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Original Article

Isolation, Purification and Characterization of Microorganisms of Brinjal Soil Rhizosphere

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Five composite rhizosphere soil samples of brinjal (*Solanum melongena* var. Dohazari) were collected aseptically from brinjal field of Zobra, Chittagong at different times. Their moisture, temperature, pH and R:S ratio were determined. Total microbial load per gram of brinjal rhizosphere soil was also recorded. 42 bacterial and 22 actinomycetes colonies were isolated from these rhizosphere soil samples. Among them, 8 species of bacteria and 8 species of actinomycetes were studied thoroughly. They were provisionally identified as, *Cellulomonas flavigena*, ($1/BR_{12}$), *Lactobacillus brevis*, ($1/BR_{18}$); *L. plantarum*, ($1/BRp_{22}$), *L. salivarius*, ($1/BRp_{20}$), *Listeria denitrificans*, ($1/BR_4$); *Listeria grayi*, ($1/BR_2$); *L. monocytogenes*, ($1/BR_6$), *L. murrayi*, ($1/BR_5$), *Micromonospora chalcea*, ($2/BR_{15}$); *Micromonospora narashinoensis*, ($2/BR_5$); *Streptomyces aburaviensis*, ($2/BR_3$); *S. albofaciens*, ($2/BR_{12}$); *Streptomyces amakusaensis*, ($2/BR_{17}$); *Streptomyces bikiniensis*, ($2/BR_8$); *S. fulvoviridis*, ($2/BR_9$) and *Streptoverticillium ardum*, ($2/BR_{16}$).

Keywords: Rhizosphere, bacteria, actinomycetes, R:S ratio, brinjal.

Introduction

Rhizosphere is a specialized region which provides a unique habitat for different types of microorganisms. Interactions between soil microorganisms and plant roots satisfy important nutritional requirements for both the plant and associated microorganisms[1-4]. The rhizosphere may be considered as a microbiological buffer zone in which the microflorae serve to protect the plant from the attack of the pathogens[5]. This region is highly favourable for the proliferation and metabolism of numerous microbial organisms. Generally, rhizosphere microbes show various types of relationship with the plant root such as symbiotic, pathogenic, commensal, protocooperation and subterranean relationship. Root exudates may have important effects on rhizosphere microbial growth have nutritional value because of the presence of a number of amino acids, organic acids, pentoses and hexoses, pyrimidines and puridines, vitamins, enzymes etc.

The root influence is often expressed as a rhizosphere effect, a stimulation which can be put on a quantitative basis by the use of the R:S relationship- a ratio of microbial numbers per unit weight of rhizosphere soil (R), to the population in a unit weight of the adjacent nonrhizosphere or control soil (S). The R:S ratio can be used to find out the degree or extent of soil microorganisms on the plant roots[6].

Rhizosphere microbes may be different in different plant species and some of them may be beneficial and others may have undesirable effects on plant growth, development and yield. Brinjal is an important vegetable crop of Bangladesh and farmers can grow it commercially throughout the year. Identification and characterization of useful microbes of the rhizosphere of brinjal (*Solanum melongena* var. Dohazari) may be helpful to get economically beneficial effect on the yield of brinjal in the long run. Keeping these views in mind, in the present investigation on characrerization and identification of brinjal rhizosphere microbes along with associated soil characters were carried out.

Materials and Methods

Composite soil samples along with brinjal (*Solanum melongena* var. Dohazari) plant were collected in sterile polythene bags from rhizosphere of brinjal at different time of the year from different places of Zobra village near Chitagong University, Chittagong. Then the samples in air tight bags were carried to the laboratory for investigation and preserved.

pH of each sample was determined in the laboratory using pH meter (pH Hanna Instrument Ltd. & 3310, pH meter Jenway, UK) by mixing with distilled water in the proportion of 1:2, respectively according to Jackson[7].

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Moisture content (M%) of the soil sample was determined by drying the sample in oven at 100°C following Jackson[7], as:

$$M\ \% = \frac{WI - WF}{WF} \times 100$$

Where WI and WF are initial and final weights, respectively.

To determine the R:S ratio of the developed colonies in rhizosphere soil and non-rhizosphere soil samples were counted by a colony counter and the R:S ratio was determined following Alexander[5].

For temperature determination, a bulb thermometer was inserted into the rhizosphere soil in each site of sampling and the temperature was recorded.

Microorganisms were isolated by dilution plate technique using various culture media. During the period of isolation of microorganisms 8 different types of media were used, e.g. nutrient agar, modified nutrient agar, starch casein agar, yeast extract malt extract agar, inorganic salt starch agar, glycerol asparagin agar, peptone yeast extract agar, tyrosine agar, potato dextrose agar, czapek dox agar media for isolating rhizosphere bacteria, actinomycetes and fungi.

For determination of total microbial population, the selected plates were placed on a colony counter and the colonies were counted. The count of colonies per ml or g was calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution. The calculated results would be as colony forming units (cfu) per ml or gm of sample.

The selected bacterial and actinomycetes colonies were transferred to slope of slants prepared with the corresponding plating media for further studies. For bacteria and actinomycetes, isolates were purified by repeated streaking on to nutrient agar plate and starch casein agar plate, respectively.

Morphological, cultural, physiological and biochemical characters of the isolates were determined. Following Bergey's Manual of Determinative Bacteriology, identification of the isolates were done[8].

Some special cultural studies were done for actinomycetes: determination of growth response and chromogenesis of the selected isolates in different ISP media, determination of melanin production by the selected isolates, determination of growth response of the selected isolates in different nitrogenous sources using basal media.

Results and Discussion

The date, time, temperature, pH and moisture content of collected soil samples were recorded. The temperature, pH and moisture of the collected rhizosphere soil samples were found to vary from 27°C to 33°C, 6.50 to 7.30 and 2.74 to 5.07%, respectively (Table 1). The total bacterial, fungal and actinomycetes count of the samples were varied from $95x10^3$ to $152x10^3$ cfu/g, $4x10^3$ to $56x10^3$ cfu/g and $11x10^3$ to $48x10^3$ cfu/g, respectively (Table 2).

The date, time, temperature, pH and moisture content of collected soil samples are recorded and shown in Table 1.

Table 1. *The date, time, temperature, pH and moisture content of collected soil samples*

No. of sample	Date of collection	Time of collection	Temperature (°C)	pH	Moisture content
1.	16.02.08	11:30 a.m.	27°C	7.1	3.45
2.	23.03.08	10:45 a.m.	27.5°C	7.3	2.40
3.	30.03.08	12:30 p.m.	33°C	7.2	3.45
4.	12.09.08	12:30 p.m.	31.5°C	6.6	2.74
5.	15.09.08	1:00 p.m.	31°C	6.5	5.07

The R:S ratio of the collected samples are recorded and shown in Table 2. The R:S ratio of the collected samples vary from 1.6:1-6.2:1 for bacteria, 2:1-14:1 for fungi and 1.1:1-24:1 for actinomycetes.

Table 2. *R:S ratio for bacteria, fungi and actinomycetes.*

Sample No.	Bacteria			Fungi			Actinomycetes		
	No. of Colony per gm of soil ($x 10^3$)		R:S ratio	No. of Colony per gm of soil ($x 10^3$)		R:S ratio	No. of Colony per gm of soil ($x 10^3$)		R:S ratio
	R	S		R	S		R	S	
1.	130	21	6.2:1	56	4	14:1	48	2	24:1
2	144	79	1.8:1	22	11	2:1	25	22	1.1:1
3	121	69	1.9:1	4	2	2:1	22	20	1.1:1
4	152	93	1.6:1	10	3	3.3:1	11	9	1.2:1
5	95	31	1.9:1	52	26	2:1	32	30	1.1:1

R= Rhizosphere soil, S= Non-rhizosphere soil.

Primarily, a total number of 42 bacterial colonies and 22 actinomycetes colonies were isolated on the basis of their colony morphology. Out of the 64 isolates, 18 bacterial colonies and 18 actinomycetes isolates were selected for further study on the basis of their staining properties and cultural characteristics. Finally, 8 bacteria and 8 actinomycetes were selected for detail study. The selected bacterial isolates were $1/BR_2$, $1/BR_4$, $1/BR_5$, $1/BR_6$, $1/BR_{12}$, $1/BR_{18}$, $1/BR_{P20}$, $1/BR_{P22}$ and the selected actinomycetes isolates were $2/BR_3$, $2/BR_5$, $2/BR_8$, $2/BR_9$, $2/BR_{12}$, $2/BR_{15}$, $2/BR_{16}$, and $2/BR_{17}$. Selected bacterial and actinomycetes isolates were microscopically observed. Size and arrangement of vegetative cells, gram reaction, acid fast reaction and spore staining were observed under microscope. All the isolates were found to be gram positive and non-acid fast.

The bacterial isolates were characterized on the basis of their morphological, cultural and physiological characteristics. All the isolates of bacteria were found to belong to 3 genera, such as, *Cellulomonas*, *Lactobacillus* and *Listeria*. An attempt was made to identify them up to species and provisionally identified as; *Cellulomonas flavigena*, ($1/BR_{12}$), *Lactobacillus brevis*, ($1/BR_{18}$); *L. plantarum*, ($1/BR_{P22}$), *L. salivarius*, ($1/BR_{P20}$), *Listeria denitrificans*, ($1/BR_4$); *Listeria grayi*, ($1/BR_2$); *L. monocytogenes*, ($1/BR_6$), *L. murrayi*, ($1/BR_5$). (Table 3).

All the isolates of actinomycetes were found to belong to 3 genera, such as, *Micromonospora*, *Streptomyces* and *Streptoverticillium*. An attempt was made to identify them up to species and provisionally identified as *Micromonospora chalcea*, (2/BR_{15}); *Micromonospora narashinoensis*, (2/BR_4); *Streptomyces aburaviensis*, (2/BR_3); *S. albofaciens*, (2/BR_{12}); *Streptomyces amakusaensis*, (2/BR_{17}); *Streptomyces bikiniensis*, (2/BR_8); *S. fulvoviridis*, (2/BR_9) and *Streptoverticillium ardum*, (2/BR_{16}) (Table 4).

Cow dung, litter, compost, etc. are the common source of *Listeria* sp. The farmer may use these materials as sources of organic manure and from these sources *Listeria* sp. could be incorporated itself into the rhizosphere soil. Helene *et al.* reported the behaviour of pathogen *L. innocua* during production of parsley in fields, fertilized with contaminated amendments[9]. Stefan *et al.* reported about the colonization of barley (*Hodeum vulgare*) roots with *Listeria* sp[10]. They found it to colonize in the root hair zone but did not in other parts of the root surface.

Table 3. *Morphological, physiological and biochemical characteristics of bacterial isolates.*

Characteristics	Isolate No.							
	1/BR_2	1/BR_4	1/BR_5	1/BR_6	1/BR_{12}	1/BR_{18}	1/BR_{P20}	1/BR_{P22}
Colony shape	Punct-iform	Punct-iform	Irregular	Circular	Circular	Punct-iform	Filame-ntous	Circular
Colony elevation	Flat	Convex	Flat	Flat	Raised	Raised	Raised	Raised
Colony margin	Entire	Entire	Undulate	Entire	Entire	Entire	Filame-ntous	Entire
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram staining	+	+	+	+	+	+	+	+
Endospore formation	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+
Urease test	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	_
Starch hydrolysis	+	+	+	+	+	-	+	+
Methyl red test	-	-	+	-	-	+	-	-
Indole test	-	-	+	-	-	-	-	-
VP test	-	-	-	-	+	+	+	+
Nitrate reduction test	-	+	-	-	-	+	+	+
H_2S production	+	-	-	+	-	-	+	+
Deep glucose agar test	Aerobic	Aerobic to microaer-ophilic	Aerobic to microaer-ophilic	Aerobic to microaer-ophilic	Aerobic to microaer-ophilic	Aerobic to microaer-ophilic	Aerobic to microaer-ophilic	Aerobic
Acid from sucrose	-	-	-	-	+	+	-	-
Acid from lactose	-	-	+	+	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+

Note: + = Positive, - = Negative.

Table 4. *Morphological, physiological and biochemical characteristics of actinomycetes isolates.*

Characteristics	Isolate No.							
	$2/BR_3$	$2/BR_5$	$2/BR_8$	$2/BR_4$	$2/BR_{12}$	$2/BR_{15}$	$2/BR_{16}$	$2/BR_{17}$
Colony shape	Circular	Irregular	Circular	Circular	Circular	Circular	Circular	Circular
Colony elevation	Raised	Raised	Flat	Flat	Umbonate	Umbonate	Raised	Raised
Colony margin	Entire	Undulate	Entire	Entire	Entire	Entire	Entire	Entire
Mycelium	Whitish, septed, branched	Light yellow, septed, branched	Whitish, septed, branched	Whitish, non-septed, branched	Whitish, non- septed, branched	Whitish, septed, branched	Whitish, septed, branched	Whitish, non- septed, branched
Spore	Grey, smooth	Grey, smooth	Grey, smooth	Grey, smooth	Grey, smooth	Grey, smooth	Grey, smooth	Blue, smooth
Gram staining	+	+	+	+	+	+	+	+
Nitrate reduction test	-	-	-	+	-	+	+	-
Melanin production test	-	-	+	+	-	+	-	+
Growth in ISP-II media	Mycelium Light yellow, Spore: Grey	Mycelium : Cream, Spore: White	Mycelium : Cream, Spore: Greyish white	Mycelium: Whitish, Spore: Grey	Mycelium: Off white, Spore: White	Mycelium: Light brown, Spore: Whitish	Mycelium: White, Spore: White	Mycelium: White, Spore: Astral blue
Growth in ISP-IV media	Mycelium: Whitish, Spore: Grey	Mycelium : Cream, Spore: Grey	Mycelium : Whitish, Spore: Ash	Mycelium: Off white, Spore: Grey	Mycelium: Off white, Spore: Whitish	Mycelium: Light cream, Spore: Light grey	Mycelium: White, Spore: White	Mycelium: Light grey, Spore: blue/ dark grey
Growth in ISP-V media	Mycelium: Off white, Spore: Grey	Mycelium : Cream, Spore: Grey	Mycelium : Cream, Spore: Grey	Mycelium: Whitish, Spore: Grey	Mycelium: Off white, Spore: White	Mycelium: Light yellow, Spore:White	Mycelium: White, Spore: White	Mycelium: Off white, Spore: Light grey
Growth in ISP-VI media	Mycelium: Cream, Spore: Grey	Mycelium : Cream, Spore: Grey	Mycelium : Cream, Spore: Grey	Mycelium: Off white, Spore: Grey	Mycelium: Milk white, Spore: Light brown	Mycelium: Light brown, Spore: Grey	Mycelium: White, Spore: White	Mycelium: Off white, Spore: Light grey
Growth in ISP-VII media	Mycelium: Whitish, Spore: Grey	Mycelium : Whitish, Spore: Grey	Mycelium : Whitish, Spore: Grey	Mycelium: Whitish, Spore: Grey	Mycelium: Off white, Spore: White	Mycelium: Cream, Spore: Grey	Mycelium: White, Spore: White	Mycelium: Whitish, Spore: Bluish green
Deep glucose agar test	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic to micro-aerophilic	Aerobic to micro-aerophilic	Aerobic
Acid from fructose	+	-	-	+	+	+	+	-
Acid from glucose	-	-	+	+	-	_	+	-

Note: + = Positive, - = Negative.

Milillo *et al.* reported the growth and persistence of *Listeria monocytogenes* isolates on the plant model *Arabidopsis thaliana*[11]. The present work on the rhizosphere microorganisms of brinjal are in concurrence with the above reports.

In the rhizosphere soil cellulytic materials can be present. The sources of this cellulytic materils may be compost, green fertilizers etc. which are used as organic amendments. Even the slought-off cells of the root may be a good source of cellulytic compounds from which *Cellulomonas* sp. can be benefited. Sarathchandra *et al.* reported the growth patterns of *Cellulomonas communities* in rhizosphere of white clover (*Trifolium repens* L.) and perennial rye grass (*Lolium perenne* L.)[12]. Egamberdiyeva and Höflich reported the root colonization and growth promotion of winter wheat and pea by *Cellulomonas* spp. at different temperatures[13]. The present work on the rhizosphere microorganisms of brinjal are in conformity with the above reports. *Lactobacillus* generally found in the rumen of cattle and cowdung. From these sources the organism could be colonized in the rhizosphere soil when the farmer used these as sources of manure. Fujitoshi *et al.* reported about the isolation and characterization of bacteriocin-producing lactic acid bacteria in soil[14]. The present work on the rhizosphere microorganisms of brinjal corroborated with the above reports.

Tokala *et al.* reported that rhizosphere of pea plant was colonized by *Streptomyces* species such as *S. lydicus* WYEC108[15]. Sahin isolated and characterized mesophilic oxalate degrading *Streptomyces* from plant rhiosphere and forest soils while Kortemaa *et al.* (2008) reported the distribution of antagonistic *Streptomyces griseoviridis* in the rhizosphere soil of turnip rape and carrot[16,17]. Shirokikh *et al.* noted the presence of actinomycetes in the rhizosphere of barley (*Hordeum vulgare*) grown on acid soddy podzolic soil[18]. Merzaeva and Shirkikh reported the colonization of plant rhizosphere (winter rye) by actinomycetes (*Streptomyces, Micromonosperma*)[19]. The present work on the rhizosphere microorganisms of brinjal are in concurrence with the above reports.

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