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SCREENING OF PHOSPHATE SOLUBILISERS FROM SOME SELECTED WETLANDS OF NORTH BIHAR AND THEIR ASSESSMENT AS POTENTIAL BIOFERTILISERS

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ABSTRACT
Phosphorous is an essential element for plant development and growth making about 0.2% of dry weight.95-99% of phosphorous present in soil is insoluble and cannot be utilized by plants. Plants acquire phosphorous from soil solution as phosphate anions. However phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{++}, Mg^{++}, Fe^{+++} and Al^{+++} depending on the particular properties of a soil. Several scientists have reported the ability of different bacterial species to solubilise insoluble inorganic phosphate compounds such as tri-calcium phosphate, di-calcium phosphate, hydroxyapetite and rock phosphate. Phosphate solubilisers must contain phosphate solubilising bacteria or fungi. Detection and estimation of the phosphate solubilisation ability of microorganisms have been possible using plate screening methods. Phosphate solubilisers produce *"clearing zones"* around the microbial colonies in the media. The principle mechanism for mineral phosphate solubilisation is the production of organic acids, and *"acid phophatases*" play a major role in mineralization of organic phosphorous in soil. It is generally accepted that the major mechanism of mineral phosphate solubilisation is the action of organic acids synthesized by soil microorganisms. Production of organic acids results in *"acidification"* of microbial cells and its surroundings. In the present paper experimental investigations were carried out to isolate & screen the phosphate solubilisers from some selected wetland sites of North Bihar and also to assess their potential as biofertilisers on some selected plants in various consortia's.

KEYWORDS: *phosphate anions, hydroxyapetite, clearing zones, acid phosphatases, acidification, phosphate solubilisers.*

INTRODUCTION

Plant growth promoting bacteria i.e. Phosphate Solubilising Microorganisms (PSM) is commonly used as inoculants for improving growth and yield of agricultural crops. Screening for selection of effective PSM strains is very important. The most effective phosphate solubilising microorganisms are from the genera *Bacillus*, *Pseudomonas* of bacteria and *Aspergillus* and *Penicillium* from fungi .Also strains from the genera Enterobacter and Rhizobium are among the most powerful phosphate solubilisers. Examples of these genus includes such as *Bacillus polymyxa, Aspergillus awamori, Penicillium digitatum, Pseudomonas striata.*Chelating substances and inorganic acids such as sulphuric acid, nitric acid and carbonic acid are considered as other mechanisms for phosphate solubilisation.The present investigation emphasizes on screening of effective Phosphate Solubilising Microorganisms from some selected wetland sites of North Bihar.

MATERIALS AND METHODS

Study area

In the present investigation, isolates of bacteria including a few actinomycetes, azotobacters and also some fungi were isolated and characterized morphologically, physico-chemically & biochemically from five selected wetland sites of North Bihar mainly. These were

(1) Sikandarpur Maun (Wetland), Dist-Muzaffarpur.
(2) Jubba Sahni Bird Sanctuary (Barela Chaur), Dist-Vaishali.
(3) Motijheel, Motihari, Dist-East Champaran.
(4) Devkhal, Samastipur
(5) Sareya Maun, West Champaran, Bettiah.

Method of sample collection

Soil and water samples were collected in sterile polythene bags and dropping bottles during pre monsoon and post monsoon from the three sampling sites. These were quickly brought to laboratory and kept in a refrigerator.

Isolation of microorganisms

Nutrient Agar (NA) media was used for isolation of microorganisms (bacteria and actinomycetes) as it is the most commonly used media for routine cultivation of bacteria and actinomycetes.Also Ashby's (azotobacter) media and Starch Casein Agar media was used. Method used for isolation and enumeration of microorganisms (bacteria and actinomycetes) on these selective as well as non selective medias was serial dilution method. Plates were incubated at 37º±2ºC.

COMPOSITION OF MEDIA

Nutrient Agar media; Peptone-5gm, Beef extract-3gm, NaCl-5gm, Agar-20gm, Distil water-1 litre, pH-7.0
Ashby's media (for azotobacters**)**: Glucose-10gm,Tricalcium phosphate-5gm,Diammonium sulphate-0.5gm, Sodium chloride- 0.2gm, Magnesium sulphate 0.1gm, Manganous sulphate-0.1gm,Potassium chloride-

0.2gm,Yeast extract-0.5gm,Ferrous sulphate-0.002gm,Agar-15gm,Distil water-1 litre,pH-7.0

Starch Casein Agar media

Soluble starch-1gm,Casein-0.3gm,Potassium nitrate-2gm,Sodium chloride-2gm,Dipotassium hydrogen phosphate-2gm,Magnesium sulphate-0.5gm,Calcium carbonate-0.02gm,Ferrous sulphate-0.01gm,Agar-15gm,Distil water-1 litre,pH-7±0.2.

Media used for biochemical characterisation of isolates

Following medias were used for characterizing the isolates biochemically.

1.**Pikovaskay'smedia**

Glucose-10gm,Tricalcium phosphate -5 gm,Diammonium sulphate-0.5gm,Sodium chloride-0.2gm,Magnesium sulphate-0.1gm,Manganous **su**lphate-0.1gm,Potassium chloride-0.2gm,Yeast extract-0.5gm,Ferrous sulphate-0.002gm,Agar-15gm,Distil water-1 litre,pH-7.0

2) **Starch Agar Medium:** Soluble starch-20gm, Peptone-5gm, Beef extract-3gm, Agar-15gm, Distil water-1 litre, pH-7.0

3)**Czapek Mineral Salt Agar Media(Cellulase Producing media**)

Sodium nitrate-2gm,Dipotassium hydrogen phosphate-1gm,Magnesium sulphate-0.5gm,Potassium chloride-0.5gm,Peptone-2gm,Carboxy methyl cellulose-5gm,Agar-20gm,Distil water-1litre,pH-6.5

4) **Skimmed milk agar media (Casein hydrolyzing media**)

Skimmed milk agar powder-50gm, Distil water-1litre, pH-7.2

5) **Gelatin medium (Nutrient gelatin broth)**

Peptone-5gm, Beef extract-3gm, Gelatin-20gm, Distil water-1litre, pH-6.8

6) **Indole Broth**

Beef extract-10gm, Peptone-10gm, Distil water-1 litre, pH-7.4

Kovac's reagent: p-dimethylaminobenzaldehyde-5gm, Iso amyl alcohol-75ml, Conc HCl- 25ml.

7) **Methyl Red-Voges Proskauer Broth**-Peptone-7gm,Dextrose-5gm, Di-potassium hydrogen phosphate-5gm,Distil water-1 litre,pH-6.9

MR Reagent-0.425gm, Distil water-1litre
VP Reagent 1-0.425gm, Distilwater-1litre
VP Reagent 2-40%KOH.

8) **Simmon's citrate agar media**

Ammonium dihydrogen phosphate-1gm,Dipotassium hydrogen phosphate-1gm,Sodium chloride-5gm,Sodium citrate-2gm,Magnesium sulphate-0.2gm,Bromothymol blue-0.08gm,Agar-15gm,Distil water-1 litre,pH-6.8 to 6.9

9) **Sulphide Indole Motility (SIM) Agar media**

Peptone-30gm, Beef extract-3gm, Ferrous ammonium sulphate-0.2gm, Sodium thiosulphate-0.025gm, Agar-3gm, Distil water-1 litre, pH-7.3

10) **Nitrate broth**: Peptone-5gm, Beef extract-3gm, Potassium nitrate-5gm, Agar-0.1%, Distil water-1 litre, pH-7.2

11) **Catalase test reagen**t-by using 3%H_2O_2.

12) **Trypticase soy agar media** (for oxidase test): Trypticase-15gm, phytone-5gm, NaCl-5gm, Agar-15gm, Distil water-1litre, pH-7.3

13) **Urea agar media**:Peptone-1gm,NaCl-5gm,Potassium monohydrogen(or dihydrogen)phosphate-2gm,Glucose-1gm,Phenolred(0.02%)6gm,Urea(20%aqueous solution)-100ml,Distil water-1litre,pH-6.8

14) **Media for acid production**: Dipotassium sulphate-2gm,Di-potassium hydrogen phosphate-0.25gm,Magnesium sulphate-0.25gm,Potassium chloride-0.1gm,Yeast extract-0.1gm,Bromocrescol purple-4 to 8 drops of 0.8%aqueous soln for 1 1litre,distil water-1litre,pH-6.8

RESULTS AND DISCUSSIONS

All the 84 isolates were characterized morphologically, physico-chemically and biochemically. Also test for acid production from carbohydrates was performed using particular sugar discs.

At morphological level colony morphology, configuration, margin, surface, density, Gram's reaction, cell shape, size, spore staining and motility was studied.

At physico-chemical level growth of isolates at different temperature, pH, and NaCl concentration was studied.

At biochemical level a no. of important biochemical tests were performed such as starch hydrolysis, casein hydrolysis, gelatin hydrolysis, IMVIc test, hydrogen-sulphide production test, nitrate test,catalase test,oxidase test, urea hydrolysis,phosphatase test,cellulase test and acid production from some sugar discs such as of arabinose,fructose,galactose,glucose,mannitol,mesoinositol,raffinose,rhamnose,sucrose,salicin and xylose.

Screening of Phosphate Solubilisers

Among the 84 isolates only a few of the test isolates showed very good results for phosphatase test Finally among these isolates, only 7 isolates were selected on the basis of their biochemical characteristics as they produced *clearing zones* around the microbial colonies in media as they showed remarked growth on phosphatase medium (Pikovaskay's medium). The strains were designated as SM4, SM8, SM10, SM2A, SM18A, BC7A and BC15A.These isolates were ultimately tested on various paddies and pulses in different consortia's to assess their potential as biofertilisers/biomineralisers.

Potting experiments

Potting experiments were conducted on some paddies and pulses. These were *Zea mays*(maize),*Triticum aestivum*(wheat)*Phaseolus aureus*(green gram) and *Vicia faba(*bakla daal).Also a single pot with market biofertiliser "**Biozyme**" and one pot with control was used.Consotria's of above mentioned isolates was also used for assessment of their potential as biofertilisers.Results were noted in tabular form.

The measurement of plants with respect to their growth/height (in cm), no. of seeds and other parameters were studied.

TABLE 1: Table showing Biofertiliser potential of selected isolates of bacteria on *Zea mays* (Maize). (A-SM4, B-SM8, C-SM10)

Sl. no.	Soil type	Date of potting	Ht. on 20/04/08 (in cm)	Ht. on 28/04/08 (in cm)	Ht. on 05/05/08 (in cm)	Ht. on 12/05/08 (in cm)	Ht. on 19/05/08 (in cm)	Ht. on 26/05/08 (in cm)
1.	Control	13/04/08	0.5,0.5	1.5,2.0	3,5	13,17	18,28	23,32
2.	Biofertilizer	13/04/08	1,1.5	3,5	7,7	17,20	20,33	26,39
3.	A	13/04/08	2,2.5	9,11	19,20	29,30	42,48	47,55
4.	B	13/04/08	2.5,3	10,12	20,23	31,40	48,50	52,55
5.	C	13/04/08	5	16	29	43	56	60
6.	AB	13/04/08	4,9,10	12,16,18	22,26,27	31,35,38	45,50,53	50,55,57
7.	AC	13/04/08	8,10,12	14,15,19	24,25,30	34,35,39	55,58,59	60,63,65
8.	BC	13/04/08	7,9,11	16,18,20	26,28,30	36,38,40	56,57,59	61,62,65
9.	ABC	13/04/08	8,9	20,22	32,34	44,48	60,62	66,68

TABLE 2: Table showing Biofertilizer potential of selected isolates of bacteria in *Phaseolus aurerus* (Moong). (A-SM4, B-SM8, C-SM10).

Sl. no.	Soil type	Date of potting	Ht. on 20/05/08 (in cm)	Ht. on 28/05/08 (in cm)	Ht. on 05/05/08 (in cm)	Ht. on 12/05/08 (in cm)	Ht. on 19/05/08 (in cm)
1.	Control	13/05/08	1,1	2,4	6,9	10,13	15,18
2.	Biofertiliser	13/05/08	2,2.5	5,8	9,13	12,16	16,20
3.	A	13/05/08	3,3.5	8,9.5	15,20	26,30	35,38
4.	B	13/05/08	3.5,4	8,9	18,21	30,35	40,46
5.	C	13/05/08	5	11	25	39	55
6.	AB	13/05/08	3,4,6	8,9,11	19,20, 22	33,38, 38	53,54, 56
7.	AC	13/05/08	3.5,5,8	8,12,13	20,21, 24	35,37, 42	52,55, 57
8.	BC	13/05/08	7,9,11	16,18, 20	25,29, 31	36,45, 45	53,55, 56
9.	ABC	13/05/08	9,13	16,20	28,32	40,43	56,59

Observation

Isolate SM4,SM8 and SM10 were used to test their biofertiliser potential on *Triticum aestivum ,Zea mays and Phaseolus aureus* and *Vicia faba.*Also isolate SM2A,SM18A,BC7A and BC15A were used to test the biofertiliser potential on *Triticum aestivum* and *Vicia faba*.

In case of *Zea mays,* best growth was observed in plants growing in control with combination of strain SM4,SM8 and SM10,followed by control with SM4 and SM10,then with SM8 and SM10.While minimum growth was observed in case of control with combination of biofertiliser, "*biozyme*".

In case of *Triticum aestivum*, best growth was observed for plants growing in control with combination of strains SM2A, SM18A, BC7A and BC15A, followed by control with combination of strains SM2A, SM18A and BC15A, then BC7A, SM2A and BC 15A.Some notable growth was also observed in case of consortia of 2 bacterial isolates such as in case of SM2A and BC15A, and SM18A and BC7A.While minimum growth was observed in case of control with combination of biofertiliser.

In case of *Phaseolus aureus*, best growth was observed in plants growing in control with combination of strain SM4, SM8 and SM10 and then SM4 and SM10.While minimum growth was observed in case of control with combination of biofertiliser biozyme.

In case of *Vicia faba*, best growth was observed in plants growing in control with combination of strain SM2A, SM18A, BC7A and BC15A followed by control with combination of strains SM2A, BC7A and BC15A, and then on SM2A, SM18A and BC7A.Some notable growth was also observed in case of consortia of two bacterial isolates such as, SM18A and BC15A, SM18A and BC7A.While minimum growth was observed in case of control with combination of biofertiliser biozyme.

Overall it can be concluded that the best combination of microbes used as biofertiliser for better growth of crop plants are

(1) SM8 and SM10,
(2) SM2A, SM18A and BC7A,
(3)SM2A,BC7A and BC15A
(4) SM2A,SM18A,BC7A and BC15A.

TABLE 3: Table showing biofertiliser potential of selected isolates of bacteria on *Triticum aestivum* (Wheat). (A-SM2A, B-SM18A, C-BC7A & D-BC15A).

Soil type	Date of potting	Height (in cm)							
		09/01/08	23/01/08	30//01/08	06/02/08	15/02/08	22/02/08	01/03/08	08/03/08
CONTROL	25/12/08	2,1,1	10,17,20	22,24.5, 26.5	25,30,31	35,39,40	38,42,46	45,47,53	49,54,55
BIOFERTILISER	25/12/08	2,2.5,2	12,11,12	23,26,28	32,34,34	40,42,43	47,49,50	48,51,52	52,55,56
AB	25/12/08	2.5,3,3,3.5	18,20,20,21	30,34,35,35	38,40,41,42	41,46,46,47	45,48,48,50	46,49,50,55	46,52,53,58
AC	25/12/08	1.5,1.5,2.5,3	15,17,19,20	27.5,29.5,32,33	33,35,37,39	41,42,44,47	43,47,49,51	47,51,53,56	53,56,58,62
AD	25/12/08	2,2.5,3,3.5	17,18,20,21	27,26.5,28,30	333,37.5,38,40	38,42.5,44,49.5	43.5,48,50,58.5	47,52,55,64	52,56,60,66
BC	25/12/08	2,2.5	15,21	27,29	37,40	44,46	53,56	60,64	68,70
BD	25/12/08	2,3	19,20.5	28,29.5	37,39.5	45,46.5	53,56	62,65	68,70
CD	25/12/08	3,3,3.5,3.5	18,19,19,20.5	27,29,29,30	34,36,37,39	43,44,44.5,45.5	52,53,55,56.5	60,63,64,63	66.5,65,67,68
ABC	25/12/08	2,3,3	16,18,18.5	26,28,29	36,38,38	43,44.5,46	53,55,56	61,61.5,62.5	65,66.5,68
ABD	25/12/08	2,2.5,3,3.5	14,15.5,16,18	23,25.5,26.5,27	33,36.5,38,38	40,43.5,45,46	50,52,54,55.5	55,58,61.5,62	60,64,65,67
CAD	25/12/08	3,3.5,3,3	15,16,16,15.5	23,25,24,26	32,34,35.5,36.5	40,43,44,45.5	50,52,54,56	58,60,60,63	65,65,66,69
CBD	25/12/08	2.5,2.5	15,16.5,	24,25	34,36	42,45	51,53	59,62	66,66
ABCD	25/1/08	3,3.5,4,4.5	17,18,19.5,20	26,27,30,31	35,38,39,40	43,45,47, 49	53,55.5,56.5,	61,65,67,69	68,70,72,74

TABLE 4: Table showing biofertiliser potential of selected isolates of bacteria on *Vicia faba* (Bakla daal)

Soil Type	Date of potting	17/06/09	25/07/09	08/08/09	15/08/09	22/08/09	08/09/09	15/09/09	No.of seeds.
CONTROL	25/05/09	1,0.5,0.5	2,1.5,1.5	8,8.5,8.5	9,9.5,9.5	12,12.5,12.5	18.5,19,19.5	20,22,22.5	2,2
BIOFERTILISER	25/05/09	1,1.5	2,2.	8,8.5	9.5,10	12.5,13	19.5,20	22.5,24	2,2,2
AB	25/05/09	1,1.5,2.5	2,2,5,3	8,8.5,9	12,12.5,12.5	17,17.5,18	22,22.5,23	25.5,26,26	2,3,3
AC	25/05/09	1.5,2	2.5,2.5	8,8.5	12,12	16.5,17	22,23	25,26	2,3
AD	25/05/09	1.5,1.5,2	2,2.5,2.5	8,8.5,9	12,12.5,12.5	16,16.5,17	23,24,25.5	25,26.5,27	1,2,3
BC	25/05/09	0.5,1.5	2,2.5	8,8.5	12,12.5	16,17	23,23.5	26,26.5	2,2
BD	25/05/09	1.5,1.5,1.5	2,2.5,2.5	8,8.5,8.5	12,12,12.5	16,16.5,17	22.5,23,24	26,26.5,27	2,2,3
CD	25/05/09	2,1.5,1.5	2,2.5,2.5	8,8.5,8.5	12,12,12.5	16,16.5,17	23,23,23.5	26,26,26.5	2,3,4
ABC	25/05/09	1,1.5,2	2,2.5,2.5	8,9,9	12,12.5,13	16,16.5,18	23,24.5,26	26,27,27.5	3,2,3
ABD	25/05/09	1.5,2.5	2.5,3	9,9	12.5,12.5	18,19	24,25	26.5,27	2,3,3
CAD	25/05/09	1.5,2,2.5	2.5,2.5,3.5	9.5,10,10	12.5,13,13	18,18,18	24,24.5,24.5	26.26,26.5	3,2,3
CBD	25/05/09	1.5,2,2.5	3,3,3.5	9,9,9.5	14,14,14.5	19,19,19	25,25,25	26.5,26,27	2,2,3
ABCD	25/05/09	2,2,2.5,2.5	3.5,3.5,4,4	10.5,10.5,11,11	15,15.5,16,17	20,20.5,21,22	26,26.5,27,28	28,29,29,30	2,2,3,

FIGURE 1. Plants of *Vicia faba* grown in control, biofertiliser and combination of bacterial isolate ABCD (SM2A, SM18A, and BC7A & BC15A).

FIGURE 2. Plants of *Triticum aestivum* grown in combination of bacterial isolate AB (SM4 & SM8), AC (SM4 & SM10) and BC (SM8 & SM10)

FIGURE 3. Plants of *Triticum aestivum* grown in biofertiliser, combination of bacterial isolates AC (SM4 &SM10) and BC (SM8 & SM10)

FIGURE 4. Plants of *Triticum aestivum* grown in combination of bacterial isolate AC (SM4 & SM10), BC (SM8 & SM10) and ABC (SM4, SM8 & SM10)

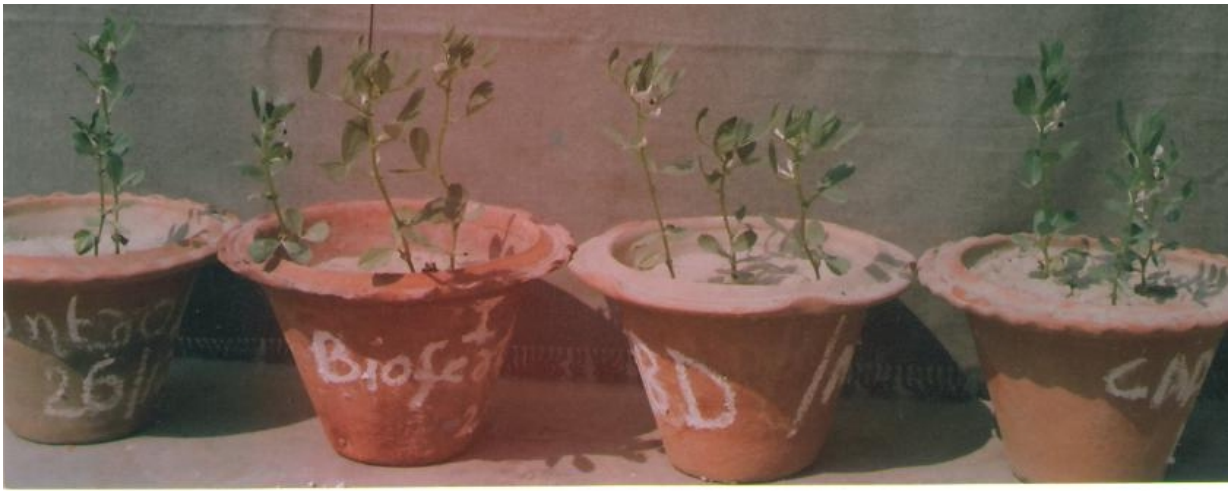

FIGURE 5. Plants of *Vicia faba* grown in control, biofertiliser and combination of bacterial isolate CBD (BC7A, SM18A&BC15A) and CAD (BC7A, SM2A & BC15A)

FIGURE 6. Plants of *Phaseolus aureus* grown in control, biofertiliser and combination of bacterial isolate BC (SM8 & SM10)

FIGURE 7. Plants of *Vicia faba* grown in control, biofertiliser and combination of bacterial isolate ABD (SM2A, SM18A & BC15A) and CAD (BC7A, SM2A & BC15A)

FIGURE 8. Plants of *Vicia faba* grown in control, biofertiliser and combination of bacterial isolate ABC (SM2A, SM18A & BC7A) and CBD (BC7A, SM18A & BC15A)

FIGURE 9. Plants of *Vicia faba* grown in combination of bacterial isolate ABC (SM2A, SM18A & BC7A), ABD (SM2A, SM18A & BC15A), and CAD (BC7A, SM2A & BC15A).

FIGURE 10. Plants of *Vicia faba* grown in biofertiliser, control and combination of bacterial isolate AB (SM2A & SM18A) and AD (SM2A & BC15A)

Figure 11. Plants of *Vicia faba* grown in biofertiliser, control and combination of bacterial isolate AB (SM2A & SM18A) and BD (SM18A & BC15A)

FIGURE 12. Plants of *Triticum aestivum* grown in biofertiliser, control and combination of bacterial isolate ABD (SM2A, SM18A & BC15A) and CAD (BC7A, SM2A & BC15A).

FIGURE 13. Plants of *Triticum aestivum* grown in biofertiliser, control and combination of bacterial isolate ABCD (SM2A, SM18A, BC7A & BC15A)

Figure 14. Plants of *Triticum aestivum* grown in biofertiliser, control and combination of bacterial isolate AD (SM2A & BC15A) and BC (SM18A & BC7A).

The morphological, physico-chemical and biochemical characterisation of the selected best five test isolates used for assessing their potential as biofertilisers are as follows:

TABLE 5 Morphological characterization of selected Bacterial isolates.

	SM8	SM10	SM2A	SM18A	BC7A
Colony morphology	Round	Round	Round	Round	Round, with scalloped margin
Configuration	Smooth, shiny(entire)	Smooth, shiny(entire)	Filamentous	Filamentous	Wavy
Margin	Raised	Raised	Ciliate	Irregular	Raised
Surface	Shiny	Smooth	Smooth	Smooth	Smooth
Density	Opaque	Opaque	Opaque	Opaque	Opaque
Gram's reaction	-ve	+ve	+ve	+ve	+ve
Cell shape	Mycelium	Rods	Rods	Rods	Rods
Size	Small	Small	Long	Long	Small
Spore staining	-ve	+ve	+ve	+ve	+ve
Motility	-ve	-ve	+ve	+ve	-ve

TABLE 6. Physico-chemical characterization of selected Bacterial isolates.

	SM8	SM10	SM2A	SM18A	BC7A
Growth at temp.					
25ºC	+	+	+	+	+
37ºC	+	+	+	+	+
42ºC	+	+	+	+	+
55ºC	-	-	+	+	+
Growth on NaCl%					
0.87%	+	+	+	+	+
2%	+	+	+	+	+
5%	+	+	+	+	+
7%	-	+	-	-	-
10%	-	-	-	-	-
Growth on pH					
5.2	+	+	+	+	+
7.0	+	+	+	+	+
8.0	+	+	+	+	+
9.0	+	+	+	+	+
11.0	+	+	+	+	+

TABLE 7- Biochemical characterization of selected Bacterial isolates.

	SM8	SM10	SM2A	SM18A	BC7A
Starch hydrolysis	_	+	_	_	_
Casein hydrolysis	+	+	_	_	_
Gelatin hydrolysis	_	_	_	_	_
Indole test	_	_	_	_	+
Methyl-Red test	+	+	_	_	_
Voges-Proskauer test	_	_	_	_	_
Citrate test	_	_	+	+	_
H_2S production test	_	_	_	_	_
Nitrate test	+	+	+	+	+
Catalase test	+	+	+	+	+
Oxidase test	_	_	_	_	_
Urea hydrolysis	_	_	+	+	_
Phosphatase test	+	+	+	+	-
Cellulase test	+	+	+	+	+
Acid production from					
Arabinose	+	_	+	_	+
Fructose	_	+	+	_	_
Galactose	_	_	+	_	_
Glucose	+	+	+		
Mannitol					
Meso-inositol	_	_	_	_	_
Raffinose	_	_	_	_	_
Rhamnose	_	_	_	_	_
Sucrose	_	+	_	_	_
Salicin	_	_	_	_	_
Xylose	_	_	+	_	_

Finally among the 7 test isolates only 5 isolates viz SM8, SM10, SM2A, SM18A and BC7A were send to IMTECH for identification and MTCC accession no.(Microbial Type Culture Collection).The MTCC no. allocated to these isolates are as follows:

Serial no.	Strain Designation	Isolate identified (Genus and species)	MTCC no.
1.	SM8	*Nocardiopsis flava*	MTCC 9525
2.	SM10	*Bacillus species*	MTCC 9524
3.	SM2A	*Virgibacillus pantothenticus*	MTCC 9479
4.	SM18A	*Lysinibacillus sphaericus*	MTCC 9523
5.	BC7A	*Lysinibacillus sphaericus*	MTCC 9526

CONCLUSION

From the overall investigation it can be concluded that micro-organisms (bacteria, actinomycetes as well as fungi) can be used as suitable biofertilisers for enhanced and better growth of plants. The use of these microorganisms as biofertilisers represents an attractive environment friendly alternative. The use of biofertiliser or microbial inoculants for increasing the efficacy of chemical fertilizer can be effective in reducing the cost of cultivation and maintaining the natural fertility of soil. Ultimately it can also replace the use of chemical fertilizer in future and can be an attractive eco friendly alternative.

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