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The Isolation and Molecular Identification of Bacterial and Fungal Species from Potato Fields of Indore District of Madhya Pradesh

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ABSTRACT

The microorganisms of rhizosphere play an important role in development of healthy plant. The rhizosphere is the specific fine region of soil that is directly in contact with soil microorganisms and significantly influenced by root secretions. In present study we have studied the microflora of Potato fields of Indore district of Madhya Pradesh. The sampling of soil from rhizosphere using a soil corer with a diameter of 3 cm at a depth of 0–20 cm and sampling of plant root materials as rhizoplane were collected three times: during seedling stage, peak of vegetative growth and fruiting of potato (October/November to February/March) from potato fields of Indore district of Madhya Pradesh. The samples were serial diluted and streaked over the Nutrient Agar and PDA media Plates for the isolation of Bacterial and Fungus. For the identification of Cultured bacterial and fungal species were further analyzed by morphological and Molecular analysis using PCR and sequencing of 16S and ITS region of Bacteria and Fungus species. We reported various types of bacterial (*Pseudomonas fluorescens*, *Pseudomonas aerogenosa*, *Bacillus subtilis*, *Pseudomonas putida etc.)* and fungal species (Species of Penicillium and Trichoderma etc.) which are required for the development of healthy plant and protection of potato plant from other pathogens.

Keywords: Bacterial species, Fungal species, Molecular identification, 16S Gene.

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INTRODUCTION:

The current human population of our planet is around 7.7 billion as reported up till July, 2019 (Worldometer, n.d.) and it is increasing drastically every second. India is the second leading country in the world in terms of population statistics and majority of its inhabitants belongs to rural area. It's a natural requirement of every individual to receive a meal twice daily. For this most of the people around the globe depends on the staple food crops, viz. rice, wheat and maize in order to meet their daily needs of hunger. Following these cereals comes the fourth) most important and staple food crop worldwide i.e. Potato (Solanum tuberosum)[1]. The bacterial and fungal species of rhizosphere play a crucial role in development of healthy plant.

The rhizosphere is the specific fine region of soil that is directly in contact with soil microorganisms and significantly influenced by root secretions. The word rhizosphere originates from a Greek word 'rhiza', meaning root. It was for the first time when Lorenz Hiltner coined and described the term 'rhizosphere'. He called it as the area around the roots which is populated by diversified micro-organisms present in the soil who gets affected by the exudates of root system. The exudates include both active and passive releases. The active ones are called secretions and the passive ones are called diffusates. While rhizosphere is a combination of endorhizosphere, rhizoplane and ectorhizosphere.specifically is the surface area of root which is in direct contact with soil[2]. This region, Rhizosphere, thus is a unique environment and an encouraging habitat for the growth and multiplication of a number of micro-organisms[3]. In present study our aim to the isolation and identification of microflora of Potato fields of Indore district of Madhya Pradesh using morphological ,biochemical and molecular techniques for the further use of these for the management of other disease in Potato crop.

MATERIALS AND METHODS

In present study the soil samples from rhizosphere and plant root materials as rhizoplane were collected. The sampling were done three times: during seedling stage, peak of vegetative growth and fruiting of potato (October/November to February/March) from potato fields (Depalpur, Sanwar and Indore) of Indore district of Madhya Pradesh. A total of 27 field soils (rhizosphere) and 27 samples of plant root materials (rhizoplane) from three different locations. The collected samples were divided into two parts, one part of each samples were analyzed for bacterial analysis remaining second part of fungal identification. The serial dilution method was opted for the soil bacterial samples inoculation on Nutrient Agar Medium, appeared bacterial colony were further subjected to streaked on Nutrient Agar Medium for pure culture. Morphological characterization (Cell Size, shape, Gram staining, Arrangement, Motility and Staining of capsule) of pure culture were done and for further validation, we have isolated the genome from Bacteria and fungus using Phenol chloroform and CTAB methods.

We have performed PCR for the amplification of 16S and ITS region of Bacteria and Fungus species using universal primers and PCR product subjected to DNA sequencing.

Table 1: Universal Primer sequence used in present study are given below

Primer	Sequence	PCR product	Reference
16S rRNA Fw	AGAGTTTGATCMTGGCTCAG	919 bp	4
16S rRNA Rw	CCGTCAATTCATTTGAGTTT		
ITS1 Fw	TCCGTAGGTGAACCTGCGG	620 bp	5
ITS4 Rv	TCCTCCGCTTATTGATATGC		

The sequences of DNA were further analyzed using basic local alignment tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for identification and Clustal W software was used to get genetic similarity.

RESULTS AND DISCUSSION

In present investigation we have successfully isolated and prepared pure culture of bacterial species of rhizosphere and rhizoplane of potato fields of bacterial species from Depalpur, Sanwar and Indore tehsils of Indore district of Madhya Pradesh. The isolated bacterial colonies were subjected to obtain pure culture, which were successfully achieved as given in figure 1.

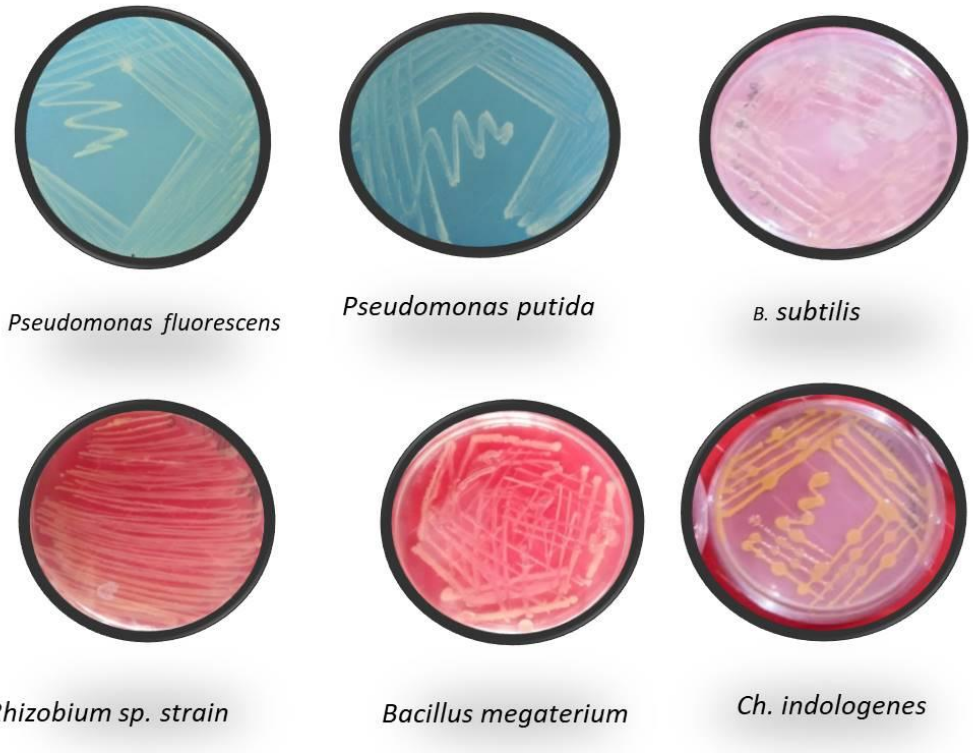

Figure 1: The figure showing selected images of isolated bacterial pure cultures during present study.

We further validate the pure culture molecularly as given in figure 2, We have sequenced all the PCR products and obtained sequence were used for the blast on NCBI database for the accurate Identification.

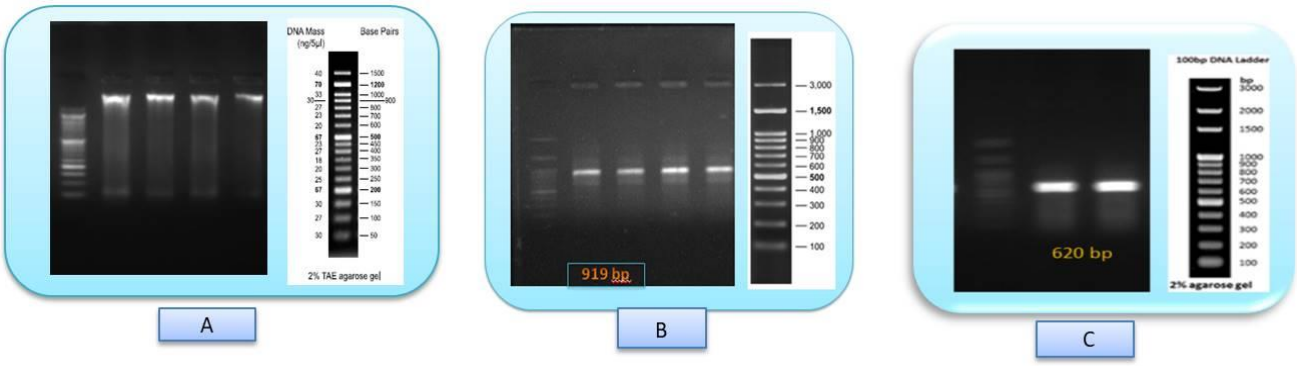

Figure 2: Figure A showing genome of Bacteria and Fungus, Figure B showing PCR amplification of 16S RNA and Figure C, showing PCR amplification of ITs gene of Fungus.

On the basis of aforementioned study various types of bacteria species (*Chryseobacterium indologenes, Pseudomonas fluorescens, Pseudomonas aerogenos, Pseudomonas aerogenosa, Bacillus subtilis, Bacillus subtilis, Pseudomonas putida, Brevibacillus brevis, Bacillus megaterium, Rhizobium daejeonense, Bacillus endophyticus*) were reported from fields of Potato of Indore district.

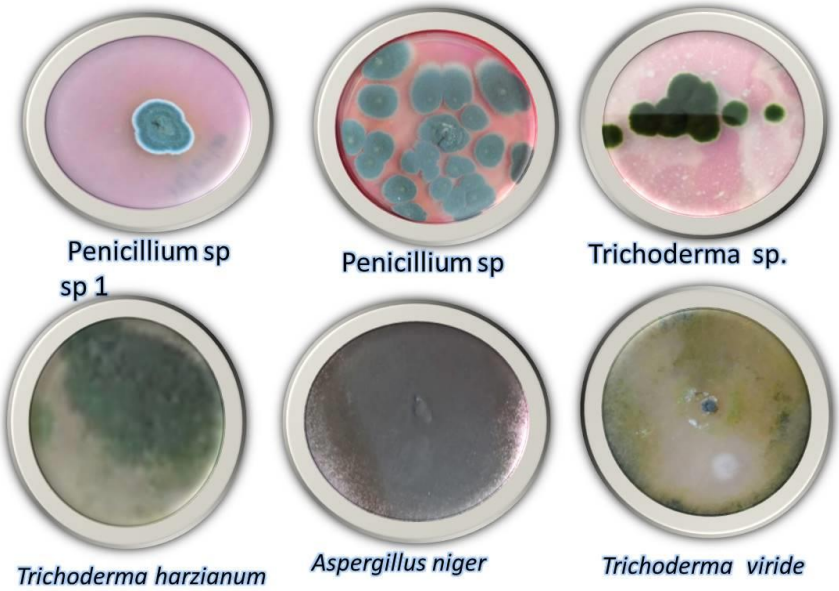

Figure 3: The figure showing selected images of isolated fungal pure cultures during present study.

On the other hand we have reported fungal species ,also prepared their pure culture (figure 3) and were morphologically and molecularly validated and identified as *Penicillium sp 1, Penicillium sp 2, Trichoderma viride, Trichoderma harzianum, Aspergillus niger, Myrothecium verrucaria.*In present investigation we have reported microbial species which showed antagonistic effectagainst plant pathogens[6].

CONCLUSION:

Present investigation has confirmed the various types of isolates from selected genera of Bacillus and Pseudomonas, and Trichoderma in fungus, which have higher antagonistic activity against plant pathogens.

REFERENCES

1. Spooner DM, Solanum tuberosum (Potatoes). Brenner's Encyclopedia of Genetics (Second Edition),2013 ;481-483.
2. HiltnerL, Uberneuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berucksichtigung der Grundungung and Brache," Arbeiten der Deutschen Landwirt schaft lichen Gesells chaft, 1904; 59-78.
3. Das B B, Rajak N, Nakhro N, Dkhar, M S, Rhizosphere microflora of potato as affected by organic treatments. Agricultural Journal,2010; 5(3),181-185.
4. Lane D, J In E, Nucleic acid techniques in bacterial systematics. John Wiley, Chichester, England, 1991; p. 115–175.
5. Hite TJ, Bruns T, Lee S, Taylor JW,Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. San Diego, CA: Academic Press;PCR protocols: a guide to methods and applications,1990.
6. Mardanova AM, Hadieva GF, Lutfullin MT, Khilyas IV, Minnullina LF, Gilyazeva AG, Bogomolnaya LM, Sharipova MR Bacillus subtilis strains with antifungal activity against the phytopathogenic fungi. Agr. Sci. 2017; 8: 1–20.