CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA ISOLATED FROM ROOT SYSTEM OF SUNFLOWER (*HELIANTHUS ANNUS* L) GROWN UNDER SALT AFFECTED AREA OF PAKISTAN

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ABSTRACT:- Plant growth promoting rhizobacteria (PGPR) directly promote plant growth by providing indole-3-acetic acid (IAA), solubilization of inorganic phosphates, nitrogen fixation and siderophores and other organic acid production, whereas indirectly support plant growth by suppressing plant pathogens. The objective of this study was isolation and characterization of bacterial strains from rhizosphere, endosphere and rhizoplane of sunflower. Thirty six bacterial strains were selected out of 44 from plant root samples along with rhizospheric soil, collected from different salt affected areas of Central Punjab (Pakistan). Selected bacterial strains were characterized morphologically as well as biochemically at National Agricultural Research Centre, Islamabad during 2011-13. It was observed that all isolates produced IAA, whereas14 strains were declared as phosphate solubilizing bacteria (PSB), eight isolates exhibited antifungal characteristics, 30 were nitrogen fixer and all of them were gram -ve. During biochemical characterization of bacterial isolates KS 15 and KS 8 produced the highest indole acetic acid whereas KS 15 and KS 17 indicated maximum phosphate solubilization (PS) among all isolated strains. The bacterial strains KS 10 and KS 44 showed maximum bio-control activity (fungal growth inhibition) than other isolated strains.

Key Words: Helianthus annus; Plant Growth Promoting Rhizobacteria; Characterization; Indole-3-acetic acid; Bio-control; Pakistan.

INTRODUCTION

Research of past 15 decades continuously demonstrated that among microbial population rhizobacteria and fungi have very close relationship with plant roots and also have ability to enhance plant growth as well as anti-pathogenic effect on soilborne diseases (Whipps, 2001). In the rhizospheric region of roots microbial population is large as compared to rhizoplane and endosphere. After isolation and characterization in vitro microbial testing shows that two third of the population exhibit plant growth promotional activities whereas 1% - 35% suppressed the growth of pathogens (Berg et al., 2006; Furnkranz and Muller, 2009).

Many of PGPR strains have been known to play an essential role in improving crop growth. Certain

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groups of PGPR strains belonging to Bacillus, Enterobacter, Burkholderia, Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Beijerinckia, Erwinia, Flavobacterium, Rhizobium and Serratia are being used worldwide to enhance the crop productivity (Burd et al., 2000 ; Nelson, 2004; Chaiharn et al., 2008; Yang et al., 2009; Bharti et al., 2013).

Environmentally safe and sustainable agriculture production is a major concerns of 21st century. An increased crop production is essential for feeding the escalating population of the world. Agriculture is a natural process and more related to natural activities. No doubt chemical fertilizers and pesticides are important components of green revolution but, these chemicals are posing serious threats to ecological system (soil, water and air) and more significantly human health. The increasing cost of agricultural inputs is also a financial issue of farmers. Biofertilizers contain specific strains of bacteria, fungi and algae that perform particular function like, nitrogen fixation, phosphorus solubilization, mineralization of organic material, production of phytohormones, chelation of iron (Fe) by siderophore production. These natural fertilizers are cost effective and environment friendly. Bacterial groups (rhizobium, azospirilum, Azotobacter and pseudomonas) and fungi Vascular-Arbuscular Mycorrihizae (VAM) are commonly used in the formulation of biofertilizers. Biofertilizers are innovative and renewable source of plant nutrients. This study was designed to isolate and characterize bacterial strains from soil samples collected from salt affected area of Central Punjab.

MATERIALS AND METHOD

Samples Collection

Five soil samples of sunflower plants along with rhizospheric soil were collected from moderate to high saline area (EC 5-9 dS m^{-1}) from different locations of Central Punjab-Pakistan (Soil salinity research area Pindi Bhattian, Hafizabad and Sheikhupura) and were stored in Soil Biology and Biochemistry Laboratory, Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC), Islamabad at 4^{0}C in 2011.

Isolation of Plant Growth Promoting Rhizobacteria (PGPR)

Isolation was made from rhizosphere, rhizoplane and endosphere of sunflower roots by using dilution plate technique (Brierly et al., 1928).

Purification of Rhizobacterial Strains

The colonies were singled out and purified by four ways streaking. Thirty six strains out of 44 were characterized on the basis of colony morphology (elevation, margin, color and opacity) and cell morphology under microscope.

Gram Staining

Vincent (1970) method was followed for gram staining of purified rhizobacterial isolates. Bacteria showing pink color were gram-ve whereas purple color isolates were gram+ve.

Biochemical Characterization of PGPR

Determination and Quantification of IAA

IAA production ability of isolated rhizobacterial strains were measured

by using spectrophotometer. All bacterial isolates were inoculated separately in 100 ml flasks containing Luria Bertani (LB) broth medium and 1 ml tryptophan. These bacterial cultures were incubated for one week on shaker at 28±2°C. After incubation, the cultures were centrifuged for 30 min at 3000 rpm. Supernatant (2 ml) was taken and 4 ml of Solawaski's reagent (1 ml 0.5% $FeCl_3$, 50 ml and 35% perchloric acid) was added to it, with two drops of orthophosphoric acid. The development of pink color indicated IAA production. At spectrophotometer 530 nm wave length was adjusted to read optical density (O.D). A standard graph (Ahmed et al., 2005) was used for quantification of IAA.

Phosphorus Solubilization and Quantification

Purified colonies of rhizobacterial strains were inoculated to Pikovskaya agar medium (Pikovskaya, 1948) plates having tri-calcium phosphate as insoluble source of phosphorus, under gnotobiotic condition and then incubated at 28±2°C for seven days. These plates were arranged in complete randomized design (CRD) with three replications. Data regarding colony diameter and halozone diameter were recorded up to seven days.

Solubilization Index (SI)

The index of phosphate solubilizing bacterial strains was determined by using formula of Edi-premono et al. (1996).

$$SI = \frac{\text{Halozone diameter + Colony diameter}}{\text{Colony diameter}}$$

Nitrogen Fixer PGPR

Petri plates having nitrogen free media (Okon et al., 1977) were inoculated with purified strains under aseptic condition. These plates were arranged as complete randomized design (CRD) and incubated at 28±2°C for 3-4 days. Those bacterial colony exhibited growth in nitrogen free media were declared as nitrogen fixer.

Dual Culture Assay

Antifungal characteristic were also studied of eight selected isolates (Table 3). Five (5) mm fungal mycelial disc was placed in the middle of the petri plate having potato dextrose agar (PDA) medium. Bacterial inoculum prepared in LB broth was streaked around the fungal disc on the same petri plate. These petri plates were incubated for 5 days at 28°C. Whereas petri plates inoculated with fungal disc only served as controls. Comparison was made between the mycelial growth of the pathogen and that of control. The experiment was repeated thrice for each treatment and CRD experimental design was used. The percentage growth inhibition (PGI) of the fungal pathogen was calculated by using formula (Sivan et al., 1987).

$$(PGI) = 1 - \frac{\text{Fungal growth}}{\text{control growth}} \times 100$$

RESULTS AND DISCUSSION

Thirty six rhizobacterial strains were selected out of 44 and were allotted code name ranging from KS 1 to KS 44. In first step, morphological and physical characterization was carried out (Table 1). In second step biochemical characterization of isolated strains were made for IAA, PSB, qualitative nitrogen fixation and

antifungal activity. All of 36 isolate demonstrated IAA production, 14 were declared as PSB and 30 were found as nitrogen fixer, whereas all of them were gram-ve. Maximum IAA was produced by KS 8 (36.52 mg l^{-1}) while minimum was found in KS 6 (5.63 mg l^{-1}). Similarly the highest phosphate solubilization index (SI) was exhibited by strains KS 15 (4.13) whereas minimum was observed in KS 42 (2.20) (Table 2). The bacterial isolates KS 10 and KS 44 exhibited the highest antifungal activity (Table 3).

For effective PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce the beneficial effects. The exact mechanism by

Table 1. Morphological and physiological characterization of plant growth promoting bacteria isolated from sunflower

Isolate	Isolation source	Gram staining	Bacteria shape	Colony shape	Elevation	Margin	Opacity
KS 1	Rhizosphere	-ve	Rod	Circular	Flat	Entire	Transparent
KS 2	Rhizosphere	-ve	Rod	Circular	Flat	Entire	Transparent
KS 3	Rhizosphere	-ve	Rod	Circular	Flat	Entire	Translucent
KS 4	Rhizosphere	-ve	Rod	Filamentous	Raised	Undulate	Translucent
KS 6	Endosphere	-ve	Rod	Irregular	Flat	Curled	Transparent
KS 7	Endosphere	-ve	Spiral	Circular	Raised	Entire	Opaque
KS 8	Rhizosphere	-ve	Round	Circular	Raised	Entire	Translucent
KS 9	Rhizosphere	-ve	Round	Circular	Pulvinate	Entire	Transparent
KS 14	Rhizoplane	-ve	Round	Regular	Raised	Entire	Translucent
KS 15	Endosphere	-ve	Rod	Regular	Pulvinate	Entire	Translucent
KS 16	Rhizoplane	-ve	Round	Irregular	Flat	Undulate	Transparent
KS 17	Endosphere	-ve	Round	Regular	Raised	Entire	Transparent
KS 18	Endosphere	-ve	Rod	Irregular	Raised	Undulate	Translucent
KS 23	Rhizoplane	-ve	Round	Regular	Pulvinate	Entire	Translucent
KS 24	Rhizoplane	-ve	Rod	Irregular	Raised	Undulate	Translucent
KS 25	Rhizosphere	-ve	Rod	Irregular	Flat	Entire	Transparent
KS 26	Endosphere	-ve	Rod	Regular	Flat	Entire	Transparent
KS 27	Rhizoplane	-ve	Round	Regular	Flat	Entire	Transparent
KS 28	Rhizoplane	-ve	Round	Irregular	Flat	Undulate	Opaque
KS 29	Rhizoplane	-ve	Rod	Filamentous	Flat	Lobate	Translucent
KS 30	Rhizoplane	-ve	Rod	Filamentous	Raised	Lobate	Translucent
KS 31	Rhizoplane	-ve	Round	Irregular	Flat	Entire	Translucent
KS 32	Rhizosphere	-ve	Rod	Regular	Flat	Entire	Transparent
KS 33	Rhizosphere	-ve	Rod	Filamentous	Flat	Lobate	Translucent
KS 34	Rhizosphere	-ve	Round	Regular	Flat	Entire	Transparent
KS 35	Endosphere	-ve	Spiral	Regular	Flat	Entire	Opaque
KS 36	Rhizosphere	-ve	Round	Regular	Raised	Entire	Translucent
KS 37	Rhizosphere	-ve	Rod	Regular	Raised	Entire	Transparent
KS 38	Rhizosphere	-ve	Round	Regular	Flat	Entire	Transparent
KS 39	Rhizosphere	-ve	Rod	Irregular	Flat	Undulate	Translucent
KS 40	Rhizosphere	-ve	Rod	Regular	Flat	Entire	Transparent
KS 41	Rhizosphere	-ve	Rod	Irregular	Raised	Undulate	Opaque
KS 42	Endosphere	-ve	Rod	Irregular	Flat	Undulate	Transparent
KS 43	Endosphere	-ve	Rod	Circular	Pulvinate	Entire	Opaque
KS 44	Endosphere	-ve	Rod	Circular	Raised	Entire	Transparent

Table 2. Biochemical characterization of isolated PGPR strains from sunflower

Sr. No.	Isolate	IAA (mg l^{-1})	PSB (SI)	Nitrogen fixer
1	KS 43	14.38	-	+
2	KS 6	5.63	-	+
3	KS 9	15.31	-	+
4	KS 32	15.82	-	-
5	KS 33	16.31	-	+
6	KS 34	14.58	-	+
7	KS14	8.01	-	+
8	KS 16	10.42	-	+
9	KS 17	16.84	3.74	-
10	KS 23	13.98	3.59	+
11	KS 24	15.54	3.60	+
12	KS 25	14.45	3.36	+
13	KS 26	7.23	-	+
14	KS 29	13.69	-	-
15	KS 30	12.64	2.3	+
16	KS 35	15.26	-	+
17	KS 36	7.56	-	+
18	KS 37	17.09	-	+
19	KS 38	11.31	-	+
20	KS 39	6.48	-	+
21	KS 40	16.74	-	+
22	KS 1	6.80	-	+
23	KS 2	8.22	-	-
24	KS 3	15.56	-	+
25	KS 4	15.66	2.27	+
26	KS 5	9.85	-	-
27	KS 11	7.65	-	+
28	KS 12	16.61	-	-
29	KS 44	25.54	2.28	+
30	KS 7	23.44	2.70	+
31	KS 41	17.04	3.35	+
32	KS 42	19.20	2.20	+
33	KS 28	32.35	3.48	+
34	KS 8	36.52	3.12	+
35	KS 10	17.92	3.00	+
36	KS 15	34.89	4.13	+

Each entries in table has three replicates

which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved (Herman et al., 2008).

Many researchers have reported the phytohormones production from PGPR, isolated from rhizosphere, rhizoplane and endosphere (Khan and Zaidi, 2002; Khalid et al., 2004; Su et al., 2005; Nadeem et al., 2010; Saleemi, 2011. Similar results were reported by Fenice et al. (2000); Aslam et al. (2002); Maliha et al. (2004); Gull et al. (2004). Saleemi (2011) also reported similar finding from nine isolated strains that solubilize inorganic P within a range of 37-130 mg l^{-1}, whereas three isolates (WPR- 42, WPR-51 and WM-3) also showed antifungal activity. Whereas, eight isolates showed antifungal characteristic (Table 3), Maximum selected strains exhibited nitrogen fixation ability by growing on nitrogen free media.

Table 3. Antifungal Activity of PGPR isolated from sunflower

Isolate	Mean	PGI	Isolate	Mean	PGI
KS 15	3.06	43.34	KS 10	1.83	66.10
KS 08	3.96	26.67	KS 44	3.06	43.34
KS 28	3.20	40.75	KS 42	2.10	61.12
KS 07	4.10	24.08	KS 41	4.46	17.30

PGI= Percentage Growth Inhibition

It was obvious from above results that all rhizospheric strains produced IAA but in varying amount. However 14 isolates solubilize inorganic tri-calcium phosphate and only eight showed antifungal activity. The varying performance of isolates during characterization is due to these strains collected from diverse places having different micro-environment. The promising PGPR strains (KS 10, KS 15, KS 44 and KS 17) could be recommended to use as inoculants for preparation of biofertilizer to enhance the sunflower growth.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Mr. Muhammad Zahid Kiani	Conceived the idea, Overall management of the article.
2.	Dr. Arshad Ali	Technical input at every step, Write up
3.	Dr. Tariq Sultan	Technical input at every step
4.	Dr. Muhammad Munir Ahmad	Conceived the idea, Conducted research and write up

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