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Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan

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The present work deals with the isolation and characterization of microorganisms from polyhouse agriculture soil of Jharna village (Rajasthan). Several bacteria and fungi were isolated from polyhouse soil, using serial dilution method. These bacterial isolates were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella* sp., *Proteus mirabilis, Bacillus anthracis, Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis* species which were further identified on the basis of colony morphology, Gram staining, biochemical tests and using selective and differential media. Identification of fungi such as *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Trichoderma* sp. and *Rhizopus* sp. was carried out by culturing on potato dextrose and sabouraud's dextrose agar media and microscopic method. Microorganisms play an important function in biodegradation of solid agriculture waste and also help in the crop production.

Key words: Bacteria, fungi, agriculture, polyhouse.

INTRODUCTION

Microorganism are frequently present in soil, manure and decaying plant tissues which are able to degrade wastes that are correlated with the substrate organic matter (Alexander, 1977). Agriculture soil is a dynamic medium in which a large number of pathogenic and non-pathogenic bacterial and fungal flora live in close association. Microbes in the soil are the key to carbon and nitrogen recycling. Microorganisms produce some useful compounds that are beneficial to soil health, plant growth and play an important role in nutritional chains that are important part of the biological balance in the life in our planet (Paul and Clerk, 1966; Kummerer, 2004). Polyhouses are basically naturally ventilated climate controlled. Polyhouse cultivation has been evolved to create favorable micro-climates, which favours the crop production and could be possible all through the year or part of the year as required. Wherein, off season crops are also grown under a favorable controlled environment and other conditions viz. temperature, humidity, light intensity, ventilation, soil media, irrigation, fertigation and other agronomical practices throughout the season irrespective of the natural conditions outside. Therefore, the soil of polyhouse may have different conditions for growth of microorganism than natural environment. The present study aimed to isolate effective microorganisms that are present in polyhouse soil and find out the optimize culture

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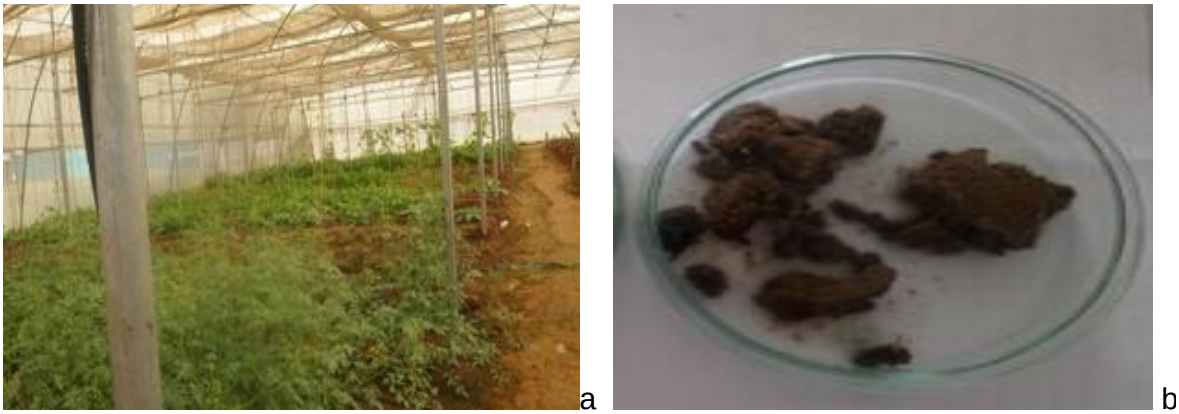

Figure 1. (a) and (b) Collection of soil sample from polyhouse of Jayoti Vidyapeeth Women's University, Jaipur.

conditions.

MATERIALS AND METHODS

Collection of soil samples

Agricultural soil sample were collected from polyhouse of Jayoti Vidyapeeth Women's University campus, located at East Longitude-75° 27' 38", North Latitude-26° 49' 34", 450-500 m above sea level, weather: Temperature - 28- 34° C in summer, 12°- 18° C in winter, Jaipur, Rajasthan (Figure 1). Soil samples were taken with the help of sterile spatula, in sterile plastic bags. The samples were brought to the microbiology laboratory.

Determination of physiochemical properties of soil

Freshly collected soil samples were taken for determination of physiochemical properties. The moisture content of the sample was measured in a hot air incubator at 105°C to constant weight. The pH, temperature, humidity, and air pressure was determined using digital pH meter, thermometer, hygrometer and barometer, respectively (Pramer and Schmidt, 1964; Iyengar and Bhave, 2005).

Isolation of microorganism

The microorganisms were isolated by serial dilution technique on Potato Dextrose Agar (PDA) and Nutrient Agar Media (NAM). In this technique, a sample suspension was prepared by adding 1.0 g sample to 10 ml distilled water and mixed well for 15 min and vortexed. Each suspension was serially diluted 10^{-1} to 10^{-6}. 0.1 ml was pipetted onto plates with PDA and NAM media, spread with a glass spreader and incubated at 28°C for fungal and 37°C for bacterial observation. Each colony that appeared on the plate was considered as one colony forming unit (cfu) (Waksman, 1927; Nazir, 2007).

Identification of fungi

The fungal isolates were identified by morphological examination and its characteristics. Morphological characteristics were examined under microscope (Onion et al., 1981).

Optimization of culture condition for fungi

For the determination of optimum condition of isolated fungi, three media were used (Potato dextrose agar media, Sabouraud's dextrose agar, Czapack Dox agar). The media were adjusted to pH 4 to 7. For optimization of the incubation period, the culture plates were incubated at 28°C for 4 to 7 days (Bhattacharyya and Jha, 2011; Azzaz et al., 2012).

Identification and characterization of Bacteria

Gram staining was performed to check the morphology of the cells and spore chain morphology was identified by spore staining technique. The pure culture was grown on nutrient agar medium and transferred to Mac-conkey agar medium, EMB agar medium, Endo-Agar medium and mannitol salt agar medium for differentiate and identified bacteria The plates were incubated at 37°C in the incubator and readings were taken 24 h after inoculation. The bacterial isolates were biochemically characterized by catalase test, oxidase test, urease test, motility test, TSI test, nitrate reduction test and IMVIC test (Collins and Lyne, 1989; Harold, 2002; Zaved et al., 2008).

RESULTS AND DISCUSSION

This study revealed that polyhouse soil samples were analyzed with respect to different types of bacteria and fungi. The common bacteria, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella* sp., *Proteus mirabilis, Pseudomonas aeruginosa, Bacillus anthracis, Bacillus subtilis,* and *S. epidermidis* are dominating species in the soil samples. Similarly, when the soil samples were tested for different types of fungi, *Aspergilllus niger, Aspergillus flavus, Trichoderma* sp., *Fusarium oxysporum, Rhizopus* sp. were dominating species in soil samples. The physiochemical properties of soil play an important role in the growth of microorganism. The polyhouse soil was slightly acidic. The humidity, moisture content, and temperature were 35, 45.5 and 32°C, respectively (Table 1).

In the present study, the isolated fungi were identified on the basis of cultural, microscopic and morphological characteristics (Figure 2). Earlier work reported that for maximum growth of fungi, potato dextrose agar was most favorable (Maheshwari, 2000). The isolated fungi were

Table 1. Physiochemical properties of soil samples.

Physiochemical properties of soil	Observation
pH	6.5 ±1.0
Temperature	32^0C
Humidity	35% Fair
Moisture content	45.5 %
Air pressure	743 mb

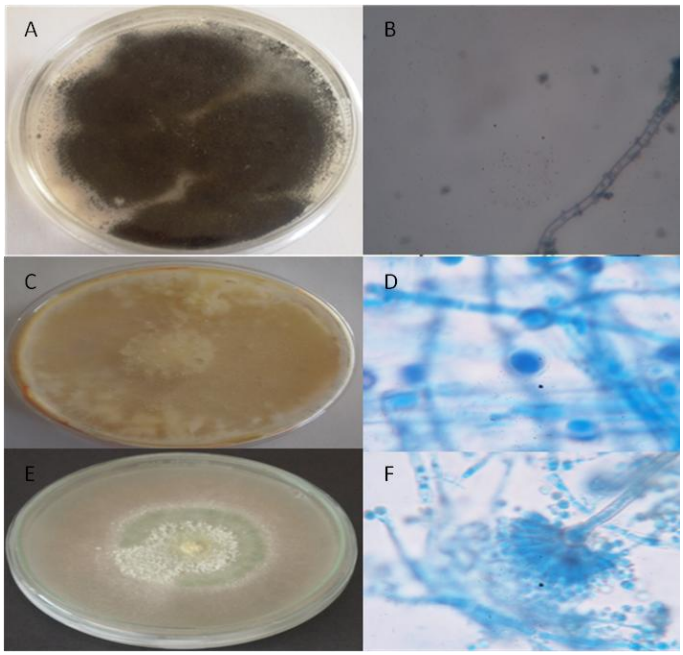

Figure 2. Identified fungi and their microscopic image. A, B; *Aspergillus niger*, C, D; *Fusarium* sp., E, F; *Aspergillus flavus*.

cultured in PDA, SDA and Czapeck Dox agar media. It was observed that the PDA and SDA media were most suitable for good growth of *A. niger*, *A. flavus* and *Trichoderma* sp., *Rhizopus* sp. and *Fusarium oxysporum* at optimum range of pH 6 to 8 and a suitable incubation period was 4 to 7 days, respectively. However, czapeck dox agar medium was also suitable for *A. niger, A. flavus*, *Trichoderma* sp., *Rhizopus* sp. and *F. oxysporum*. Most of the fungi show favorable growth in alkaline side of neutral pH but they can also tolerate the pH range from 6 to 8 (acidic to basic) (Table 2). The enzymatic activities and use of bacteria, actinomycetes and fungi in the decomposition of organic matter provides beneficial metabolic products to the soil (Tiquia et al., 2002; Singh et al., 2003). Similarly, Karthik et al. (2011) also reported the isolation, identification of microorganisms such as *Bacillus* species from agricultural waste dump soil for screening of pectinase producing activity. The isolation of various fungal, bacterial species showed that the agricultural soil is quite rich in microbial flora. In agriculture process, soil microorganisms such as bacteria and fungi may play important roles in soil fertility and in the form of loss and gain in the production of grains, fruits, vegetables. Moreover, it also helps to maintain or enhance the environment quality and conserve natural resources. Most of the bacteria such as *Bacillus anthracis*, *B. subtilis*, and *S. aureus* and *S. epidermidis* isolated in this study have been reported by other researchers (Amir and Pineau, 1998; Okoh et al., 1999). Several bacteria were isolated from agriculture soil samples. Identification and characterization of isolated bacteria were performed by morphological, microscopically (Figure 3), biochemical tests such as shape, arrangement, colonies, temperature, growth, indole production test, ethyl red and Voges-proskauer test, citrate utilization test, urease test, catalase test, Mac-conkey's test, TSI test, growth at 37°C (Table 3). Further, selective and differentiate media were prepared and the isolated bacteria were inoculated under sterilized conditions incubated at 37°C for 18 to 24 h and the results were recorded (Table 4). In different fermentation media, isolates showed different level of growth. The growth of *S. aureus* was observed on mannitol salt agar medium. In the presence of high salt concentration, *S. aureus* fermented mannitol producing acid which changes the pH turning phenol red to yellow (Figure 4). The present study, concluded on isolation and identification of bacterial and fungal floras with optimum pH, temperature and other cultural condition. These isolated polyhouse soil bacteria and fungi are also used in the seasonal and off season crop production with different environment condition. These microorganisms are to supply nutrients to crop, to encourage plant growth; for example, through the production of plant hormones and to control or inhibit the activity of plant pathogen. This study provides knowledge on microorganisms of Rajasthan semi arid soil which is used in polyhouse and produce seasonal and off seasonfoods and vegetables which are not able to grow in normal environment condition.

Conclusion

The environment where we live is the habitat for various microorganisms; mostly bacteria and fungi which are used for various industrial applications like enzymes production, fabric manufacturing, bioremediation, pharmaceutical production, etc. Micro-organisms play an important role in composting of organic waste and can be an important contributor to optimal agricultural waste. This study revealed the isolation and identification of diversity of microorganisms which are present in agricultural soil habitat.

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Table 2. Effect of pH and incubation period on the growth of isolated fungi on PDA, SDA, Czapek - Dox agar media at 28°C temperature.

pH of the Medium	Isolated fungi	PDA Media				SDA Media				Czapek Dox agar Media			
		Incubation period (days)				Incubation period (days)				Incubation period (days)			
		4	5	6	7	4	5	6	7	4	5	6	7
6	*Aspergillus niger*	+1	+2	+3	+4	+2	+2	+3	+4	+2	+3	+4	+4
	Aspergillus flavus	+1	+2	+3	+4	+1	+2	+3	+4	+2	+2	+3	+4
	Trichoderma sp.	+2	+3	+4	+4	+2	+3	+4	+4	+2	+2	+3	+4
	Fusarium oxysporum	+1	+1	+2	+3	+1	+2	+3	+4	+2	+3	+3	+4
	Rhizopus sp.	+1	+2	+3	+4	+1	+2	+3	+4	+1	+2	+3	+4
7	*Aspergillus niger*	+2	+3	+4	+4	+2	+3	+4	+4	+2	+3	+4	+4
	Aspergillus flavus	+2	+2	+3	+4	+2	+3	+3	+4	+1	+2	+3	+4
	Trichoderma sp.	+3	+3	+4	+4	+2	+3	+4	+4	+1	+2	+3	+4
	Fusarium oxysporum	+1	+2	+3	+4	+1	+2	+3	+4	-	-	-	-
	Rhizopus sp.	+1	+2	+3	+4	+1	+2	+3	+4	+1	+1	+2	+3
8	*Aspergillus niger*	+1	+2	+3	+4	+2	+2	+3	+4	-	+2	+3	+4
	Aspergillus flavus	+2	+2	+3	+4	+1	+2	+3	+4	-	+1	+2	+3
	Trichoderma sp.	+3	+3	+4	+4	+2	+3	+3	+4	-	+2	+3	+4
	Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-	-	-
	Rhizopus sp.	+1	+1	+2	+2	+1	+1	+1	+2	-	-	+2	+3

- No growth, +1 poor growth, +2 moderate growth, +3 good growth, +4 massive growth.

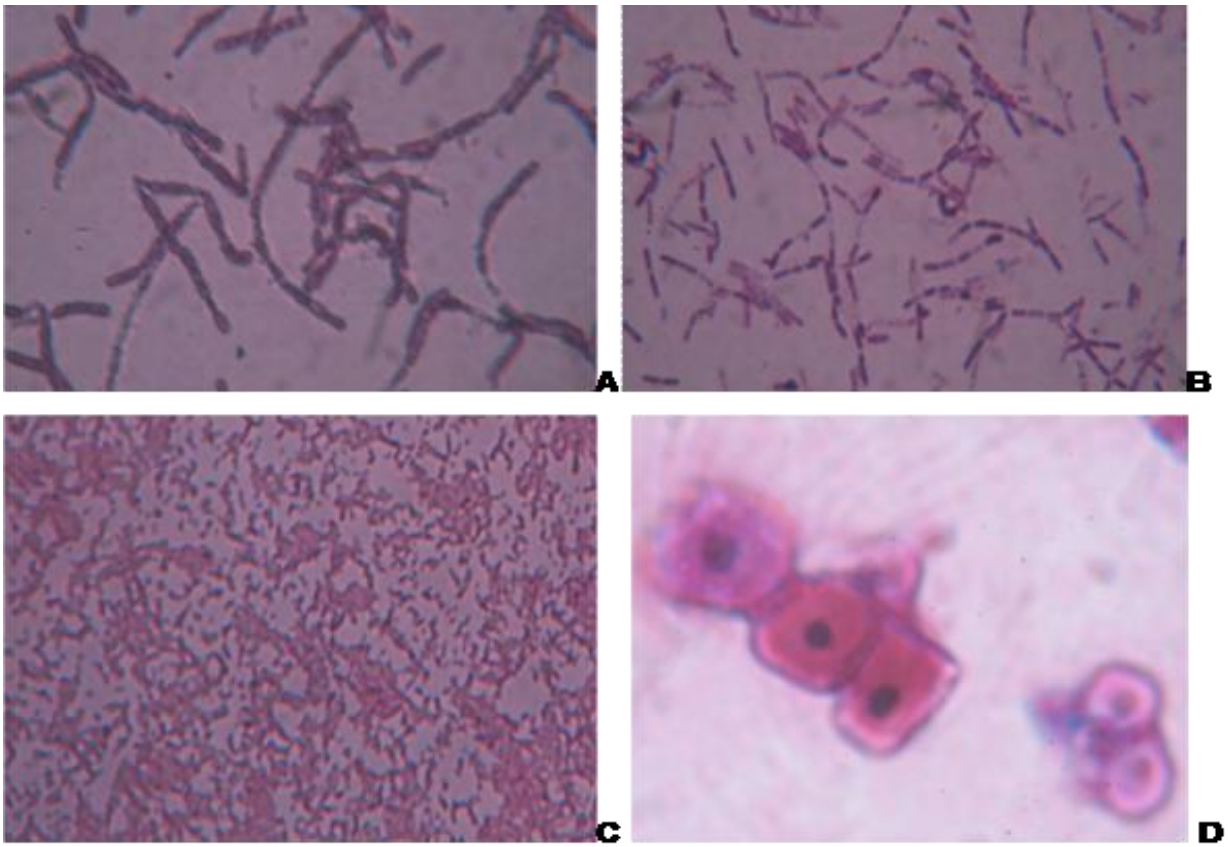

Figure 3. Microscopic images showing A. *Bacillus anthracis,* B. *Bacillus subtilis* C. *Pseudomonas aeruginosa*, D. *Klebsiella pneumoniae*.

Table 3. Biochemical characterization of bacterial isolates.

Identified bacteria	Gram stain	Oxidase	Catalase	Nitrate reductase	VP	MR	Ind	Citrate	Motility	Urease
E. coli	-ve bacilli	-ve	+ve	+ve	-ve	+ve	+ve	-ve	Motile	-ve
Klebsiella pneumoniae	-ve bacilli	-ve	+ve	+ve	+ve	-ve	-ve	+ve	Non motile	+ve
Enterobacter aerogenes	-ve, bacilli	-ve	+ve	+ve	+ve	-ve	-ve	+ve	Motile	+ve
Shigella sp.	-ve rods	-ve	+ve	+ve	-ve	+ve	-ve	-ve	Non motile	-ve
Proteus mirabilis	-ve, bacilli	-ve	+ve	+ve	-ve	+ve	-ve	-ve	Motile	+ve
Pseudomonas aeruginosa	-ve, bacilli	+ ve	+ve	+ve	-ve	-ve	– ve	+ve	Motile	-ve
B. anthracis	+ve, bacilli	-ve	+ve	-ve	+ve	-ve	-ve	-ve	Non motile	+ve
B. subtilis	+ve, bacilli	-ve	+ve	-ve	+ve	-ve	-ve	+ve	Motile	+ve
Staphylococcus aureus	+ve , cocci in clusters	-ve	+ve	-ve	-ve	-ve	-ve	-ve	Non motile	+ve
Staphylococcus. epidermidis	+ve , cocci	-ve	+ve	-ve	-ve	-ve	-ve	-ve	Non motile	+ve

+ve Positive, -ve negative, VP: Voges- Proskauer test, MR: Methyl Red test, In: indole test.

Table 4. Growth of bacterial isolates on different media.

Identified bacteria	Mac- Conkey agar media	EMB agar media	TSI agar media
E. coli	LF	Metallic sheen	A/A, Gas (+), H_2S (-)
Klebsiella pneumoniae	LF	Dark	A/A, Gas (+), H_2S (-)
Enterobacter aerogenes	LF	Dark	A/A, Gas (-ve), H_2S (-)
Shigella sp.	NLF	Colorless	A/ALK Gas (-), H_2S (-)
Proteus mirabilis	NLF	Colorless	A/Alk, Gas (+), H_2S (+)
Pseudomonas aeruginosa	NLF	Light	Alk /no change, Gas (-), H_2S (-)

LF: Lactose fermentation, NLF: lactose non fermentation, A: yellow slant or butt, Alk: slant or butt red.

Figure 4. (A). *Staphylococcus aureus* and (B). *Staphylococcus epidermidis* on mannitol salt Agar media.

REFERENCES

Alexander M (1977). Introduction to Soil Microbiology. 2nd Edition. John Wiley & Sons, New York. pp. 423-437.

Amir H, Pineau R (1998). Influence of plants and cropping on micro-biological characteristics of some new Caledonian Ultramafic soils. Aust. J. Soil Res. 36:457–470.

Azzaz HH, Murad HA, Kholif AM, Hanfy MA, Abdel Gawad MH (2012). Optimization of culture conditions affecting fungal cellulase production Res. J. Microbiol. 7:23-31.

Bhattacharyya PN, Jha DK (2011). Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a subsurface *Aspergillus* strain TSF 146". Int. J. App. Biol. Pharma. Technol. 2:133 – 143.

Collins CH, Lyne PM, Grange GM (1989). Collins and Lyne Microbiological methods, 6th Edition. Butterworth, London.
Harold JB (2002). Microbiological Applications. Laboratory Manuals in General Microbiology, 8th Edition. McGraw - Hill Higher Education.
Iyengar S, Bhave PP (2005). In-vessel composting of household wastes. Waste Management, pp.1-11.
Karthik JL, Kumar G, Rao Bhaskara KV (2011). Screening of pectinase producing microorganisms from agricultural waste dump soil. Asian J. Biochem. Pharma. Res. 2:329- 337.
Kummerer K (2004). Resistance in the Environment. J. Antimicrob. chemother. 45:311- 320.
Maheshwari R, Bharadwaj G, Bhat MK (2000). Thermophilic fungi: Their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64:461-488.
Nazir N, Mirza JH, Akhtar N, Bajwa R, Nasin G (2007). Some studies of thermophilic and thermotolerant fungi from Lahore, Pakistan. Mycopath. 5:95-100.
Okoh LA, Badejo MA, Nathaniesl IT, Tian G (1999). Studies on the bacteria, fungi and springtails (collembola) of an agroforestry arboretum in Nigeria. Pedobio. 43:18-27.
Onion AHS, Allsop D, Eggins HOW (1981). Smiths introduction to industrial mycology. 7th Edition. Edward-Arnold Publishers Ltd. London p.398.
Paul EA, Clark FE (1996). Soil Microbiology and Biochemistry. 2nd Edition. New York. Academic press. pp. 225-229.
Pramer DE, Schmidt EL (1964). Experimental soil microbiology burges publishing company Minneapolis, 15. Minnesota, USA.Sons Ltd. Beecles and London. pp. 31-32.
Singh A, Sharma S (2003). Effect of microbial inocula on mixed solid wasted composting, vermicomposting and plant response. Compost Sci. Uti. 11:190-199.
Tiquia S, Wan J, Tam N (2002). Microbial population dynamics and enzyme activities during composting. Compost Sci. Util. 10:150-161.
Waksman SA (1927). Principles of soil microbiology, Batimore; William & Wilkins co.
Zaved HK, Rahman Mizanur M, Rahman Mashir M, Rahman A, Arafat SMY, Rahman MS (2008). Isolation and characterization of effective bacteria for solid waste degradation for organic manure KMITL Sci. Tech. J. 8:44-55.