

Screening of Phosphate Solubilising Bacteria Isolated from Tea Soil of Brahmaputra Valley Showing Antagonism Against *Fomes lamoensis* and *Ustulina zonata*

ABSTRACT : A total of 12 phosphate solubilising bacteria were isolated from top soil sample of 17 tea gardens of three different tea growing regions of Brahmaputra valley. The soil samples were collected from rehabilitated, non-rehabilitated, and virgin sections from each 15 tea gardens and from other two tea gardens, soils were taken from disease infested and Non-Infested areas. All the isolated phosphate solubilising bacteria, showed marked clearing zone and were evaluated for their antagonistic activities against two primary root disease causing pathogen of tea, *Fomes lamoensis* (Causing Brown root rot) and *Ustulina zonata* (Causing Charcoal stump rot). Against *Ustulina zonata* MM/PH/KMP, MM/PH/BST MM/PH/DLJB-1, MM/PH/DLJB-2 and MM/PH/TY and Against *Fomes lamoensis* MM/PH/KMP, MM/PH/BST MM/PH/DLJB-1 and MM/PH/DLJB-2 showed more than 35% inhibition. Among them two strains MM/PH/KMP and MM/PH/BST were found to inhibit the pathogens more than 55% under study in invitro condition. Physiological study was also conducted for the two most potential antagonist, to see the effect of different pH, carbon and nitrogen sources.

Key words: Antagonistic activity, Rehabilitated, Non-rehabilitated and virgin areas, phosphate solubilising bacteria, *Ustulina zonata*, *Fomes lamoensis*.

Tea, the most popular and inexpensive beverages is manufactured from young shoots comprising two to three leaves and a bud of commercially grown tea plant (*Camellia sinensis*(L) O.Kuntz.)¹. Being a monocultured and perennial plant it has been facing serious threat from different pests and diseases. Among different diseases Charcoal stump rot caused by *Ustulina.zonata* and Brown root rot caused by *Fomes lamoensis* are recognized traditionally as primary root diseases particularly in N.E india. The residual toxicity due to the use of various toxic, health hazardous chemicals as pesticides and fungicides are creating havoc during the recent years. In order to escape from this serious problem eco-friendly biocontrol agents are being recommended as plant protection tool in controlling the major pest and diseases of tea throughout the world. Tea soil are rich in phosphate². It contains a variety of microorganisms and most of them are beneficial to plant. Presence of many phosphate solubilizing bacteria

has been reported in the tea soil³ which makes the phosphate available, to be utilized by the tea plants for its growth and development. As biological means for controlling diseases are gaining momentum, an approach can be made to exploit these phosphate solubilizing bacteria for controlling the two primary soil borne root disease causing pathogens of tea.

Materials and Methods : Rhizospheric soil sample from top soil at a depth of (1-6 inch) were randomly collected from rehabilitated, non-Rehabilitated and virgin areas of five selected tea gardens each from three different regions of Brahmaputra valley and from other two gardens of south zone³ soil samples were collected from root disease infested areas and were allowed to air dry. Quantification of Microbial population was enumerated using 10-fold serial dilutions⁴ and expressed as CFU g-1. Serial dilutions were prepared by peptetting out 10 ml of the original suspension with sterile pipette and transforming into 90 ml of the sterile water blank and this was repeated to get suitable dilutions. When suitable dilutions were prepared, 1 ml of the suspension was transferred to sterile Petri dish, to which 10-15 ml of appropriate medium was added. Triplicate plates were maintained for each treatment and numbers of cfu per gm of soil were recorded after 48 hours and 15 days for bacterial populations and others respectively. Phosphate solubilizing microorganisms are routinely screened by a plate assay method using Pikovskaya (PVK) agar⁵. The test of the relative efficiency of isolated strains is carried out by selecting the microorganisms which are capable of producing a halo/clear zone on plate due to the production of organic acids into the surrounding medium⁶. The assay for antagonism in invitro condition was performed on PDA Petri dishes by the dual culture method⁷. A mycelia agar disc of 5 mm from pathogen cultures was placed on the one side of a Petri dish containing PDA medium. The dishes were incubated at 25°C for 72 h. A loopful of phosphate solubilizing bacteria for test was then streaked 3 cm away from the disc of *Fomes lamoensis* or *Ustulina zonata* on the same dish. Paired cultures were than incubated at 25°C. Dishes inoculated only with test pathogens served as controls. The experiment was repeated twice with three replications of each treatment. The percent growth inhibition (PI) was calculated using the formula: $PI (\%) = \frac{KR-R1}{KR} \times 100$, where *KR* represents the distance

(measured in mm) from the point of inoculation to the colony margin on the control dishes, and *RI* the distance of fungal growth from the point of inoculation to the colony margin on the treated dishes in the direction of the antagonist⁸.

Results and Discussion : From our study it was revealed that population of phosphate solubilizing bacteria

is more dominant in rehabilitated soil of summer season. Maximum population is found in soil of Upper Assam. Clearing zone of more than 0.2 cm is observed in all the isolated strains (Table 1). Among the 12 isolated strains, 6 strains namely MM/PH/DLJB-1, MM/PH/DLJB-2, MM/PH/BST, MM/PH/TY, MM/PH/KMP and MM/PH/BZL showed their antagonism to both the fungal pathogen taken

TABLE 1: Population density and zone clearing of isolated phosphate solubilizing bacteria.

Sl No.	Strain code	Name of the T.E	Type of land pattern	Season	Population density ($\times 10^5$ cfu/gm)	Phosphate Solubilizing zone(cm)
1	MM/PH/DLJB-1	Dalowjan	NR	Winter	2.4	0.51
2	MM/PH/DLJB-2	Dalowjan	R	Summer	1.2	0.54
3	MM/PH/GPB	Gabroopurbat	R	Winter	2.2	0.39
4	MM/PH/BST	Ghillidary	R	Summer	0.6	0.82
5	MM/PH/KC	Kakojan	CA	Winter	1.67	0.27
6	MM/PH/TY	Tyroon	BA	Winter	0.67	0.49
7	MM/PH/KMP	Kolony	R	Summer	1.42	0.78
8	MM/PH/MB-1	Monobug	R	Summer	4.40	0.25
9	MM/PH/ MB-2	Monobug	V	Summer	3.4	0.20
10	MM/PH/BZL	Bazaloni	R	Summer	2.6	0.46
11	MM/PH/LKS	Lankasi	NR	Summer	5.4	0.20
12	MM/PH/SV	Sookerating	V	Summer	4.4	0.37

R=Rehabilitated areas, NR=Non-Rehabilitated areas, V=Virgin areas, CA=Charcoal stump rot affected areas, BA=Brown root rot affected areas.

TABLE 2. Inhibition zone and percent inhibition of isolated antagonists with root disease causing fungus (*Ustulina zonata* and *Fomes lamoensis*)

SINo.	Strain code	<i>Ustulina zonata</i>		<i>Fomes lamoensis</i>	
		ZOI	PI	ZOI	PI
1	MM/PH/DLJB-1	9.2	44.44	6.3	40.0
2	MM/PH/DLJB-2	11.3	53.33	9.5	39.33
3	MM/PH/GPB	**	-	2.5	11.11
4	MM/PH/BST	20.04	62.1	21.04	66.06
5	MM/PH/KC	-	-	-	-
6	MM/PH/TY	7.2	37.78	5.1	22.89
7	MM/PH/KMP	17.34	56.98	19.52	64.44
8	MM/PH/MB-1	-	-	-	-
9	MM/PH/MB-2	**	-	**	-
10	MM/PH/BZL	2.1	6.67	2.7	12.22
11	MM/PH/LKS	**	-	**	-
12	MM/PH/SV	3.3	20	**	-

ZOI=Zone of inhibition, PI=Percent inhibition.

** =Just near the edge.

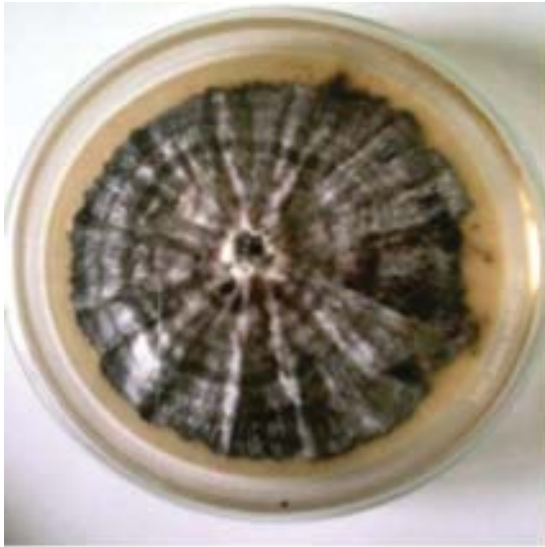


Fig 1. Pure culture of *Ustilina.zonata*



Fig 2. Pure culture of *Fomes.lamoensis*



Fig 3. MM/PH/KMP showing antagonism against *U.zonata*

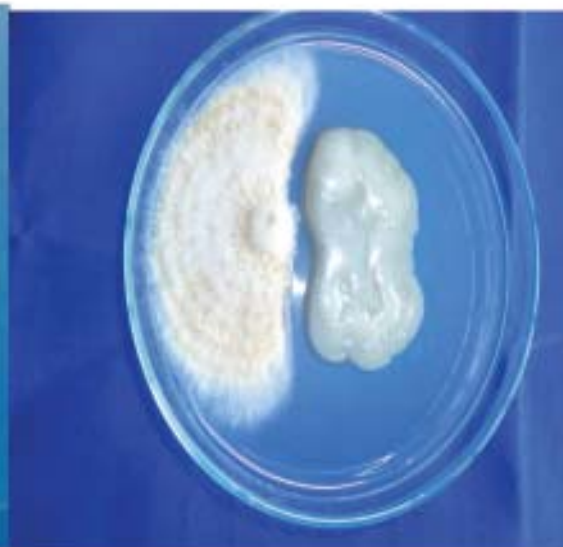


Fig 4. MM/PH/BST showing antagonism against *U.zonata*

under study, while MM/PH/SV and MM/PH/GPB only inhibited *U.zonata* and *F.lamoensis* respectively (Table 2). Two strain MM/PH/KMP and MM/PH/BST showing more than 55% inhibition of the growth of *U.zonata* and *F.lamoensis* were considered to be highly potential and can be utilized for further study.

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