ISOLATION AND CHARACTERIZATION OF ROOT ASSOCIATED BACTERIA FROM *CURCUMA LONGA* PLANT AND ITS ANTIBACTERIAL ACTIVITY

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Abstract:

Those microorganisms which grow in, on or around plant root and influence healthy plant growth and yield are known as plant growth promoting Rhizobacteria (PGPR). These microorganisms can act and parasitize on other harmful condition or microorganisms populations by antagonistic behavior. Curcumin, the active ingredient of turmeric, is known for its antioxidant, anti-inflammatory, anti-fatigue, antiparasitic , antiallergic, anti-microbial, anti-mutagenic and anticancer properties. It exhibits wide therapeutic potential due to the multi targeting nature against variety of different cancers. Microbes are also associated with medicinal plant roots which have antimicrobial activity. This study involves isolation and characterizations of root associated bacteria with their applications in therapeutics and used of this potential novel isolate for potential PGPR activities. After bacterial isolation, primary screening was done by observe inhibition against four different well known pathogens (*E. coli*, *S. aureus*, *B. cereus*, and *P. aeruginosa*).

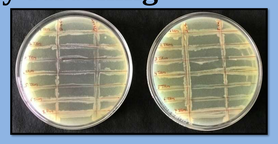
1. Introduction:

Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents . Different plant parts are used for medicinal purposes i.e., bulb, gel, leaves, roots, barks, peels etc. The use of plants to treat illness is found throughout human culture . The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds **[1].** *Curcuma Longa* commonly known as turmeric is traditionally used as a spice in Indian food. A wide range of biological activities e.g. anticancer, antimicrobial, anti-inflammatory and free radical scavenging activity of the plant suggests a logical basis for its traditional use in foodstuff. Various phototherapeutic uses of *Curcuma Longa* have been reviewed. *Curcuma Longa* Linn. Syn. *Curcuma Domestica* Val. (Family: *Zingiberaceae*) has been used as an ethanomedicine from time immemorial in Ayurvedic system and is also used as a dietary spice and colouring agent. Turmeric is used as a colouring agent to dye wool, silk and unmordent cotton. It is used as an antacid, carminative, stomachic, blood purifier wound healing and anti-inflammatory in Indian medicinal system. Turmeric was the second most active spice among 23 spices studied for antioxidant activity**[2]**. The active constituents of turmeric are the flavonoid Curcuminoids which is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bisdesmethoxycurcumin Curcumin makes up approximately 90% of the curcuminoid content in turmeric. Other constituents include sugars, proteins, and resins. The best researched active constituent is curcumin, which comprises 0.3-5.4% of raw turmeric. Turmeric is comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. The Curcumin is a lipophilic polyphenol that is nearly insoluble in water but is quite stable in the acidic pH of the stomach. Water and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity, comparable to vitamins C and E. A study of ischemia demonstrated that curcumin pretreatment decreased ischemia-induced changes in the heart. Curcumin is anti-inflammatory properties and therapeutic benefit have been demonstrated for a variety of gastrointestinal disorders, including dyspepsia, *Helicobacter pylori* infection, peptic ulcer, irritable bowel syndrome, Crohn's disease, and ulcerative colitis**[3]**.This study will involve isolation and characterizations of root associated bacteria with their applications in therapeutics.

2. Materials & Methods:

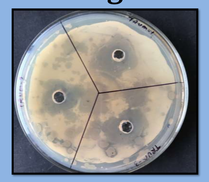
1. Isolation and characterization was completed with spread plate technique by using Nutrient agar medium.
2. Screening for inhibition assays was completed by streak plate and agar well diffusion methods by using Nutrient agar medium.
3. For PGPR activity test was completed by streaking method by using Pikovskayas agar and Lowenstein-jensen medium.
4. Comparison of antimicrobial activity of methanolic extraction with isolated bacteria
5. DNA sequencing

Results : Preliminary screening



Secobndary screnning

E.coli

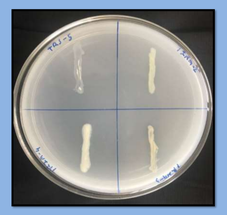


S. aureus

B.cereus

PGPR activity test

Phosphate solubilization



Nitrogen fixation

3. Discussion

The main source of the isolation of bacteria is Rhizoplane of the root of the *Curcuma longa* plant. There are two types of root samples were collected (Mature & Immature). There are successfully different types of bacteria were isolated on nutrient agar medium from two types of root sample as mature and immature and also isolated from soil sample (rhizosphere) from the *curcuma longa* plant roots there are 19 well isolated colonies found from the sample.There are four types of pathogenic organisms were used for the primary sceening (*E.coli, S.aureus, P. aeruginosa, B.cereus)* to check the antimicrobial activity of the isolates. Preliminary screening was carried out by cross streaking inhibition method and there are 5 isolates from the soil sample and 4 isolates of the root sample were gives antimicrobial activity against pathogen which are inhibit the growth of pathogens. Secondary screening was done by agar well diffusion method and there 3 isolates (TRIM 2, TRIM 3, TRIM 4) of immature root were gives zone of inhibition against *E.coli, S.aureus and B.cereus.* from mature root sample 2 isolates (TRM 2 & TRM 3) were gives positive results against pathogens. There are no any of positive results against *P.aeruginosa.* In PGPR activity test ,there are sort outed from secondary screening out of 4 isolates 1 isolate(TRIM3) gives zone of clearance on the phosphate solubilization and nitrogen fixation test. After that, by biochemical tests results compared with the Bergy's Mannual of identification and confirmed that the bacteria were gram negative and from the genus "*Enterobacter"*. Then, DNA sequencing report confirmed that sample of bacterial culture was *Pantoea spp.* from family of Erwiniaceae which are recently separated from the genus Enterobacter.

4. Summary

The aim of this study to development of new antibiotics to overcome the problems associated with the existing antibiotics and used of this potential novel isolate for potential PGPR activity. Microbes are generally interact with plant root for carbon source. Continuous interaction of microbes to plant root make them resistant against chemical secreted by that plant. *Curcuma longa* commonly known as turmeric is traditionally used as a spice in Indian food. Curcumin is known for its antioxidant, anti-inflammatory, anti-fatigue, antiparasitic, antiallergic, anti-microbial, anti-mutagenic and anticancer properties. Isolation of root associated bacteria from *curcuma longa* plant was carried out on the nutrient agar medium. Turmeric have very good ability to reducing tumors and excellent capacity to fight againt cancer. *Curcuma longa* is best known for the medicinal applications and different methods like as cross streaking and agar well diffusion method and methanolic root extract from that result there is gram negative *" Enterobacter"* were found in the study. Efforts are now being made to investigate mechanism of action of some of these plants using model systems.

5. References

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