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Actinobacteria : A Biological Tool for Maize Crop Improvement, Nutrient Acquisition and Soil Health

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Abstract

Actinobacteria are most important group of soil microorganisms which performs multifarious functions in soil like decomposition of organic materials, production of phytoharmones and suppression of soil borne pathogens. In our study the positive influence of Actinobacteria on soil fertility and crop improvement has been tested using maize as a test crop. All the Actinobacterialisolates proved better in influencing germination percentage, plant growth and yield parameters, chemical and biological properties. Among the different isolates, Actinobacterial isolate A6 (*Streptomyces*) showed higher germination percentage (94%), plant height (197.83 cm) at 120 DAS,less days taken to 50% tasselling (40.65 days), plant dry weight (340.9gm/plant), kernel yield (42.34 q ha^{-1}), kernel test weight **(**35.9 g 100 kernels^{-1}) stover yield (36.65 q ha^{-1}), NPK uptake (79 kg N, 23 kg P, 36 kg K ha^{-1}) available NPK (80, 26, 40 kg ha^{-1}) enzyme activities (17.9µg TPF/gm/day, 94.4µg p-nitrophenol/g of soil/hour, 164.3µg p-nitrophenol/g of soil/hour, 5.7µg fluorescein/g of soil/hr of dehydrogenase, Acid phosphatase, Alkaline phosphatase and FDA respectively). Similarly the Actinobacterial isolates also increased the population of general (Bacteria, fungi, Actinobacteria) and beneficial microbial population and beneficial microflora*viz.,Azotobacter*compared to control treatments.

Key words : *Actinobacteria, biological tool, maize, nutrient acquisition, soil health.*

Introduction

The microbial activity in crop rhizosphere is greatly influenced by root activities such as exudation of organic substrates like amino acids, sugars/carbohydrates, enzymes and vitamins. These substances as well as microbial interactions help in releasing crop nutrients and make it available to plant growth (1). Thus the Crop productivity can be improved by manipulating the beneficial rhizosphere microorganisms through addition of appropriate soil amendments and by identifying the effective strains of beneficial microorganisms suitable to different agro ecological conditions. Actinobactreria are one such beneficial microorganisms involved in the decomposition of resistant components of plant and animal wastes, and perform their task even at high temperatures. It exhibit various properties that are useful for promotion of plant growth and in improving soil health (2,,3). It also act as excellent biocontrol agent in combating diseases through liberation of antibiotics (4) production of siderophores, ammonia, hydrogen cyanide and chitinase and are important features of Actinobacteria useful for combating biotic and abiotic stresses in plants. These organisms can be exploited as potential tools for agriculture and as beneficial inoculants for the future agriculture (5). Plant growth promotion potential of *Streptomyces* was reported in Bean (6), Pea (7), Wheat (8) and in Rice (9). They also promote mycorrhizal colonization rate including spore germination (10).

In the present Research work, Actinobacteria were isolated from rhizosphere of different crops like Sorghum, Pearl millet, Pigeon pea, Finger millet and Groundnut grown in arid and semi arid regions of Karnataka, Andhra Pradesh and Rajasthan. The potentiality of Actinobacterial isolateson growth and yield of Maize and their influence on soil chemical and biological properties was evaluated.

Materials and Methods

Location : The field studies were conducted at the experimental site of All India Network project on Soil Biodiversity and Biofertilizer, Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur (M.P).

Climate : Jabalpur is in semiarid zone having subtropical climate with a characteristic feature of dry summer and cold winter. Jabalpur is situated between 22^{o} 21'and 24^{o} 8' North latitude, 78^{o} 21'and 80^{o} 58' east longitude and at an altitude of about 411.8 meters above the mean sea level.

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In winter season *i.e.,* from November to February the temperature ranges from 4 to 33^{0}C and the relative humidity varies from 70 to 90%. Dry and warm weather usually persists during the month of March to June. Monsoon season extends from mid-June to mid-September. The temperature during this period varies between 25^{0}C to 35^{0}C and the relative humidity ranges from 70 to 80%. The total annual rainfall varies from 1200 to 1500 mm.

Soil characteristics : The soil of the experimental site was Vertisol belonging to montmorillonite, hypothermic family of *Typichaplusterts* popularly known as "Black cotton soil". Before starting the field experiment soil samples upto a depth of 15 cm was collected from the experimental site and a composite sample was prepared and analyzed for important physical and chemical properties as well as enzyme and microbial analysis was done by employing standard methods.

Isolates used : Seventeen isolates of Actinobacteria which proved to be highly efficient in initial screenings under glass house conditions on maize and chickpea were used for field studies. These isolates were A1, A5, A6, A7, A15, A16, A25, A30 which were highly efficient, that produced an increase in dry matter of >35% in maize; and isolates A2, A10, A11, A17, A27, A28, A35, A36 and A40 that produced an increase in dry matter of >30% in chickpea. All these 17 isolates were selected for evaluation on growth and yield of Maize in the field.

Preparation of the inoculants : Actinobacterial isolates were grown separately in 100 ml starch casein broth. The flasks were incubated at 28±2°C on a reciprocatory-shaker and shaken intermittently for 7 days at 125 rpm.

Land preparation and layout : The experimental site was brought to a fine tilth by ploughing once with tractor using iron plough followed by two harrowings. There were 19 treatments (including two controls-Fertilized Uninoculated (FUI) and another Unfertilized Uninoculated (UFUI) with three replications and the experimental design followed was RCBD.

Fertilizer application : The recommended dose of 120:60:40 kg NPK per ha for maize crop was applied in the form of urea, single super phosphate (SSP) and murate of potash (MoP). SSP and MoP were applied as basal dose to each plot, while urea was applied in three equal splits *i.e.,* at the time of sowing, 21 days and 42 days after sowing.

Seeds and sowing : The maize variety JM-216 @ 20 kg seed ha^{-1} wascalculated to net plot area of the experimental plot (5 2 m^2) which worked out to approximately 40 g/plot. The seeds were obtained from farm section of JNKVV, Jabalpur. The seeds were treated separately with different Actinobacterial isolates using grown broth added with 1% CMC. The inoculated seeds were air dried and sown immediately. After germination, thinning was done to maintain the required plant population. Necessary plant protection measures were taken as per recommended package of practices.

Estimation of soil physical, chemical and biological properties of soil : Observations on germination (%) was recorded on 6th day after sowing (DAS) by counting the germinated seeds in each plot and expressed as per cent germination.The growth parameters like plant height (cm), number of leaves, days taken to 50% tasseling, were observed in five tagged plants in each net plot which were selected randomly leaving the border row for taking periodical observations. Cobs from the five randomly selected plants at the time of harvest were used for recording observations on yield components like length of cob, girth of cob, kernel lines per cob, number of kernels per line, number of kernels per cob, number of cobs per plant, test weight, plant dry weight, kernel and stover yield. Plant nutrient uptake (NPK) studies, soil pH, EC and soil organic carbon were calculated by following the procedures given by (11). Soil available N (12); available P (11); available K by (13). Soil enzyme activities., *viz.,* Dehydrogense activity of the soil samples was determined by following the triphenyltetrazolium reduction test (14). Phosphatase activity of soil samples was determined by following the procedure of (15). Fluorescein diacetate (FDA) hydrolysis of soil samples was determined by following the procedure of (16). The rhizosphere soil samples collected were analyzed for microbial populations by using standard serial dilution plate count method (17) using Nutrient agar for bacteria, Martin's Rose Bengal agar for fungi, Kuster's agar (18) for Actinobacteria, Pikovskaya's medium for PO_4 solubilizers and Jensen's medium for *Azotobacter.* The population count was expressed as number of colony forming units per gram of soil.

Results and Discussion

Inoculation of Actinobacterial isolates showed a positive influence on the germination of maize.The higher germination percentage was recorded in soil inoculated with isolate A6 (*Streptomyces* isolated from arid region) (95 %) followed by A1 (*Streptomyces* isolated from soils of humid region) (94%) at 6 DAS (days after sowing) and the data is presented in Table-1. The differences in germination percentage can be attributed to the differences in the production of growth promoting hormones like IAA and GA by Actinobacterialisolates (19). Such difference in production of plant growth promoting hormones by Actinobacterial isolates has also been earlier reported by (20).

Table-1: Performance of Actinobacterial isolates on growth parameters of Maize under field conditions (Semi arid).

Treatments	Germination (%) after 6 days	Plant height (cm) at harvest (120 days)	No. of leaves at 90 DAS	Days taken to 50% tasseling
T1: A1-*Streptomyces*	94.0	190.73	13.33	41.19
T2: A2-*Streptomyces*	93.3	187.73	13.00	42.73
T3: A5-*Streptomyces*	86.0	180.80	12.67	46.12
T4: A6-*Streptomyces*	95.0	197.83	14.00	40.65
T5: A7-*Streptomyces*	90.0	184.63	12.33	44.10
T6: A10-*Streptomyces*	93.0	187.50	12.33	45.32
T7: A11-*Streptomyces*	89.0	181.83	12.00	48.12
T8: A15-*Streptomyces*	84.7	179.80	12.00	46.14
T9: A16-*Streptomyces*	90.7	186.60	11.67	48.13
T10: A17-*Streptomyces*	88.3	180.87	12.00	44.10
T11: A25-*Nocardia*	89.3	184.63	12.00	44.12
T12: A27-*Streptomyces*	90.7	184.90	11.00	46.15
T13: A28-*Nocardia*	87.3	180.80	12.00	48.10
T14: *A30-Streptomyces*	92.3	186.70	11.67	49.73
T15: A35-*Nocardia*	84.0	179.13	12.67	44.24
T16: A36-*Streptomyces*	89.3	184.10	11.00	46.13
T17: A40-*Streptomyces*	83.3	177.83	11.67	43.12
T18: FUI	82.3	153.33	10.33	50.44
T19: UFUI	78.3	135.07	7.00	54.00
S.Em ±	4.0	1.42	0.63	2.5
CD (5%)	11.8	4.10	1.82	7.8

Table-2 : Performance of Actinobcaterial isolates on yield parameters of Maize under field conditions (Semi arid).

Treatments	Length of cob (cm/cob)	Cob girth (cm/cob)	Kernel line of cob	Number of kernels per line	No. of kernels/cob	No. of cobs/plant
T1: A1-*Streptomyces*	15.0	13.8	14.0	28.3	370	1.02
T2: A2-*Streptomyces*	15.0	13.8	14.0	28.0	355	0.98
T3: A5-*Streptomyces*	13.7	13.3	12.3	24.3	295	0.90
T4: A6-*Streptomyces*	15.7	14.3	15.0	30.0	397	1.10
T5: A7-*Streptomyces*	14.2	13.5	13.3	26.3	328	0.93
T6: A10-*Streptomyces*	14.8	13.7	13.7	27.3	345	0.98
T7: A11-*Streptomyces*	13.9	13.5	12.5	25.3	314	0.92
T8: A15-*Streptomyces*	13.4	13.3	12.0	24.0	292	0.90
T9: A16-*Streptomyces*	14.5	13.6	13.3	26.7	336	0.96
T10: A17-*Streptomyces*	13.7	13.5	12.5	25.0	304	0.91
T11: A25-*Nocardia*	13.9	13.5	13.0	26.0	325	0.92
T12: A27-*Streptomyces*	14.3	13.6	13.3	26.3	329	0.94
T13: A28-*Nocardia*	13.7	13.4	12.3	24.4	302	0.91
T14: A30-*Streptomyces*	14.6	13.6	13.5	26.7	337	0.97
T15: A35-*Nocardia*	13.3	13.2	12.0	24.0	292	0.88
T16: A36-*Streptomyces*	13.9	13.5	12.7	26.0	317	0.92
T17: A40-*Streptomyces*	13.0	13.1	11.3	23.6	269	0.79
T18: FUI	12.9	13.0	11.3	20.3	257	0.73
T19: UFUI	7.90	10.6	7.30	7.0	51	0.60
S.Em ±	1.25	0.6	0.88	2.0	28.4	0.09
CD (5 %)	3.74	1.7	2.60	5.9	85.0	0.28

A6 inoculated plants also recoded highest plant height (197.83 cm) at 120 DAS and number of leaves (14.00) at 90 DAS. These results are in accordance with the findings of (21) who reported such enhancement of plant growth in wheat due to *Streptomyces* inoculation. Inoculation of Actinobacterial isolates also influenced significantly on days taken to 50% tasseling in maize. The lower number of days taken for 50% tasselling was recorded in plants inoculatedwith isolate A6 (40.65 days). Among the Actinobacterial isolate A30 (*Streptomyces*

Table-3 : Effect of Actinobacterial isolates on Kernel and Stover yield of Maize under field conditions.

Treatments	Kernel yield (kg/plot)	Stover yield (kg/plot)	Kernel yield (q/ha)	Stover yield (q/ha)	Kernel test wt. (g/100 kernels)
T1: A1-*Streptomyces*	3.89	3.43	38.85	34.30	32.9
T2: A2-*Streptomyces*	3.81	3.28	38.09	32.75	32.7
T3: A5-*Streptomyces*	3.04	2.88	30.39	28.79	26.5
T4: A6-*Streptomyces*	4.23	3.67	42.34	36.65	35.9
T5: A7-*Streptomyces*	3.53	3.10	35.31	30.95	28.2
T6: A10-*Streptomyces*	3.80	3.26	37.99	32.6	32.7
T7: A11-*Streptomyces*	3.27	3.01	32.67	30.08	28.1
T8: A15-*Streptomyces*	2.93	2.78	29.32	27.83	25.7
T9: A16-*Streptomyces*	3.66	3.19	36.60	31.90	30.0
T10: A17-*Streptomyces*	3.33	3.00	33.27	30.01	27.6
T11: A25-*Nocardia*	3.45	3.09	34.51	30.90	28.2
T12: A27-*Streptomyces*	3.62	3.10	36.18	30.99	28.4
T13: A28-*Nocardia*	3.15	2.94	31.46	29.43	27.6
T14: A30-*Streptomyces*	3.67	3.22	36.71	32.23	31.2
T15: A35-*Nocardia*	2.91	2.76	29.11	27.56	25.3
T16: A36-*Streptomyces*	3.36	3.04	33.64	30.44	28.1
T17: A40-*Streptomyces*	2.90	2.74	29.03	27.40	23.5
T18: FUI	2.82	2.68	28.16	26.82	23.2
T19: UFUI	1.94	1.87	19.41	18.73	15.8
S.Em ±	0.45	0.18	5.83	2.20	2.3
CD (5 %)	1.20	0.60	17.00	6.50	7.0

Table-4 : Effect of Actinobcaterial isolates on physico-chemicalpropertiesin post experimental soils of Maize.

Treatments	N uptake (kg/ha)	P uptake (kg/ha)	K uptake (kg/ha)	pH	EC (dS/m)	Organic carbon (g/kg)	Available N (kg/ha)	Available P_2O_5 (kg/ha)	Available K_2O (kg/ha)
T1: A1-*Streptomyces*	79	23	36	6.97	0.15	4.0	305	22.4	332
T2: A2-*Streptomyces*	79	22	34	6.99	0.16	3.9	303	22.2	332
T3: A5-*Streptomyces*	62	19	28	7.08	0.18	3.4	290	17.6	306
T4: A6-*Streptomyces*	80	26	40	6.95	0.14	4.1	308	22.8	335
T5: A7-*Streptomyces*	70	20	27	7.01	0.14	3.6	297	20.0	317
T6: A10-*Streptomyces*	75	23	31	7.09	0.16	3.7	302	21.9	330
T7: A11-*Streptomyces*	74	19	30	7.07	0.19	3.4	294	19.4	314
T8: A15-*Streptomyces*	66	17	26	7.04	0.18	3.8	280	17.4	303
T9: A16-*Streptomyces*	79	20	33	7.01	0.17	3.9	298	21.0	317
T10: A17-*Streptomyces*	72	20	31	7.09	0.17	3.5	292	18.9	312
T11: A25-*Nocardia*	75	20	28	7.10	0.16	3.3	296	20.0	316
T12: A27-*Streptomyces*	77	22	34	7.00	0.17	3.8	297	20.0	317
T13: A28-*Nocardia*	72	20	30	7.12	0.16	3.7	290	18.4	310
T14: A30-*Streptomyces*	77	21	33	7.04	0.17	3.4	301	21.2	318
T15: A35-*Nocardia*	57	17	25	7.03	0.16	3.9	279	17.4	296
T16: A36-*Streptomyces*	63	20	28	7.07	0.17	3.7	295	19.8	315
T17: A40-*Streptomyces*	62	19	25	7.03	0.16	3.4	272	17.4	290
T18: FUI	37	13	19	7.07	0.18	3.4	270	16.9	290
T19: UFUI	34	10	15	7.04	0.17	3.1	266	15.8	288
S.Em ±	8.48	3.93	5.82	NS	NS	NS	19.1	2.3	22.5
CD (5 %)	25.41	11.59	17.50	6.97	0.15	4.0	57.0	7.1	67.0

isolated form soils of semi arid regions) took more number of days to 50% tasselling (49.73 days). Data pertaining to length of cob, girth of cob, kernel lines of cob, number of kernels per line, number of kernels per cob, number of cobs per plant varied significantly with the inoculation of Actinobacteria and the data is presented in Table-2. Plants inoculated with Actinobacterialisolate A6 (*Streptomyces*) resultedin theproduction of maximum

Table-5 : Effect of Actinobcaterial isolates on enzyme activities and microbial populations in post experimental soils of Maize.

Treatments	Dehydrogenase (µg TPF/ gm/day)	Acid Phosphatase (µg p nitrophenol /g of soil/hour)	Alkaline phosphatase (µg p-nitrophenol /g of soil/hour)	Fluorescein diacetate hydrolysis (µg fluorescein/g of soil/hr)	Bacteria (10^5 cfu/g soil)	Fungi (10^3 cfu/g soil)	Actino-bacteria (10^4 cfu/g soil)	Azoto-bacter (10^5 cfu/gsoil)	PSB (10^5 cfu/gsoil)
T1: A1-*Streptomyces*	17.9	94.4	164.3	5.7	46.7	26.2	23.5	20.4	21.2
T2: A2-*Streptomyces*	16.6	93.2	164.0	5.5	45.1	25.8	22.1	19.4	20.7
T3: A5-*Streptomyces*	11.5	80.7	159.5	3.6	42.0	21.5	19.5	17.8	9.5
T4: A6-*Streptomyces*	22.3	103.9	177.0	6.7	48.2	27.5	25.1	20.8	21.5
T5: A7-*Streptomyces*	13.8	90.1	162.4	4.8	40.2	20.4	19.7	13.9	14.1
T6: A10-*Streptomyces*	16.6	91.3	163.7	5.4	42.7	23.1	21.7	18.1	17.4
T7: A11-*Streptomyces*	12.8	84.8	161.4	4.6	45.1	25.8	15.4	19.4	9.7
T8: A15-*Streptomyces*	10.7	80.1	159.2	3.5	36.7	22.1	14.2	20.4	7.9
T9: A16-*Streptomyces*	14.7	90.4	163.0	5.1	41.4	22.1	20.8	15.5	16.6
T10: A17-*Streptomyces*	12.4	82.5	160.9	4.4	38.0	26.2	20.8	15.5	12.4
T11: A25-*Nocardia*	13.8	86.6	161.8	4.8	40.1	18.9	21.7	18.1	8.1
T12: A27-*Streptomyces*	14.7	90.2	162.9	4.9	40.5	21.5	19.8	15.1	16.5
T13: A28-*Nocardia*	11.8	81.5	160.4	3.6	34.9	16.7	17.9	7.8	16.6
T14: A30-*Streptomyces*	14.8	91.3	163.1	5.1	42.0	22.4	21.7	17.8	17.0
T15: A35-*Nocardia*	10.4	79.9	159.0	3.2	31.9	19.6	15.3	6.8	12.8
T16: A36-*Streptomyces*	13.6	85.8	161.6	4.6	40.5	20.1	19.4	11.5	17.4
T17: A40-*Streptomyces*	9.8	78.5	158.5	2.9	30.4	15.1	14.2	6.6	7.9
T18: FUI	8.1	76.7	158.0	2.6	28.9	14.6	11.3	5.3	7.5
T19: UFUI	6.6	70.4	139.8	1.0	25.5	13.0	6.1	5.1	7.0
S.Em ±	1.8	1.2	2.7	0.8	0.02	0.04	0.03	0.20	0.07
CD (5%)	5.5	3.6	8.4	2.6	0.07	0.14	0.10	0.61	0.23

length of the cob (15.7cm) cob girth (14.3 cm), kernel lines in cob (15.0 kernels line^{-1}), number of kernels per line (30.0 kernels line^{-1}), number of kernels per cob (397kernels cob^{-1}), number of cobs per plant (1.10), kernel yield (42.34 qha^{-1}), Stover yield (36.65 qha^{-1}), kernel weight (35.9g/100 kernels) followed byA1. The dry weight of maize plants also differed significantly between the treatments of Actinobacterial isolates. The highest dry weight (340.9 g plant^{-1}) of maize was recorded in plants treated with A6 (*Streptomyces*) isolate. This enhanced plant growth and yield parameters can be attributed to increased production of growth promoting substances like IAA and GA, efficient P and K solubilizationby introduced Actinobacterial isolates which also enhanced enzymatic activity in soil. Such increased plant growth and yield attributes due to inoculation of efficient Actinobacterial isolates was also earlier reported by (22) in Rice and (23) in Maize.

All the isolates proved better in influencing NPK uptake, among the different isolates, Actinobacterial isolate A6 (80 kg N, 26 kg P, 40 kg K ha^{-1}) recorded highest NPK uptake in plants. The soil after harvest of crop showed no significant differences in pH, EC and organic carbon. The highest available NPK in soil (Table-4) was recorded in soils treated with isolate A6 (308 N, 22.8 P_2O_5, 335 K_2O kg Ha^{-1}). The dehydrogenase activity, acid and alkaline phosphatase, Fluorescein diacetate hydrolysis (FDA) in soil after crop harvest also followed the same trend with the highest activity in soils treated with Actinobacterial isolate A6 (*Streptomyces*) (22.3µg TPF/gm/day, 103.9µg p-nitrophenol/g of soil/hour, 177.0µg p-nitrophenol/g of soil/hour, 6.7µg fluorescein/g of soil/hr of dehydrogenase, Acid phosphatase, Alkaline phosphatase and FDA respectively).

Increased bacteria, Fungi, Actinobacterial and beneficial microflora like *Azotobacter* and PSB were recorded in soils treated with different Actinobacterial isolates (Table-5). Among the treatments not much differences were observedwith respect to general microbial populationswhereasbeneficial microflora *viz., Azotobacter* (20.8X 10^5 cfu g^{-1} soil) and PSB (*Bacillus sp.,*) (21.5 X 10^5 cfu g^{-1} soil) were found highest in the soils treated with isolate A6 (*Streptomyces*). (8) reported that application of *Streptomyces* in conjunction with beneficial microflora like *Azotobacter* has enhanced the plant nutrient uptake, increased soil NPK content and promoted enzyme activity in soil. The results of this study are in agreement with the findings of above research workers.

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