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RESEARCH ARTICLE

"Antimicrobial activity of Rhizospheric Bacteria of *Curcuma longa* Producing Metabolites against Human Bacterial Pathogens."

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Abstract

Medicinal plants are widely used all over the world for natural medicines. *Curcuma longa* are known as "mother medicine of nature"; they have chemical compounds for curing and preventing diseases. These plants have valuable antimicrobial resources and can produce a large number of metabolites which having antibacterial properties, regulating their own growth and development to encourage other organism beneficial to them and suppress organisms that are harmful. Soil microorganism provides an excellent resource for isolation and identification of therapeutically important products; Antimicrobial metabolites were produced by different bacteria present in soil. In present study 22 rhizospheric soil samples of *Curcuma longa* were collected from western Vidharbh region of Maharashtra state and were analyzed for presence of bacteria which can produce metabolites, isolation of desired bacteria were carried out by serial dilution method, Total 26 bacteria have been isolated from rhizospheric soil samples and out of 26 only 3 were potent isolates whose have been characterized on the basis of antibiogram test that revealed the activity of isolates, further characterization was done by following the Bergey's Manual of Systematic Bacteriology. Accordingly *Curcuma longa* rhizospheric characterized isolates were *Bacillus megatherium*, *Pseudomonas fluorescens* and *Globicatella sulfidifaciens*. These potent isolates could be further exploited for the production of metabolites in production media.

INTRODUCTION

India has one of the richest medical cultures in the world. Indian literature incorporated in remarkably broad definition of medicinal plant and considers all plants are potential sources of medicinal substance. The plants containing medicinal substance which substance which can be use as antifungal, antibacterial, anticancerous etc. are term as medicinal plants. Plants are primary source of medicine, among the plants known for their medicinal values

The potential importance of microbial activities associated with root system in plant nutritional and coined the term "rhizospheric" to describe the zone of intense microbial activity around the root. Rhizosphere is a narrow zone around the influenced by a plant root which inhibited by a vast population of microbes affected by a chemicals which are release from roots of plants. It is a dynamic region where various biological and chemical processes take place along with a variety of chemical release by roots and mediated by the soil microbes. Soil micro-organism constitutes world largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystem (Pandey, A. and Singh, A. 2013).

The rhizosphere is a densely populated area in which the roots must compete with the invading root systems of neighboring plant species for space, water, and mineral nutrients, and with soil-borne microorganisms, including bacteria, fungi, and insects feeding on an abundant source of organic material. Thus, root-root, root-

microbe, and root-insect communications are likely continuous occurrences in this biologically active soil zone, but due to the underground nature of roots, these intriguing interactions have largely been overlooked. Root-root and root-microbe communication can be positive (symbiotic) to the plant, such association of plant roots is in constant communication with symbiotic and pathogenic organisms (Malviya, T. and Pandey, A. 2014).

Curcuma longa has wide range of essential application like food and pharmaceutical products, cosmetics and textile industries. As agriculture and pharmaceutical are concerned it is indeed necessary to focus on plant growth, rhizosphere micro-organism and soil health. These factors are largely depending upon biological features. Stimulating effect of microorganisms is complicated and could be related to the interaction between microorganism and plant root. Certain metabolic pathways in the plant are induce by the infection with micro-organisms and can satisfy the nutritional requirement of the plant. Major activity of the chemical constituents include significant antibacterial, anti-inflammatory, antioxidant, antipyretic, antiseptic, antifungal, anticancerous capabilities

The present study is carried out by the antibacterial activity of rhizospheric bacteria of *Curcuma longa* producing metabolites against human bacterial pathogens.

Material and Methods

Sample Collection:

Soil samples were collected from rhizospheric region of *Curcuma longa* plant located in different region in Akola city western Vidharbh region of Maharashtra. Total 22 rhizospheric soil samples were collected in the sterilized polythene bag containing soil sample were transfer immediately to laboratory.

Isolation of Bacteria:

The collected Rhizospheric soil samples of *Curcuma longa* were weight 1 gm aseptically and immediately transfer to 9 ml saline suspension that is called as Stock culture. After the rhizospheric soil was added to prepare stock solution further Serial dilution method was performed to get reduce number of bacteria. Dilution was made up to 10^{-8} to reduce the load of bacteria for better isolation of colonies. After inoculating and incubation period different colonies were observed on Nutrient agar plates and Selective medium plates. Colony characteristics were observed and noted. Single colony was streak on nutrient agar slant for the isolation of pure culture.

Isolation of crude extracts producing antimicrobial substances:

For the isolation of antimicrobial crude extract the test bacterial sample was inoculated in nutrient broth fermentation medium & incubated at 37^0c for 48 hrs. Generally the antimicrobial substances produced by bacteria in their maximum stationary phage so after incubation period. The fermented broth was then treated to separate the biomass from broth. The broth was then centrifuged at 4000 rpm on for 15 minutes and then subjected to extraction with ethyl acetate by solvent extraction procedure equal volume of ethyl acetate was added to the filtrate and mixed well by vigorous shaking for 10 minutes. Tubes were allowed to settle for 5 minutes till two clear immiscible layers are formed. The upper layer containing the extracted compounds was separated and collected in another tube. This filtrate extract was evaporated to dryness in hot air oven. The extract residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4^oc to be used as stock solution for antimicrobial assay.

Antibiogram test:

Microorganisms are found in their natural habitat and are in constant exposure of undesirable chemicals, which may have antimicrobial activity against various microbes other than itself. To check the resistivity or sensitivity of a microbe against the various pathogens antibiotic sensitivity test is used to perform. This test is also termed as Antibiogram test. Nutrient agar plates were prepared. 20 µl of selected test pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*) were spread on to the solidified nutrient agar plates. Three wells were made at appropriate distance onto the agar plate with the help of gel puncture and filled using different concentration like 25 µl, 50µl and 75 µl of the bacterial isolates' broth extracts obtained from different strains. Petri plates were incubated at 37ºC for overnight (Malviya, T. and Pandey, A. 2014). Then the diameter of the zone of inhibition was measured in mm and noted. The antimicrobial activity was determined by measuring the clear zone around the wells.

Identification:

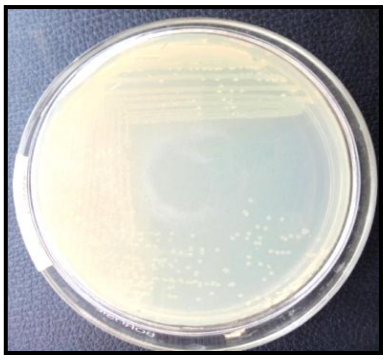
Different techniques and tests were performed such as Simple staining, Gram staining, Endospore staining, Motility, Acid fast staining, Biochemical Test, Sugar Fermentation Glucose, Lactose, Mannitol, IMViC test, Indole test, Methyl Red test, Vogas proskauer test, Citrate utilization test, Enzymes test Catalase, Oxidase, Urease, NO3 Reduction, H_2S Production, Starch hydrolysis, etc. for the identification of potent isolates (Pandey, A. and Singh, A. 2013).

Results and Discussion

Isolation, Purification and characterization of rhizospheric soil sample of *Curcuma longa.*

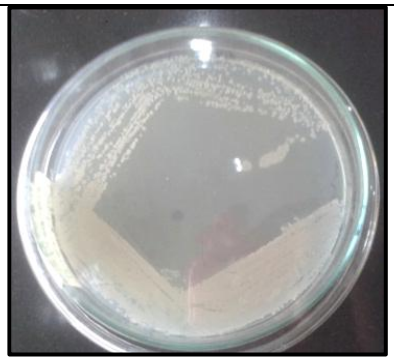
Soil samples of the *Curcuma longa* rhizosphere regions were collected from the different region in Akola City, Western Vidharbh region of Maharashtra. The bacterial culture from the soil samples were collected by the serial dilution and spread plate technique. The total 22 culture have been isolated from the soil samples and out of total 22 only 3 have been characterized which are potent isolates.

Fig: Showing results of isolated colonies on different selective media



(Fig 1). PIKOVASKY'S AGAR

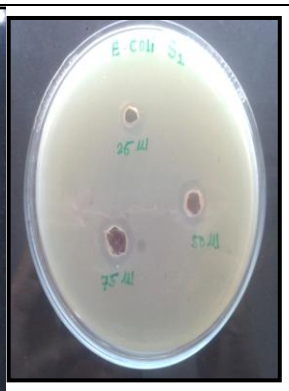
(Fig 2). KING'S B MEDIA



(Fig 3). KING'S B MEDIA

(Fig 4). ISOLATED PURE CULTURE IN FORM OF SLANTS

RESULTS SHOWING ZONE OF INHIBITION AGAINST PATHOGENIC BACTERIA



(Fig 5). Against *E.coli*

(Fig 6). Against *S.aureus*

(Fig7).Against *P.aeruginosa*

Table: 1: Antimicrobial activity of S1, S2, and S3 at different concentration

Test bacterial strains	Concentration /Diameter (µl/mm)								
	S1			S2			S3		
	25µl	50µl	75µl	25µl	50µl	75µl	25µl	50µl	75µl
E.coli	8	15	16.5	12	14	15	12	14	15
S.aureus	11	14	15	12	16	17	13	16	16
P.aeruginosa	12	13	16	11	12	14	10	12	14

- Abbreviations : S1- Sample No.1
  S2- Sample No.2
  S3- Sample No.3

Table 2: Morphological & Biochemical Characteristics

Sample	Gram Character	Motility	Endospore	Acid Fast	Sugar fermentation			IMVIC				Enzymes			No3 Reduction	H_2S prouction	Starch hydrolisis
					Glucose	Lactose	Mannitol	Indol	MR	VP	Citrate	Catalase	Oxidase	Urease			
S1	+	+	+	NA	+	+	–	–	NA	_	+	+	–	+	–	–	+
S2	–	NA	NA	NA	+	–	+	+	–	–	+	+	+	–	NA	–	+
S3	+	NA	NA	NA	+	–	–	NA	NA	–	NA	NA	NA	–	NA	+	+

Where, +: Positive, - : Negative, MR: Methyl red, S1S2S3: Sample Numbers
VP: Voges Proskaur, NA: Not applicable

On the basis of cultural, Morphological and Biochemical characteristics.

The potent isolates were identified by using Bergey's manual of systematic Bacteriology.

S1: ***Bacillus megatherium,*** **S2*: Pseudomonas fluorescence,***

S3*: Globicatella sulfidifaciens*

Discussion

Rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots (Grayston *et al*. 1996). In general, the nutrient-rich rhizosphere is naturally colonized by many beneficial or pathogenic bacteria which may have a considerable impact on plant growth, development and productivity. The numerous interactions between bacteria and roots may have beneficial, harmful or neutral effects on the plant, the outcome being dependent on the type of symbiotic interaction and the soil conditions.

In the present study, medicinal plant *Curcuma longa* has been selected, the rhizospheric region have been targeted to take the soil sample. The rhizosphere is the region adjacent to the plant root. The root exudates and the secondary metabolites secreted by the micro flora of the soil may affect each other and also to the plant health. There are total 22 cultures were isolated from these soil samples in which only 3 cultures have been screened. These 3 isolates are active against the selected pathogens, *E. coli, P. aeruginosa* and *S.aureus.* The characterized 3 cultures were *Bacillus megatherium, Pseudomonas fluroscenes and Globicitella sulfidifacein*. To characterize these cultures, Bergey's manual has been followed. According to this, gram's staining, catalase test, endospore test, acid fast staining, glucose fermentation test, mannitol fermentation test, lactose fermentation test, citrate utilization test, Oxidase test, glucose oxidation test, and nitrate reduction test have been performed. The analysis of antibiogram of the entire characterized isolates has been observed before identification against selected pathogens. This has shown the drastic change in the activity of the isolates. The potent isolates were found able to produce metabolites on the basis of their specificity and hence the metabolites have shown the many fold increment in the activity of the isolates. Further, the isolates have been tested for the activity to inhibit the growth of the selected human pathogens by antibiogram test (Malviya, T. and Pandey, A. 2014).

There are two potent isolates have been found, which has shown the best activity against selected pathogens. Those isolates are S1 (*B.megatherium*) has shown zone of the inhibition 16.5 mm against *E. coli* and S3 (*G.sulfidifaceins*) has shown zone of the inhibition 16 mm against *S.aureus*. The S2 is the much more potent culture in comparison to other one. This culture has shown the best result against *S. aureus* in contrast to other pathogens. Between both the potent isolates, the S2 culture has the maximum activity against all the selected pathogens. This has been observed by comparing all the isolates activity of *Curcuma longa* rhizospheric soil sample.

Conclusion

The present study was an attempt to identify and pick out the versatile bacterial strains that display antimicrobial activity against variety of microbial pathogens intrinsically. Total 22 cultures were isolated from rhizospheric region of *curcuma longa* out of 3 were potent isolates characterized as *Bacillus megatherium, Pseudomonas fluorescence, Globicatella sulfidifaciens.*

The Rhizospheric bacterial crude extract of *Bacillus megatherium, Pseudomonas fluorescence, Globicatella sulfidifaciens* were found to be more or less active against almost all tested pathogenic strains. Hence *Curcuma longa* can be employed as source of natural antimicrobials that can serve as an alternative to conventional medicines. It was concluded that the best activity have been shown by the *Curcuma longa* rhizospheric isolates (S2) which is of *Pseudomonas fluorescens* against all three human pathogenic organisms *(E.coli , S.aureus , P.aeroginosa*). The activity of rhizospheric isolates was showing best results against *S. aureus*

The result of this study strongly supports that the bacterial isolates produces metabolites and may be used in the management of microbial infection and the present findings highlights the important for further investigation towards the goal of obtaining novel antimicrobial agent.

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