipoferum*. *Azospirillum* spp. are gram-negative, vibro or spirillum-shaped and 1ìm diameter, possessing peritricheus flagella used for swarming and one polar flagellum used for swimming. Poly β-hydroxybutyrate

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granules fill most of the bacterial cell and colonies develop a pink pigment. This species is characterized by its tolerance for acidity with pronounced sensitivity to alkaline reaction of the medium. *A. halopraeferans* which is characterized by its high salt (3% NaCl) tolerance and was found associated with kallar grass (*Leptochla fusca*) roots, a salt-tolerant pioneer plant common in sodic soils in Pakistan. *A. irakean* was isolated from the roots of rice in Iraq which is characterized by its ability to utilize pectin as a carbon source for N_2-fixation. *A. doebereinerae* is the most recently discovered *Azospirillum* species. It has only been isolated from the C_4-plants *Miscanthus sinensis* and *M. sacchariforus*. Association between N_2 fixing-bacteria of the genus *Azospirillum* and the roots of grasses as well as other plants has been intensively studied since the first report by Dobereiner and Day at the first international Congress on N_2-fixation in 1976. Few years later, a number of pioneer reports on enhanced N_2-fixation by *Azospirillum* inoculation of cereals and other non legumes appeared [4, 5]. Nitrogen fixation was the first mechanism proposed to explain improving in plant growth following *Azospirillum* inoculation. Moreover, careful experimentation using the ^{15}N-isotope dilution technique with some cultivars of sugar cane, *Panicum maxima*, *Paspalum notatum*. Lima *et al.* [6] have demonstrated that up to 50% of the plant N is derived from BNF. This has also been demonstrated in certain wheat and maize cultivars [7, 8].

The contribution of Biological Nitrogen Fixation (BNF) to the N content (20-50%) of kallar grass grown in infertile saline soil in Pakistan, as measured by ^{15}N dilution technique, has been also described. However, Bashan *et al.* [9] reported that the contribution of N_2 fixation by *Azospirillum* was smaller than 5% increase and mutants were capable of increasing plant growth similar to that by the wild type N_2-fixer. Moreover, most investigators agree that the number of *Azospirillum* cells in roots, though considerable, is too small to enhance crop yield significantly by means of N_2 fixation [10]. It could be shown with wheat that both inoculation with *Azospirillum brasilense* Cd and application of pure IAA to the roots increased root length, numbers of lateral roots and the number of root hairs [11]. Thus the mode(s) of action of *Azospirillum* composed of more than one mechanism involved at the same time, with individual mechanisms being less significant when evaluated separated (Bashan *et al.*, 2004). Combined nitrogen generally inhibits N_2-fixation by repressing the synthesis and by inactivating existing enzyme. Information concerned with *Azospirillum* inoculation under different levels of N-fertilizer are controversial Bashan and Levanony [1] demonstrated that over than 100 plant species (C_3 and C_4 plants) were positively affected by *Azospirillum* inoculation. These authors also reported that there are more non-cereal species were successfully inoculated with *Azospirillum* than cereals, despite of *Azospirillum* was initially isolated from cereals and most of the initial inoculation has been done on cereal crops. In previous study Attitalla *et al.* [12] demonstrated the occurrence of *Azospirillum* spp in Al Jabal Al Akhdar eco-region, (Libya).

However the present study performed the Counting, isolation and Identification of indigenous *Azospirillum* strains existed in soils supporting different plants grown in El Jabal El Akhdar eco-region, Libya. We also estimated the nitrogenase activity (in vitro) for the pure *Azospirillum* cultures (by the acetylene reduction assay ARA) and their nitrate reductase activity. Finally we studied the response of two wheat cultivars (*Triticum aestivum* cultivar Giza 167 and *T. aestivum* locar cultivar) to inoculation with a selected *Azospirillum* strain individually or at different levels of wheat straw (organic matter) or at the recommended-dose of N-fertilizer (pot-experiment at 2007/2008 season). As well as the effect of such bacteria on the percentage of nitrogen derived from air (% Ndfa) the nitrogen fixed in the two cuktivars.

MATERIALS AND METHODS

The study included isolation of the N_2-fixing *Azospirillum* spp. from 23 soil and root samples collected from region and to study the effect of inoculation with *Azospirillum* spp. at different levels of organic-matter (wheat straw) on the growth and N_2-fixation as well as some physiological activities of wheat cultivars.

Determination of Soil Type: The soil type was determined by the hydrometer method, as described by Piper [13]. For the determination of total soluble salts, a known weight of each soil sample was shaken in a volume of distilled water for about 30 minutes and the mixture was left overnight to settle. The soil extract was then filtered evaporated in an oven at 105°C. The dry residue was then weighed and the amount of total soluble per gm oven-dry soil was calculated. Soil organic matter content was determined according to Walkly and Black method [14]. A certain amount of sieved soil was digested by chromic acid (for oxidation of organic matter to carbon dioxide). A Beckman pH meter was used for the determination of soil pH.s [14].

Soil Chemical Analysis

Total Soluble Salts: For the determination of total soluble salts, 100 g of each soil sample was shaken in a 500 ml of distilled water for about 30 min and the mixture was left overnight to settle. The soil extract was then filtered evaporated in an oven at 105°C. The dry residue was then weighted and the amount of total soluble per gm oven-dry soil was calculated.

Organic Matter Content: The organic content of the soil sample was determined according to Walkly and Black method [15, 16], minor modification by Sahrawat was adopted [17]. The 0.5 g of sieved soil sample was digested by chromic acid (for oxidation of organic matter to carbon dioxide) and the excess chromic acid was back titrated against standard ferrous sulphate solution using diphenylamine as an indicator.

pH Value: A Beckman pH meter was used for the determination of soil pH. The electrodes were immerse in the soil paste with water to a ratio of 1:1 to avoid the error arising through higher dilutions [16].

Isolation of *Azospirillum* spp. From Rhizosphere and Free-Soil Samples: For isolation of N_2-fixing *Azospirillum* spp., the free soil was removed from the plant roots by shaking the roots and the soils were subjected to soil-chemical analysis. Roots were cut into approximately 0.5-1 cm long segments. One hundred mg of the root pieces were collected from different localities and both the soil and roots samples were introduced into a sterile test-tubes containing 4 ml of semisolid NFb-medium [18].

In some cases the semisolid medium was inoculated with soil suspensions of 10^{-5} to 10^{-7} dilutions. After 72 h of incubation period, veil-like pellicles were observed below the medium surface which indicated presence of *Azospirillum*. It is easily recognized with the blue medium (use of malic acid lead to a pH decrease and a change in medium color). *Azospirillum* were transferred to new test tubes containing the same medium. As a new pellicle was visible, the cultures were streaked out on agar plates containing the same medium with yeast extract (20 mg l^{-1}). The small amount of yeast extract permits the growth of small colonies on the surface of the plates. Individual colonies were then maintained on NA slants for further studies.

Identification of the Bacterial Strains: Bacterial strains were streaked four times for purification on malate agar medium supplemented with (20 mg l^{-1}) yeast extract [18]. Purification and microscopic examination (using Olympus C×21 microscope) were carried out to obtain pure cultures. Fifteen strains were recognized as belonging to the genus *Azospirillum* based on the morphological, cultural and some biochemical characteristics by [19] and the schemes as described in the 9th edition of Bergey's Manual of Systematic Bacteriology [13].

The International type species, the Brazilian strain *A. brasilense* Cd. Sp7 (ATCC, 29145) and *A. lipoferum* strain 137, which was kindly supplied by Prof. Vassuyk, L.F., Academy of Science, Petersburg, Russia were used as reference for comparison with the locally isolated strains.

Cultural and Morphological Description: Putative *Azospirillum* colonies were selected on the basis of the culture plate morphology characteristics namely: opacity, pale to deep pink pigmentation, no slimy and wrinkled. Selected colonies were picked up and cultured on NA slants.

Morphological Description of the Vegetative Cells: *Azospirillum* was examined for cell-shape, Gram reaction, inclusions and motility in the semisolid malate medium after 1-3 days. Polymorphism was recorded after 2, 7 and 15 days of incubation.

Physiological and Biochemical Tests: For species determination, utilization of different carbon-sources was performed either in aerobic or anaerobic conditions. The bacterial strains were grown in semisolid malate medium containing carbohydrate together with a pH indicator (bromothymol blue).

The development of a yellow color during 96 hour incubation at 30°C indicated acidification. The tests involved catalase activity, denitrification test, growth in the presence of 3% NaCl and growth on the amino acid L-Histidine as a sole C and N source (Hartmann *et al*., 1988). Production of siderophore (growth on M-9 medium) [20] as well as starch and gelatin hydrolysis were also tested [21].

Statistical Analysis: The data were subjected to one way-analysis of variance (ANOVA) and the means were separated by the least significant difference, LSD [22].

RESULTS AND DISCUSSION

General Description of Al Jabal Al Akhdar Soils: Table 1 shows some properties of the soil samples and the plant used for isolation. The percentages of sand, silt and clay ranged from 60.68 to 90.68, 5.45 to 30.45 and 3.87 to 15.59, respectively. The texture of all soil samples

Table 1: Characteristics of the soil samples and plant used for isolation and total *Azospirillum* counts

Soil No.	Place	Partical size distribution				pH	E.C. ds/m	Organic matter %	Plant under cultivation	No. of *Azospirillum* × 10^3
		Sand %	Silt %	Clay %	Texture					
1	Kernada	64.70	26.80	8.50	Sandy Loamy	7.7	0.27	3.3	*Pimpinella anisum*	2.5
2	Albyda	66.68	20.72	12.59	Sandy Loamy	8.2	0.38	4.3	*Triticum vulgaris*	1.1
3	ElKharika	70.68	26.72	2.59	Sandy Loamy	7.9	0.41	4.1	*Phagnalon rupestre*	15.0
4	Omar Al-Mukhtar	64.69	20.35	14.95	Sandy Loamy	8.2	0.47	2.4	*Thapsia garganica*	2.1
5	El-Faidia	60.68	23.72	15.59	Sandy Loamy	7.9	0.33	2.2	*Marrubium vulgare*	26.1
6	El-Mansora	63.04	30.45	6.51	Sandy Loamy	7.7	0.43	1.9	*Portulaca oleracea*	9.2
7	Shahat	70.68	20.72	8.59	Sandy Loamy	7.8	0.41	3.1	*Thymus serpyllum*	8.5
8	Wardama	70.68	24.72	4.59	Sandy Loamy	7.6	0.30	3.0	*Marrubium vulgare*	130.2
9	Massa	64.68	24.72	10.59	Sandy Loamy	7.7	0.38	2.7	*Glycin max*	25.1
10	Belhaded	66.68	24.72	8.59	Sandy Loamy	7.8	0.27	2.0	*Ceratonia siligua*	50.5
11	El-Waseta	68.68	18.72	12.59	Sandy Loamy	8.1	0.22	3.2	*Zea mays*	2.1
12	Eslenta	68.68	19.72	11.59	Sandy Loamy	7.8	0.62	3.8	*Cucumis sativus*	14.5
13	Kandola	68.4	19.0	12.59	Sandy Loamy	7.7	0.51	1.3	*Brassica oleracea*	42.5
14	Gardas	68.68	16.72	14.59	Sandy Loamy	7.8	0.32	1.0	*Hordium vulgaris*	46.4
15	El-Khwimat	84.68	10.08	5.23	Sandy Loamy	8.3	0.26	0.9	*Artemesia herba-alba*	17.5
16	Marawa	66.68	26.72	6.59	Sandy Loamy	8.3	0.13	1.4	*Thapsia garganica*	16.5
17	Eljehad	72.68	18.72	8.59	Sandy Loamy	7.9	0.34	3.6	*Thapsia garganica*	18.4
18	Kaser Libya	73.04	26.36	0.59	Sandy Loamy	8.4	0.25	1.4	*Hordium vulgaris*	3.5
19	Zawiat Elarkob	78.68	8.72	12.59	Sandy Loamy	8.4	0.27	1.6	*Triticum vulgaris*	7.5
20	Ekfenta	56.68	34.00	9.31	Sandy Loamy	8.0	0.18	1.5	*Thapsia garganica*	8.5
21	El-Hamama	69.68	21.72	8.59	Sandy Loamy	8.0	0.26	1.2	*Artemesia* sp.	56.1
22	El-Koof	73.04	18.36	8.59	Sandy Loamy	8.3	0.31	1.8	*Paronychia argentina*	33.2
23	El-Haneia	90.68	5.44	3.87	Sandy	7.7	0.92	0.8	*Lycopersicum* sp.	6.5

Table 2: Morphological and Biochemical activities of the isolated *Azospirillum* spp.

Tests	Isolates																
	A. brasilense (Sp_7)	*A. lipoferum* (137)	F8	F9	F14	F18	R6	R7	R8	R9	R10	R17	R23	P6	P8	P9	P10
Cell-shape	curved	ovoid	ovoid	ovoid	rod	rod	curved	ovoid	curved	spiral	rod	rod	ovoid	rod	ovoid	curved	ovoid
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram-stain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polymorphism	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in																	
L-Histidine	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 3% NaCl	+/-	+	+/-	+	+/-	+	+/-	+	+	+	+	+	+	+	+	+/-	+/-
Growth in M9 medium	+	+	+/-	-	-	+	+	+	+/-	+	-	+	+	+/-	+	-	-
Growth in JNFb medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sole carbon Source:																	
Succinate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C_2H_2-reduction (nmole C_2H_4/h)	126	96.5	110	86.6	54.3	36.6	77.3	86.5	109.5	34.5	50.0	66.0	119.2	35.5	44.0	76.5	87.9

F= Isolated from free-soil. R= Isolated from rhizoshere. P= Isolated from rhizoplane

was sandy loam except for El-Haneia soil, which was sandy. The pH values were higher than 7 which means that the soils were alkaline and the highest were recorded in Kaser Libya and Zawiat Elarkob soils (with pH 8.4, Table 1). Such alkaline reaction of the Jabal Al Akhdar eco-region can be attributed to the bed rocks, which contain high concentrations of calcium carbonates (limestone) [23].

The values of EC ranged from 0.31 to 0.92 dS/m which means that the soils were not saline. However, during drought periods of this area, which is located in Mediterraean-type climate, the soil biota may influenced by the direct and indirect effects of the salts. With respect to the soil organic matter, the values ranged from 0.8 to 4.3 % which can be attributed to the quantity and quality of the crop residues [24].

Isolation, Identification and Distribution of *Azospirilum* Spp.: Twenty-eight spirillum strains were recovered from both free-soil (bulk soil), or from rhizosphere and rhizoplane of the collected plants. After several transfers for purification, 15 strains were identified as bacteria belonging to the genus *Azospirillum* based to the following common cultural and cell-morphological characteristics (Table 2).

Results presented in this study indicated that *Azospirillum* was found abundantly and dominantly in all of the tested soil-samples (23-samples) collected from different localities in Al Jabal Al Akhdar eco-region, which were from cultivated areas and areas with weed plants. *Azospirillum* densities were as high as 1.1×10^3 - 13.2×10^4 CFU/g dry soil. The size of *Azospirillum* population has been estimated at 1-10% of the total soil population [25]. Until recently, procedures used for isolation N_2 fixing bacteria were not basically different from those proposed by Beijerinck [26] and Winogradsky [27]. The procedure based on the enumeration and purification of bacteria growing in N-free media inoculated with dilutions or aggregates of soil.

The addition of certain carbon source encourage the growth of certain group of bacteria. For instance, addition of manitol or glucose in N_2-free medium leads to the frequent isolation of Azotobacteraceae [28], whereas malate leads to the isolation of *Azospirillum* [29]. In the present study, *Azospirillum* was isolated and enriched from the rhizosphere, rhizoplane or bulk soil using the nitrogen-free biotin medium (NFb) in which L-malic was the sole C-source. Although reports on the isolation of *Azospirillum* from graminaceous plants are common, other reports showed that the bacterium is a natural inhabitant of many nongraminaceous plants. *Azospirillum* was isolated from roots of coconut palms grown under diverse agronomic practices [30] and within the stem nodules, root nodules and stem of *Aeschynamene indica* [31].

In the present study *Azospirillum* was isolated from cultivated plants (crops) (*Hordium vulgaris*, *Zea mays*, *Triticum vulgaris*) as well as non-cultivated weed plants (*Thapsia garganica*, *Marrubium vulgare*, *Paronychia argentina*). Moreover, *Azospirillum* was also isolated from leguminous plants such as *Ceratonia siliqua* (Crob), *Glycin max* (Soya bean)) and non-leguminous plants such as *Hordium vulgaris* (Barley), *Zea mays* (Corn).

Thus, *Azospirillum* is a general root colonizer and is not a plant-specific bacterium [5, 32]. In the present investigation, 28 spirilla were isolated from the rhizosphere, rhizoplane and free-soil from different plants grown in Al Jabal Al Akhdar region. After several transfers, 15 strains were identified as bacteria belonging to the genus *Azospirillum* based on to their common cultural and cell morphological characteristics. The characteristics were the formation of a veil like pellicle or ballon often 10 mm below the surface of semisolid N-free media. The formation of this pellicle is due to an aerotactic response of the motile bacteria towards low levels of PO_2 that permit N_2 fixation [25]. The dissolved O_2 concentration in the media was just enough for optimal respiration rates without inhibiting N_2 fixation [2], as a result of nitrogenase inhibition.

Screening of different strains for carbohydrate utilization differed markedly with respect to the *Azospirillum* species and to the carbon source (Table 3). However, all the 15 strains effectively oxidized the tested organic acids (Succinate, Malate and Pyruvate) when used as a sole carbon source auxanotrophically [in presence of (NH_2SO_4)]. The preference of the organic acids by different *Azospirillum* species was reported earlier by Reinhold *et al.* [33]. This can be explained on the basis that organic acids were the major source of nutrients for the microflora in the rhizosphere [34].

The results of the present study also indicated that *A. lipoferum* related strains were able to utilize large group of carbohydrate, while *A. brasilense* strain (SP7) was more restricted in its use of carbon sources including glucose which was not used by *A. brasilense* (SP7). Glucose catabolized by *Azospirillum* spp. by the action of NAD (P)-glucose-6-p-dehydrogenase, is required for 6-phosphoglucanate dehydrogenase synthesis which is a key enzyme of the ED pathway for glucose catabolism. It was the first enzyme produced in high level by *A. lipoferum* but was undetectable in *A. brasilense* [35]. However, *A. amazonense* has remarkable ability to grow and fix N_2 in media containing disaccharides [36], which is a characteristic of this species. The isolated *Azospirillum* spp. in the present study was not able to utilize sucrose (Table 3).

Table 3: Oxidation and Fermentation of different carbon compounds by the isolated *Azospirillum* spp. in comparison with type species

	Carbon Source															
	Glucose		Fructose		Sucrose		Xylose		Maltose		Dextrose		Lactose		Manitol	
Isolates	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.	Ox.	Fer.
A. brasilense (Sp_7)	-	-	-	-	-	-	±	±	+	+	-	-	-	-	-	-
A. lipoferum (137)	+	+	±	±	-	-	-	-	+	+	±	±	±	±	+	+
F8	+	+	+	+	-	-	-	-	±	±	+	+	+	+	+	+
F9	+	+	±	±	-	-	-	-	+	+	+	+	+	+	±	±
F14	+	+	±	±	-	-	-	-	+	+	+	±	+	+	+	+
F18	+	+	+	+	-	-	-	-	+	+	+	±	+	+	+	+
R6	+	+	+	+	-	-	-	-	±	±	+	+	+	+	+	+
R7	+	+	±	±	-	-	-	-	+	+	+	+	+	+	±	±
R8	+	+	+	+	-	-	-	-	±	±	±	±	+	+	+	±
R9	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
R10	+	+	±	±	-	-	-	-	+	+	±	±	+	+	+	+
R17	+	+	±	±	-	-	-	-	+	+	+	+	+	+	+	+
R23	+	+	±	±	-	-	-	-	+	+	±	±	+	+	+	+
P6	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
P8	+	+	+	+	-	-	-	-	+	+	+	±	+	+	+	+
P9	+	+	±	±	-	-	-	-	+	+	+	+	+	+	+	+
P10	+	+	±	±	-	-	-	+	+	+	+	+	+	+	+	+

Ox. = Oxidation (aerobic) Fer.= Fermintation (anaerobic condition)

- = No oxidation or Fermintation

+/- = Weak oxidation or Fermintation

+ = Positive oxidation or Fermintation

Siderphores are low-molecular weight compounds produced by the microorganisms which are able to bind iron from the environment. The binding to the siderphore allow transfer of iron to the cell, enabling bacteria to compete for this otherwise unavailable element [37]. *Azospirillum* spp. produces siderophores that represent an important factor for their competition and survival in the rhizosphere [38]. The siderophore spirilobactin produced by *A. brasilense* strain RG, was also reported by Bachhawat and Gosh [20]. The siderophore iron uptake of *A. basilense* SP6 was studied by using molecular genetic approach and the *Lon* gene was found to be involved in the iron uptake of *A. brasilense* [39]. Our *Azospirillum* strains were examined for siderophore production by testing their capability to grow in iron-limiting (M-9) medium (44). Table (2) indicated that seven strains (F_{18}, R_6, R_7, R_9, R_{17}, R_{23}, P_8) were able to grow well in the later medium, whereas three other strains exhibited moderate growth and the remaining strains cannot grow in that special medium.

More studies are needed to examine the efficiency of such indigenous *Azospirillum* strains for the nitrogen fixation. Hence, the most efficient strain could be used for increasing the soil fertility and plant production. Likewise, the counts of such strains in the rhizosphere of legumes should also be further investigated.

CONCLUSIONS

In conclusion, the data presented in this study indicate that the mode of action of *Azospirillum* is most probably composed of multiple mechanisms. Further studies are needed to clarify possible interaction in *Azospirillum*-host plant association. Therefore, the increased use of the various biological processes in soil, of which some examples have been given in the present study will decisively contribute to make agriculture more productive with less harm to the environment. This fact may be of importance for developing countries where the use of fertilizers is costly. It is hoped for substantial increase in food production in order to eliminate under-nourishment and poverty, which is the main goal to be achieved by using biofertilizers.

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