EPH - International Journal of Applied Science
ISSN (Online): 2208-2182
Volume 04 Issue 04-October-2018

DOI: https://doi.org/10.53555/eijas.v4i4.124

SCREENING OF DIFFERENT ISOLATES OF AZOSPIRILLUMFOR GROWTH AT DIFFERENT SALT (NACL) CONCENTRATIONS

Amal A.A. Taher[1*], Nagah S. A. Abubaker[2], Hesham M. A. El-Komy[3]
*[*1, 2, 3]Botany Department, Faculty of Science, Omar El-Mokhtar University, El -Beyda-Libya*

[*]Corresponding author:-
***E-mail**: amal.almoher@gmail.com*

Abstract:-
Azospirillum was abundant in the rhizosphere of halophytes collected from El- Thaliga salt marsh near Benghazi, Libya. More than twenty five isolated of Azospirillum were isolated from rhizosphere by enrichement method using DN semisolid medium with or without salt. After several transfer for purification, 13 isolates were screened to select the most efficient strains in a microcosms experiment with wheat. According to the cultural, morphological and biochemical experiments Azospirillum isolated were belonging to A. brasilense and isolated was belonging to A. lipoferum, Azospirillum isolates were screened for growth at different NaCl concentration in liquid DN medium.

Keywords:- *Azospirillum, salt and concentration*.

INTRODUCTION

Azospirillum has the potential to affect many plant species so far, it has not been explained how the action of the bacteria *Azospirillum* (Bashan and Holguin 1997, El Komy and Abdel wahab 1998). The principal mechanism by which *Azospirillum* enhances plant growth is not completely determined (Bashan and Holguin 1997).However hypothesis considers multiple mechanisms rather than on mechanismis participating in this association, the contribution of an individual mechanism being lees significant when evaluated separately. The sum of their activies, when induced under appropriate environmental conditions, results in the observed changes in plant growth (Bashan *et al* 2004).

Azospirillum promote plant growth by the following mechanisms N_2 fixation, phosphate solubilization, lowering of the ethylene concentration, production of phytohormones such as auxins cytokinins and gibberellins siderphore production (Mayak *et al* 2004, El-Komy 2005). *Azospirillum* also defined on the basis of physiological and hereditary characteristics where it was defined as 15 species and the most studied species *A.brasilenes* (Tarrand *et al* 1978).

The analysis of the soil parts revealed the *A.brasilens* has preferences mainly to formation of large concentrations in ths soil (Kabir *et al* 1994).

The physical composition of the soil molecules the sex of the existing bacteria or the growth conditions of the bacteria before fertilization affect the ability of the different soils on the adsorption of the incoming bacteria (Bashan *et al* 1995).

Material and methods

More than twenty five *Azospirillum* isolates were recovered both from free and rhizosphere soil samples of 4 halophytes. After several transfers for purification, 13 isolates were identified as bacteria belonging to the genus *Azospirillum* according to the following common cultural and cell-morphological characteristics in semisolid N-free malate medium

Recently, procedures used for isolation N2 fixing bacteria were not basically different from those proposed by Beijerinck (1901) and Winogradsk (1926). They were based on the enumeration and purification of bacteria growing in N-free or N-deficient media inoculated with dilutions or aggregates of soil.

Besides the cultural and morphological tests, some biochemical tests were proceeded. For example carbohydrate utilization by *Azospirillum* isolates was different markedly with respect to the species and to the carbon source.

1. Morphological description of the vegetative cells:

Azospirillum were 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.

2. Physiological and biochemical tests:

For species determination, utilization of different carbon-sources was performed in aerobic conditions. The organisms were grown in semisolid medium containing the carbohydrate together with a pH indicator (bromothymol blue) according to Hugh and Leifson, (1953).

The development of a yellow color during 96 hour incubation at 30oC indicates acidification.

3. Other differential biochemical tests were carried out:

Biotin requirement, nitrate reductase activity, catalase activity, growth in the presence of 3% NaCl, starch and gelatin hydrolysis (Table. 1) were also tested.

- Microcosm's experiments:

In order to select the more effective *Azospirillum* strain on plant growth, a microcosms experiment was conducted in laboratory conditions. Wheat (*T. aestivum* local cultivar) was grown in small pots (500 g capacity) filled with sterilized mixture of sand and clay soil in a ratio of (2:1, wt/wt) and inoculated with different bacterial strains (106 CFU/seed). Pots were irrigated with water near the field capacity as needed. Three replicates were conducted for each bacterial isolate, and a control uninoculated pots-group was conducted.

After 3 weeks from sowing, plants were harvested, and growth parameters (shoot and root length, fresh and dry weight) were evaluated.

- Salt tolerance of *Azospirillum* isolates:

For the determination of salt tolerant ability of *Azospirillum* strains, DN liquid medium was prepared with different concentration of NaCl (100mM, 200mM, 300mM, 700mM). Four selected strains (S1, S18, S24 andR1) were inoculated separately into 100 ml conical flask, which contained DN broth, and incubated at 30oC for 48 hours. The growth level was measured by spectrometrically at 620 nm.

Results and Discussion

Table (2) shows result of acidification of different carbon sources. According to the level of acidification (i.e. the strength of the pH indicator, bromothymol-blue, to colour the reaction, three distinct notations were adopted (- , ± and +) ranging from no oxidation to marked positive oxidation.

Generally, *A. brasilense* strains were more restricted in its use of carbon sources, whereas *A. lipoferum* isolates oxidized larger number of carbohydrates including glucose.

However, all the tested isolates effectively oxidized the organic acids (Succinate, Malate and Pyruvate) when used as a sole carbon source auxanotrophically.

The preference of the organic acids by different *Azospirillum* species was reported earlier by Reinhold *et al*. (1985). This can be explained on the basis that organic acids were the major source of nutrients for the microflora in the rhizosphere (Curl and Truelove, 1986).
Morphological and biochemical tests (Table 1) indicated that three *Azospirillum* isolates were belonging to *A. brasilense* (strains R1, S1 and S18), and two *A. lipoferum* isolates (strain S24).

- NaCl resistance of *Azospirillum* spp. :

The resistance of *Azospirillum* isolates were tested for growth under different (NaCl) concentrations, and the results are presented in Fig. (A1,A2,A3). Among the tested 4 isolates, *A. brasilense* (S1) strain was found to have high degree of salt resistance, while *Azospirillum* strains (S18, S24 and R1) recorded the least salt resistance.

- Screening of *Azospirillum* isolates in a microcosms experiment: Different isolates of *Azospirillum* were screened to select the most efficient isolates on growth of wheat *(T. aestivum* L.) in a microcosmos experiment. Data of Fig (B1 , B2) showed that wheat plants generally responded positively to inoculation with most of the studied *Azospirillum* strains. This is indicated by the significant increases in the growth parameters of inoculated plants compared to the control ones. *A. lipoferum* strain S24, and *A. brasilense* S1 recorded the highest effect on plant growth. Therefore they were selected for further investigations in the pot-experiments.

Inoculation of plants with *Azospirillum* can result in significant changes in various plant growth parameters, which affect crop yield.

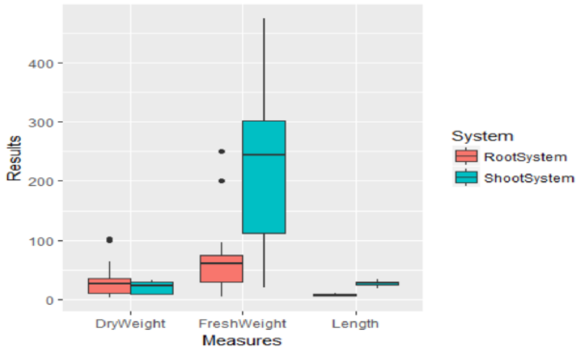

Fig (A1): Comparision of different plant system (Root system, Shoot system) using different measures (Dry weight, Fresh weight).

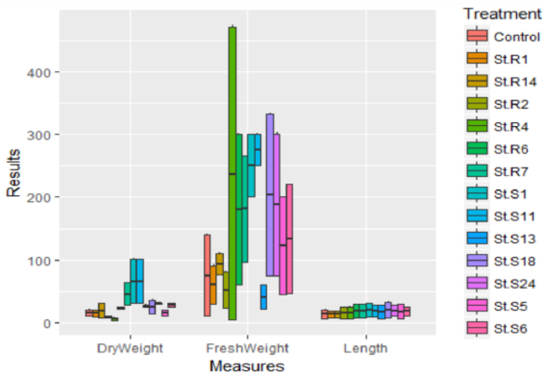

Fig (A2): Comparision of different isolayes using different measures (Length, Dry weight, Fresh weight).

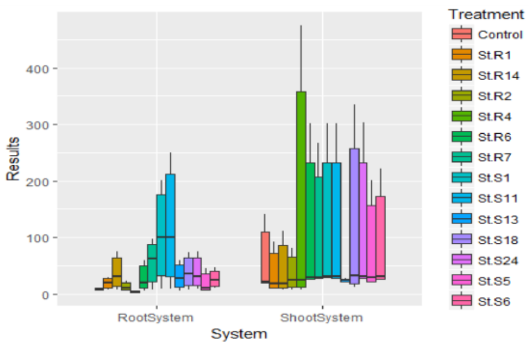

Fig (A3): Comparision of different plant system among treatment different isolates of Azospirillum

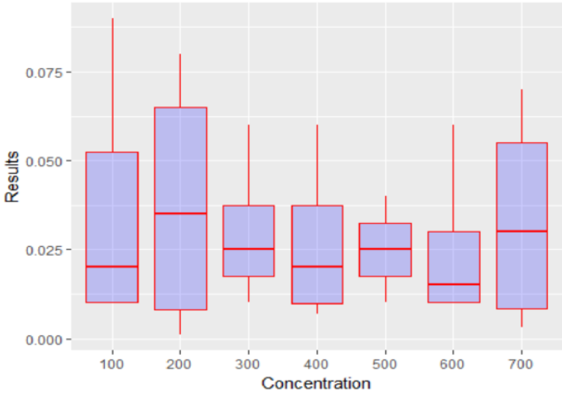

Fig (B1): Comparision of different concentration regardless of isolated

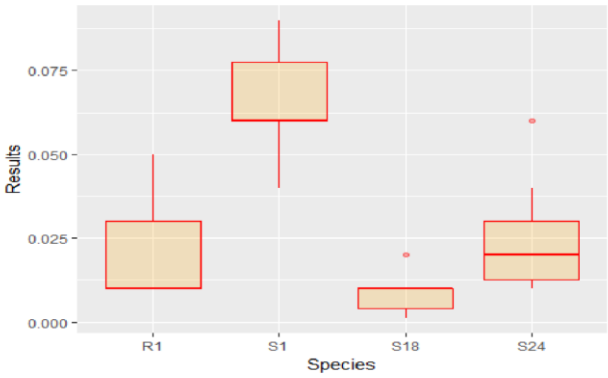

Fig (B2): Comparision of different isolated regardless of concentration

Table (1) Morphological and Biochemical activities of the isolated *Azospirillum*

Isolates / Test	*A.brasilense* (Sp7)	*A.lipoferum* (137)	R1	S1	S18	S24
Origin	-	-	*Euphorbia*	*Euphorbia*	*Retama*	*Silyene*
Cell – shape	Curved rod	Ovoid	Ovoid	Ovoid	Rod	Rod
Motility	+	+	+	+	+	+
Gram – stain	-	-	-	-	-	-
Biotin requirement	-	+	-	-	-	+
Growth in 3% NaCl	+	+	+	-	+	+
Catalase activity	+	+	+	+	+	+
Gelatin hydrolasis	-	-	-	-	-	-
Starch hydrolasis	-	-	-	-	-	-
Nitrate reductase	+	+	+	+	+	+
Growth on Succinate	+	+	+	+	+	+
Malate	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+

Table (2) Oxidation of different carbon compounds by the isolated *Azospirillum*

C-sourse / Isolate	Glucose	Arabinose	Manitol	Cellulose	Manose	Ribose	Lactose	Glycerol
A.brasilense (Sp7)	-	-	±	±	+	-	-	-
Alipoferum (137)	+	±	-	-	+	±	±	+
R1	-	+	+	±	±	+	+	+
S1	-	+	+	-	-	+	±	±
S18	-	+	-	-	+	+	±	+
S24	±	+	-	±	+	+	±	+

Conculusion:

Azospirillum isolates were screened for growth at different NaCl concentration in liquid DN medium , a.brasilense (S1) was abje to grow up to 700Mm (used as salt resistant strain)

Refrenes:

[1].Bashan, Y and Holguin, G (1997): Azospirillum plant relationships environmental and physiological advances (1990-1996); Can. J. Microbiol. 43: 103-121.

[2].Bashan, Y.; Holguin, G. and de- Bashan, L. (2004): Azospirillum- plant relationships, molecular, and environmental advances. Can. J. Microbiol. 50: 521-577.

[3].Bashan, Y.; Puente, M.E.; Rodriguez-Mendoza, M.N.; Toledo, G.; Holguin, G.; FerreraCerrato, R. and Pedrin, S. (1995): Survival of Azospirillum brasilense in the bulk soil and rhizosphere of 23 soil types. App.Environ. Microbiol. 61:1938- 1945.

[4].Beijerinck, M.W. (1901): Ueber ein Spirillum, Welches freien sticktoff binden kann Centralbl. Bakt. II Abt. 63 353-357.

[5].El-Komy, H. (2005): Coimmobilization of Azospirillum lipoferum and Bacillus megaterium for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol. Biotechnol. 43: 19-27.

[6].Elkomy, H. and Abdel. wahab, A. (1998): Effect of simultaneous inoculation of Azospirillum and Rhizobium spp. On growt and nitrogen fixation of two legumes using 15Ndilution technique and the difference method. Acta. Microbiol. Pol. 47: 283-296.

[7].Kabir, M.; Chotte, J.L.; Rahman, M.; Bally, R. and Jocteur Monrozier, L. (1994): Distribution of soil fractions and location of soil bacteria in a vertisol under cultivation and perennial grass. Plant Soil 163: 243-255.

[8].Mayak, S.; Tirosh,T. and Glick, B.R. (2004): Plant growth promoting bacteria confer resistance in tomato and pepper plants to salt stress. Plant Physiol. Biochem. 167:650-656.

[9].Tarrand, J.J.; Kreig, N.R.; Döbereiner, J. (1978): A taxonomic study of the Spirillum lipoferum group, with a descriptions of a new genus, Azospirillum gen. nov. And two species, Azospirillum lipoferum (Beijerink) comb. nov. And Azospirillum brasilense sp. nov. Can. J. Microbiol. 24:967-980.

[10]. Winogradsk, M. (1926): Sur le pourvoir fixature des terres. C. R. Hebd. Seances Acad. 907-910.