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Research Article

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Bioprospecting of bacteria from less explored ecosystem

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ABSTRACT

In this present study, for the bioprospecting aspects, bacteria isolated from shoal forest soils, Western Ghats were explored. In total 32 bacteria was isolated and screened for biological activities. Out of 32 isolates, Enzymatic activities of bacterial isolates shows 62.5% positive for protease, 28.1% for amylase and glutaminase, 65.6% for urease, 68.7% for agarase, 90.6% for pectinase, 12% for lipase and 31.5% for invertase. Biodegradation of bacterial isolates shows 40.6 % of isolates showed positive results for Malachite green, 31.2% showed positive results for crystal violet and 34.3% showed positive results for saffranin degradation. 40.6 % isolates also showed phenol degradation. Plant growth promoting substance of bacterial isolates, 21.8%, 28.1% and 15.6% shows positive results for ammonia, acetoin and phosphate solubilization, 15.6% isolates produced indole acetic acid and 18.7% isolates shows positive for N_2 fixation.

Keywords: Bioprospecting, enzyme, biodegradation, plant growth and forest

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INTRODUCTION

Bioprospecting is the exploitation, extraction and screening of biological diversity and indigenous knowledge for commercially valuable genetic and biochemical resources [1]. Bioprospecting is more frequently used to describe the collection and screening of biological material for commercial purposes [2]. The bioprospecting of plants and living organisms for pharmaceutical purpose is useful have been criticized at several forms. The biological task can be achieved by the development of competitive mechanisms such as production of toxins, enzymes and antimicrobial agents like antibiotics [3, 4].

Microbial derived enzymes are highly valued in many industrial applications. Enzymes are usually required in small amounts and can be easily obtained by manipulation of the microbes. Enzymes are often used in the processing of food and beverage, paper and pulp, textile, animal feed, detergent, cosmetic and chemical synthesis processes [5].

Forest is the natural ecosystem which is characterized by a wealthy biological diversity, millions of plants, animals and microorganisms in their natural environment [6]. Forest are increasingly important sources of valuable pharmaceuticals and other bioactive products and each different rain forest is a source for different bioactive compounds. Different organism exists within the forest layers. These organisms interact with each other and their surroundings. Each organism has a role in the sustaining the eco system. [7]. Western Ghats is one of the18-biodiversity hotspot in the world and totally unexplored from microbiological point of view [8]. Western Ghats have an average elevation of 1200 meters.

The harmful microbes and beneficial microbes compete with each other to survive in soil and other medium. To regulate the beneficial microorganisms and to prevent the harmful microorganism using bioactive compound of beneficial organisms is our motto. Isolating microorganisms of a different ecosystem to find new compounds. Forest is characterized by a wealthy biological diversity, millions of plants, animals and microorganisms [9]. In this scope and further, to understand the activity of biological compounds, we initiated the study on screening of microbial bioproducts from bacteria isolated from, Western Ghats.

EXPERIMTAL SECTION

2.1 Collection of sample and Isolation of Bacteria

In this present study, for the isolation of bacteria, soil samples were collected from the Shola forest, Kodaikanal, Western Ghats, Tamilnadu. All the samples were collected aseptically using sterile polythene bags and transport to the laboratory. About one gram of soil sample was diluted in 100 ml of sterile distilled water blank (10^{-2} dilution) and further serially diluted 10^{-3},10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilution using 9ml sterile distilled water blank . Nutrient agar was used for isolation of bacteria. After sterilization, filter sterilized nystatin 20µg/ml was added to the medium, to prevent fungal growth. About 0.1ml of aliquot from 10^{-5}, 10^{-6}, 10^{-7} dilution was plated on nutrient agar plates. Plating was done in triplicate and all the plates were incubated at 28ºC for 48hrs [9]. After incubation, morphologically different colonies were selected for further studies

2.2 Screening of forest bacteria for extracellular enzymatic activity

All the bacterial isolates were screened for various enzymes such as Amylase, Protease, lipase, invertase, pectinase, agarase, glutaminase and urease by Plate Method. All the isolates were spot inoculated on respective enzyme screening media and incubated at 28ºC for 5-7days. [10].

Enzyme	Media	Method of detection	Reagents used
Amylase	Starch Agar	Zone of clearance	1% iodine
Protease	Skim Milk agar	Zone of clearance	-
Lipase	Tween 80 agar	Opaque zone	1% cuso4
Invertase	Minimal agar+1% sucrose	Growth	-
Pectinase	Minimal agar+1% pectin	Growth	-
Urease	Christensonºs urea agar	Pink colour	-
Agarase	Artificial agar medium	Zone of clearance	1% Iodine
Glutaminase	Nutrient agar+1%glutamine	Pink color	-

2.3 Screening for Biodegradation activities:

Dye degradation: All the bacterial isolates are tested for dye degradation using different dyes such as Crystal violet, Saffranin and Malachite green by Plate method. All the bacteria were spot inoculated into screening medium supplemented with Crystal violet (0.01%), Malachite green (0.01%), Saffranin (0.01%) in respective plate and incubated at 28ºC for 5 days. Clear zone around the bacterial spot indicates dye degradation [11].

Phenol degradation: All the bacterial isolates were screened for phenol degradation. The isolates were spot inoculated on mineral agar medium supplemented with phenol (0.05%) as sole carbon source. The plates were incubated at 28ºc for 5 days. Presence of growth on the mineral agar medium indicates phenol degradation [12].

2.4 Effect of Sodium Chloride on growth of bacteria

To study the effect of sodium chloride, bacterial isolates, were inoculated onto nutrient agar medium supplemented with different concentrations of NaCl(0-10%). All the plates were incubated at 28ºc for 5days and observed for every 24 hrs. [10].

2.5 Screening for plant growth promoting substances

Acetoin production: The Voges-Proskauer test was used as a qualitative method for the detection of acetoin, a precursor for 2,3 butanediol. All the bacterial isolates were inoculated on each 2ml of MR-VP broth and incubated at 28ºc for 5 days. After incubation, to the 1ml bacterial culture 3ml of freshly prepared 5% α-Napthol in absolute ethanol and 1ml of 40% KOH were added and the mixture was stirred vigorously. The formation of a red colour is indicative of the presence of acetoin [13].

Ammonia Production: For the screening of ammonia production, all the bacterial isolates were inoculated into each 5ml of sterile peptone broth and incubated at 28ºc for 48 hours. After incubation, about 0.5ml of Nessler's reagent was added into all the tubes. The production of ammonia is indicated by the appearance of pink colour.

Phosphate solubilization: All the isolates were screened for phosphate solubilization by the method described [14]. All the bacterial isolates were spot inoculated on Pikovoskaya's agar plates and incubated at 28ºc for 5-7days. After incubation the plates were observed for clear halo formation around the bacterial growth for every 24hours.

Nitrogen fixing bacteria: Nitrogen fixing activity of bacterial isolates was studied by inoculating them into Jensen medium and incubating them into 5 days for 28ºc. Presence of growth on Jensen medium indicates nitrogen fixation.
Indole acetic acid (IAA): For the screening of IAA production, all the bacterial isolates were inoculated into each 5ml of sterile nutrient broth supplemented with 3mg/ml of L-tryptophan and incubated at 28ºc for 5 days. After incubation, cell free supernatant was obtained by centrifuging at 10,000rpm for 10min and to that a drop of orthophosphoric acid and 2ml of Solwaski's reagent was added and kept for 20min at room temperature. The development of pink colour indicates the production of IAA.

2.6 Preliminary screening for biological activity

Antimicrobial substances from bacterial isolates were produced by submerged fermentation using fermentation medium. For the preparation of 18hrs cultures, Nutrient broth was prepared and all the bacterial isolates were inoculated and incubated at 28ºc. For the production of antimicrobial compound, 10% inoculum were transferred into fermentation medium and incubated in rotary shaker at 280C for 120 hours. Cell free supernatant was prepared by centrifuging fermentation medium for 10,000 rpm for 10minutes. Antimicrobial activity of culture supernatant was tested by agar well diffusion method using nutrient agar medium. Test bacterial strains used in this study include *Staphylococcus aureus, Bacillus spp, klebsiella, Pseudomanas spp, E. coli*. [9].

2.7 Antibiotic susceptibility test for bacterial isolates

Antibiotic susceptibility pattern for bacterial isolates were determined by Kirby-Bauer disc diffusion method [15]. 18 hrs broth cultures of bacterial isolates was prepared in nutrient agar and adjusted to 0.5 McFarland standards. All the cultures were inoculated into nutrient agar plates using sterile cotton swab. Standard antibiotic disc streptomycin, vancomycin, trimethoprin, bacitracin were placed on nutrient agar plates and incubated at 37°C for 24 hours. After incubation antibiotic susceptibility pattern was determined by measuring zone of inhibition.

RESULTS AND DISCUSSION

Isolation of Bacteria

Totally 32 morphologically different bacterial colonies were selected from forest soil samples after incubation. All the strains were selected for further studies based on their fast growth. Maruthu *et al*., [16] isolated microorganisms from Kodaikannal hills and studied antimicrobial activity of isolated microorganisms. Many reports are there for forest ecosystem from various plants but there is less report of Western Ghats.

Characterization of bacterial isolates

In the microscopic characteristics studies, out of 32 isolates, 16 gram negative rods, 5 Gram Positive cocci and 11 gram positive rods were present. Totally 5 isolates showed positive results for Endospore staining and they were suspected as *Bacillus sp.* [10]. Gayathri *et al*., isolated Gram positive, gram negative organisms with spore producing bacteria from his studies.

Enzymatic activity of Bacterial isolates

Enzymatic activities of bacterial isolates shows majority of isolates exhibit positive activity. Maximum number of strain showed positive activity, Protease (62.5%), Amylase (28.1%), Glutaminase (28.1%), Urease (65.6%), Agarase (68.7%), Pectinase (90.6%), Lipase(12%), Invertase (31.5%). Number of bacteria showing different enzymatic activities are given in fig 1.

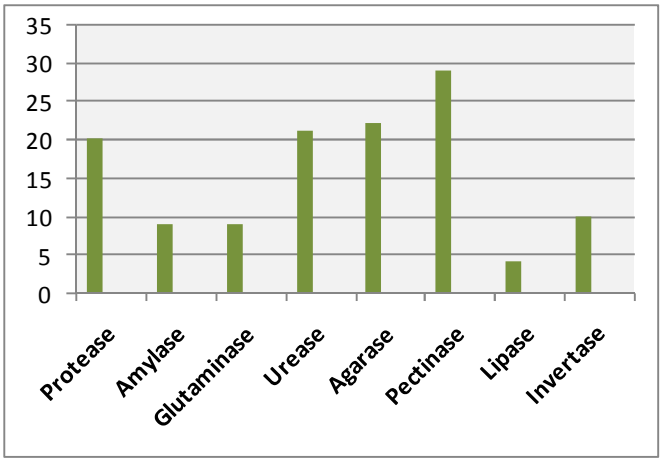

Figure 1. Number of bacterial isolates showing enzymatic activity

Biodegrading activity of Bacteria

Out of 36 isolates 12 strains showed degradation against malachite green degradation and 20 strains showed phenol degradation [10]. In this study, out of 32 isolates tested, 40.6 % (13 strains) of isolates showed positive results for Malachite green, 31.2%(10 strains) showed positive results for crystal violet and 34.3%(11 strains) showed positive results for saffranin degradation. 40.6 % (13 strains) isolates also showed phenol degrading activity. (Fig 2)

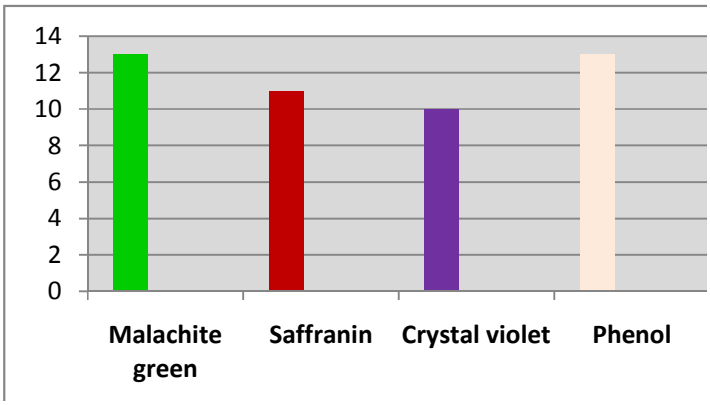

Figure 2. Number of bacterial isolates showing biodegradation activity

Effect of Sodium Chloride on the growth of bacteria

All the 32 isolates showed good growth at 0% to 5% NaCl concentration. 26 isolates showed good growth upto 5% NaCl concentration and 20 isolates showed growth at 7.5% NaCl concentration and 12 isolates showed good growth at 10% NaCl concentration. The growth rate is decreases when the NaCl concentration is increased.

Plant growth promoting substances

Plant growth promoting substances of bacterial isolates in this study shows positive results. Out of 32 isolates 7 (21.8%), 9 (28.1%) and 5(15.6%) shows positive results for ammonia, acetoin and phosphate solubilization. Seven isolates (15.6%) produced indole acetic acid and 6 (18.7%) isolates shows positive for N_2 fixation.(Fig 3)

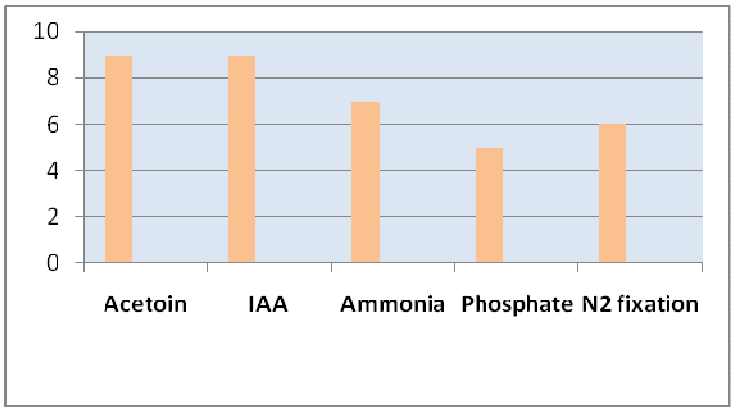

Figure 3. Number of bacterial isolates showing Plant growth substances

Antimicrobial Activity

A great part of the compounds are in the group of antibiotics. 60, 30 and 5% of the compounds are inhibiting Gram positive, Gram negative and mycobacteria, respectively. Majority of the antibiotic compounds are active against Gram positive bacteria [17]. Berdy et al. However in this present study, out of 32 isolates tested, 20 showed inhibition against *Bacillus sp*., 11 showed inhibition against *S. aureus*, 5 showed against *Klebsilla sp*., 7 showed against *Pseudomonas sp*., and 6 showed against *E.coli*.

CONCLUSION

The present study concludes that bacteria isolated from less explored ecosystem region were potential for pharmaceutical and environmental application. Out of 32 isolates maximum strains shows positive for enzyme production, antimicrobial activity and producing plant growth promoting substance. The present study proves that forest ecosystem with less explored are the sources of bacteria with bioprospecting potential which deserves further studies.

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REFERENCES

[1] P Kumar; N Tarui, *Bridging scales and epistemologies conference*, **2004**, 17-20.
[2] M Synnes, *Clean Techn. Environ. Policy*, **2007**, 9, 53-59.
[3] S Courtis; C Capellano; M Ball; F Fracois; G Helynck; A Marutuzez; S Kolvek; J Hopke; M Osburne; P August; R Nalin; M Guerineau; P Jeannin; P Simonnet, *J. Prenodet. Appl. and Environ.*, **2003**, 69, 49-55.
[4] E Gillespie; S Brady; A Bettermann; N Cianciotto; M Lies; M Clardy; R Goodman; J Handelson, *Appli. and Environ. Microbial*. **2002**, 68, 4301-4360.
[5] A Ting; D Cruz; HY Chan, *J. of App. Biosci.*, **2008**, 3, 67-75.
[6] AR Srividhya; GS Saritha; B Suresh, Indn. *J. of pharma. Sci.*, **2009**, 70(6), 812-815.
[7] AD Azaz; O Pekel, *Turk. J. Bot*., **2002**, 26, 409-416.
[8] YJ Megha; R Alagawadi; PU Krishnaraj, *Karnataka J. Agric. Sci.*, **2007**, 20(2), 305-309.
[9] D Saravanan; S Bharathi; M Radhakrishnan; R Balagurunathan, *J. of Applied Pharm. Sci.*, **2012**, 2(3), 120-123.
[10] S Gayathri; D Saravanan; M Radhakrishnan; R Balagurunathan; K Kathiresan, *Indian J. of Biotechnol.*, **2010**, 9, 397-402.
[11] V Gopalakrishnan, M Radhakrishnan, R Balagurunathan. Microbial Degradation of Textile Dyes – An inventory, Natl Symp on Recent Trends in Microbial Biotechnolgy, Sri Sankara Arts and Science College, Kanchipuram, Tamilnadu, **2005**, 57-64.
[12] M Rigo; RM Algegre, *Folio Microbiol*, **2004**, 49, 41-45.
[13] RV Houdt; P Moons; MH Buj; CW Michiels, *J. of Bacteriol*, **2006**, 188, 1570-1572.
[14] A Pandey; N Das; B Kumar; K Rinu; P Trivedi, *World J. of Biotechnol.*, **2006**, 24, 97-102.

[15] AW Bauer; M Kirby; JC Sherris; M Turck, *Am. J. Clin. Pathol.*, **1966**, 45, 493-497.
[16] R Marudhu; MK Balakrishnan; AD Cynthiya; M Manisankar; M Panimalar; T Raghuraman; RSG Karthikeya, *Current Science*, **2007**, 726-727.
[17] J Berdy, *The Journal of Antibiotics*, **2012**, 65, 385–395.