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SCREENING OF MICROORGANISMS FOR ANTIMICROBIAL PROPERTY FROM THE LACHHIWALA RESERVE FOREST OF HIMALAYAS – A BIODIVERSITY HOTSPOT

Nidhi Srivastava, Ahongshangbam Ibeyaima and Indira P. Sarethy*

Department of Biotechnology, Jaypee Institute of Information Technology A-10, Sector-62, Noida-201309, India.

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***Corresponding Author**
Indira P. Sarethy
Department of Biotechnology, Jaypee Institute of Information Technology A-10, Sector-62, Noida-201309, India.

ABSTRACT

Lachhiwala Reserve Forest, a subtropical deciduous forest of the biodiversity-rich Himalayas in India, is an under-explored forest for microbial diversity studies. This study was undertaken with the objective of characterization of microbial diversity and investigates their potential for production of antimicrobial and other compounds. Microorganisms were isolated from the study area on different media (Nutrient agar, Potato Dextrose agar and Glucose Yeast Extract), amended with antibiotics, and further characterized for antimicrobial activity against Gram positive and negative bacteria and the yeast *Saccharomyces cerevisiae*. Production of extracellular enzymes (protease, lipase, amylase and cellulase) was also studied. A total of sixty eight distinct bacterial and colonies of bacteria and fungi were obtained. UK-201, UK-204, UK-205, UK-207, UK-208, UK-217, UK-220, UK-222 and UK-223 showed broad-spectrum antimicrobial activity against bacteria and yeast. Results show that this habitat harbors a diverse microbial population. Isolation using amended media resulted in a larger diversity of cultivable microbial population. The selected isolates are being studied for taxonomic novelty.

KEYWORDS: Multidrug-resistant; natural product; actinomycetes; antimicrobial.

INTRODUCTION

Unrestricted use, easy availability and self medication of antibiotics has lead to an increasing threat of multidrug-resistant (MDR) pathogens, which are non responsive to existing antibiotics.[1] Therefore, new drug discovery is urgently needed to counter and reverse the

expansion of antibiotic resistant pathogens.[2] Natural products have been a major source of therapeutic agents and approved drugs due to their vast structural diversity[3] although in recent times there has been less interest shown by large pharmaceutical companies.[4] However, it is becoming increasingly difficult to discover commercially significant bioactive compounds from well-known microorganisms as it leads to wasteful rediscovery of known compounds, hence there is a need to isolate, screen and characterize microbial isolates from under or unexplored habitats. Such habitats are found to be a rich source of novel microorganisms producing hitherto unknown bioactive compounds, including antibiotics.[5,8]

Forests are one such understudied habitat. Many studies have documented the microbial diversity in Amazon tropical forest,[9] evergreen forest,[10] agroforestry systems,[11] and tropical mangrove forest.[12] The Himalayan forests constitute an important part of the megabiodiverse hotspots of the world. Lachhiwala Reserve Forest is a subtropical deciduous forest in the Siwalik area of the Himalayas. It constitutes a part of the Doon valley of Uttarakhand state of India. The average maximum and minimum temperatures of the location are 27°C and 13°C respectively and the average annual rainfall is~200 cm.[13] The soil of this area is derived from the Doon alluvium, consisting of very fine clay and some quantity of sand.[13]

To our knowledge, no studies pertaining to microbial diversity have been reported from the Lachhiwala Reserve Forest. The major objectives of this study were to (i) characterise bacterial and fungal diversity of this forest and (ii) investigate their potential for production of antimicrobial compounds and other compounds of industrial interest.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from Lachhiwala Reserve forest, Dehradun (30.24º N; 78.08º E). The top 5cm of soil was collected in triplicates using a sterile scoop and placed in sterile polycarbonate bottles. The samples were stored at 4°C until analysis, within a maximum period of one week.[14] All experiments were carried out three times with three experimental replicates each. Mean and SD values of the results were generated after plotting on Excel version 2007.

Physicochemical characterization

The pH of the soil samples was determined following the method of Reed and Cummings (1945).[15] Electrical conductivity measurements were performed as per Avery and Bascomb (1982).[16] The organic carbon content of the soil was determined by using wet combustion method.[17]

Selective isolation and enumeration of microorganisms

Soil samples (10 g) were pooled and suspended in quarter strength Ringer's solution. Aliquots of each dilution were spread over Nutrient Agar (NA), Glucose Yeast Extract agar (GYE) and Potato Dextrose Agar (PDA) media. NA was used for isolation of bacteria. GYE was supplemented with 20 µg ml^{-1} Rifampicin to preclude growth of fast growing bacteria and favour that of slow growing actinomycetes. PDA was used for isolating fungi. Subsequently the plates were incubated at 37ºC (NA and PDA) and 30ºC (GYE) for 1-7 days. Total cultivable bacteria and fungi were counted and the results expressed as mean Colony Forming Units (CFU) per gram of soil.

Characterization

Morphological characterization

Distinct bacterial and fungal isolates were coded as UK series. Bacteria were Gram stained, visualised under microscope (OLYMPUS BX51) and their characteristics were noted. Actinomycetes were assigned to colour groups[18] and their morphological characteristics were recorded as per Shirling and Gottleib (1966).[19] Fungi were stained using Lactophenol Cotton Blue and observed under the microscope (OLYMPUS BX51).

Activity characterization

Antimicrobial activity

Isolates were further characterised for production of antimicrobial compounds. Agar plugs of the cultures were used to assess antimicrobial activity as per Bauer et al. (1966)[20] against the following panel of target microorganisms: *Bacillus subtilis* (MTCC-121), *Staphylococcus epidermidis* (MTCC-435), *Escherichia coli* (MTCC-1679) and *Saccharomyces cereviseae* (MTCC-151). These plates were kept at 4ºC for 2 hours for diffusion of any antimicrobial compound and then incubated at 37ºC for 24-48 hrs. The diameters of zone of inhibition were subsequently measured and expressed in cm.

Enzyme production

Agar plugs were inoculated and plate assays were used to assess production of protease,[21] lipase,[22] amylase,[23] and cellulase.[24]

RESULTS

Physicochemical characterisation of soil

Soil samples from Lachhiwala Reserve Forest were analysed for physicochemical parameters (pH, electrical conductivity and organic carbon) as shown in Table 1.

Table 1: Physicochemical characteristics of soil sample from Lachhiwala Reserve Forest, Dehradun.

Characteristics	Value (Mean ± SD)
pH	6.78 ± 0.43
Electrical conductivity (µs)	166.7 ± 0.21
Organic carbon (%)	1.4 ± 0.52

Selective isolation and enumeration of microorganisms

Microbial colonies were enumerated on three different media; NA, GYE and PDA (Table 2). Maximum number of colonies was obtained on NA. A total sixty eight distinct colonies were obtained in which thirty nine distinct bacterial colonies were on NA, twenty seven (Twenty two actinomycetes, one bacterium and four fungi) on GYE and two fungal colonies on PDA respectively. Maximum and minimum microbial diversity were obtained on NA and PDA respectively.

Table 2: Enumeration of microorganisms from Lachhiwala Reserve Forest on various media.

	CFU (g^{-1}) Mean ± SD	Number of distinct colonies
Nutrient Agar	$1.45x10^4$ ± 2.62	39
Glucose Yeast Extract	$6.3X10^2$ ± 1.24	27
Potato Dextrose Agar	$1.0\ X10^2$ ± 2.16	2

Fig. 1 Shows some of the isolates obtained on NA, GYE and PDA media.

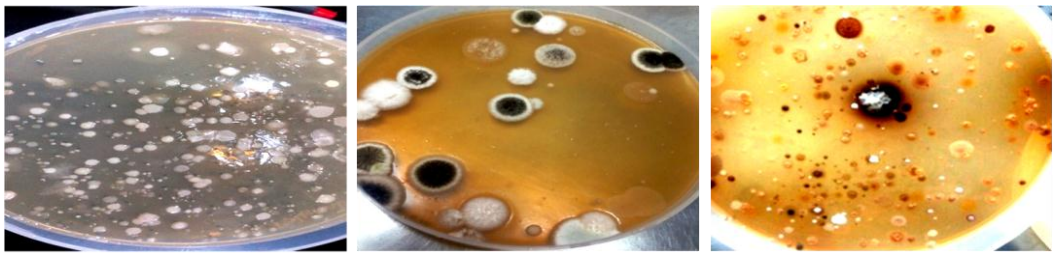


Fig. 1. Selective isolation of microorganisms on (a) nutrient agar (b) potato dextrose agar and (c) glucose yeast extract.

Morphological characterization

The colony characteristics and morphological features of the isolates were recorded. The characteristics of bacterial and fungal isolates are shown in Table 3 and that of actinomycetes in Table 4. It was observed that overall 59% isolates were bacteria, 32% actinomycetes and 9% isolates were fungi. In terms of morphology, 45% isolates were rods, 10% isolates were coccus, 4% were coccobacilli, 32% isolates were filamentous bacteria and 9% were filamentous fungi. All of the bacterial isolates were Gram positive. Cultural and sporulation pattern of isolates UK-400 and UK-401 (from PDA) showed that they belonged *Aspergillus* sp. UK-204, UK-205, UK-222 and UK-228 were unidentified fungi. All the actinomycetes isolates (twenty two) were Gram positive and with filamentous nature. Five actinomycete isolates belonged to white colour group, one belonged to red and eight isolates belonged to each yellow and brown colour group. Fourteen isolates had crystalline colony texture and remaining was leathery (Table 4). UK-201, UK-203, UK-217, UK-220 and UK-223 produced diffusible brown pigments.

Table 3: Morphological characteristics of bacterial and fungal isolates obtained from Lachhiwala Reserve forest.

Media	Isolate code	Colony Characteristics	Microscopic characteristics	
			Gram/Lactophenol Cotton Blue staining	Features
Nutrient Agar	UK-1000	Rounded regular shiny white large opaque	Gram positive	Large rods arranged in clusters
	UK-1001	Rounded regular centrally raised white opaque	Gram positive	Large rods, some in chains
	UK-1002	Rounded irregular margin cream large opaque	Gram positive	Large rods
	UK-1003	Rounded regular large cream opaque	Gram positive	Rods
	UK-1004	Rounded regular medium sized yellow	Gram positive	Rods, some in chains

		opaque		
	UK-1005	Rounded large regular shiny white opaque	Gram positive	Coccus
	UK-1006	Rounded regular large cream opaque	Gram positive	Large rods
	UK-1007	Rounded irregular large cream opaque	Gram positive	Rods with endospores
	UK-1008	Rounded regular large white opaque	Gram positive	Coccobacillus cells, some in chains
	UK-1009	Rounded regular white opaque	Gram positive	Large rods
	UK-1010	Rounded regular white translucent	Gram positive	Rods
	UK-1011	Rounded regular translucent	Gram positive	Coccobacillus
	UK-1012	Rounded regular white	Gram positive	Large rods
	UK-1013	Rounded regular cream opaque	Gram positive	Rods in chains
	UK-1014	Rounded regular white	Gram positive	Rods, some in chains
	UK-1015	Rounded regular medium sized opaque	Gram positive	Coccus
	UK-1016	Rounded irregular off white opaque	Gram positive	Rods in chains
	UK-1017	Rounded regular off white centrally opaque marginally translucent	Gram positive	Rods, some in chains
	UK-1019	Rounded regular cream small opaque	Gram positive	Large rods
	UK-1020	Rounded regular shiny cream	Gram positive	Very small rods
	UK-1021	Rounded irregular shiny cream	Gram positive	Rods
	UK-1022	Rounded regular medium sized light orange opaque	Gram positive	Coccus
	UK-1025	Regular rounded small translucent	Gram positive	Small rods
	UK-1026	Rounded regular opaque yellow small	Gram positive	Rods some in chains
	UK-1027	Rounded regular large translucent	Gram positive	Small rods
	UK-1028	Rounded regular medium sized cream opaque	Gram positive	Coccus
	UK-1029	Rounded irregular white opaque	Gram positive	Coccus
	UK-1030	Rounded regular cream large opaque	Gram positive	Rods
	UK-1031	Rounded regular small white translucent	Gram positive	Large rods
	UK-1032	Rounded regular cream medium sized opaque	Gram positive	Large rods
	UK-1033	Rounded regular cream opaque	Gram positive	Rods
	UK-1037	Rounded regular yellow opaque	Gram positive	Coccus
	UK-1038	Rounded regular small translucent	Gram positive	Coccobacillus
	UK-1039	Rounded regular medium sized white opaque	Gram positive	Rod
	UK-1040	Rounded regular small shiny white opaque	Gram positive	Small rod
	UK-1041	Rounded regular small shiny light pink opaque	Gram positive	Coccus
	UK-1042	Rounded regular small translucent	Gram positive	Rod
	UK-1043	Rounded regular small translucent	Gram positive	Rod
	UK-1045	Rounded regular medium sized translucent	Gram positive	Rod
Potato Dextrose Agar	UK-400	White mycelia black spores	Filamentous	*Aspergillus* sp.
	UK-401	White mycelia brown spores	Filamentous	*Aspergillus* sp.

Glucose Yeast Extract	UK-204	White aseptate mycelium, sporulation not observed	Filamentous	Fungus
	UK-205	White aseptate mycelium, sporulation not observed	Filamentous	Fungus
	UK-218	Rounded regular yellow shiny opaque	Gram positive	Rod
	UK-222	White septate mycelium, sporulation not observed	Filamentous	Fungus
	UK-228	White septate mycelium sporulation not observed	Filamentous	Fungus

Table 4: Morphological characteristics of actinomycetes obtained from Lachhiwala Reserve forest.

Colour group		Colony Characteristics			
	Isolate code	Aerial Mycelium	Substrate Mycelium	Diffusible Pigment	Texture
White	UK-206	White	Yellow	-	Crystalline
	UK-208	White	Dark brown	-	Leathery
	UK-211	White	Yellow	-	Crystalline
	UK-216	White	Yellow	-	Crystalline
	UK-229	White	Yellow	-	Crystalline
Red	UK-209	Orange	Yellow	-	Crystalline
Yellow	UK-202	Cream	Yellow	-	Crystalline
	UK-210	Cream	Dark yellow	-	Crystalline
	UK-213	Cream	Yellow	-	Crystalline
	UK-214	Cream	Yellow	-	Crystalline
	UK-221	Cream	Yellow	-	Crystalline
	UK-224	Cream	Yellow	-	Crystalline
	UK-225	Cream	Yellow	-	Crystalline
	UK-230	Cream	Yellow	-	Crystalline
Brown	UK-201	Light brown	Dark brown	Brown	Leathery
	UK-203	Light brown	Dark brown	Brown	Leathery
	UK-207	Light brown	Brown	-	Leathery
	UK-212	Brown	Dark brown	-	Leathery
	UK-215	Light brown	Yellow	-	Crystalline
	UK-217	Brown	Dark brown	Brown	Leathery
	UK-220	Brown	Brown	Brown	Leathery
	UK-223	Brown	Dark brown	Brown	Leathery

In Fig. 2, representative actinomycete isolates are shown.

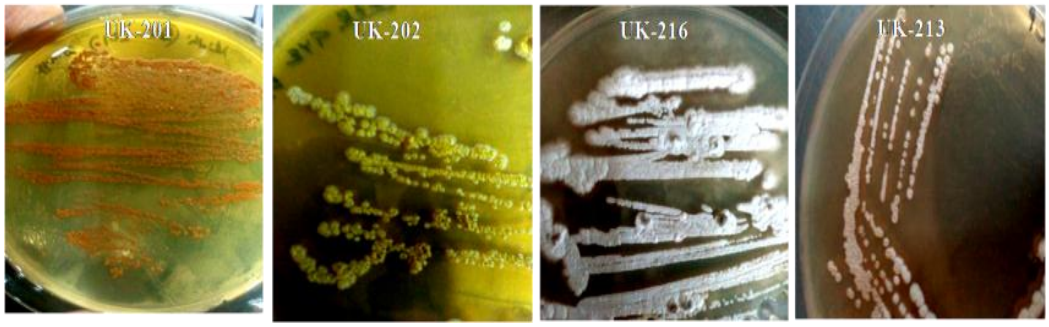


Fig. 2. Representative actinomycete isolates from Lachhiwala Reserve forest.

Characterization

Antimicrobial activity

Except for isolates UK-400 and 401 (*Aspergillus* sp.) the others were tested for antimicrobial activity (Table 5).

Table 5: Antimicrobial activity of isolates obtained from Lachhiwala Reserve Forest.

Media	Isolate code	Antimicrobial activity (Zone of Inhibition in cm)*			
		B. subtilis	*S. epidermidis*	*E. coli*	*S. cereviseae*
Nutrient Agar	UK-1000	+	+	+	++
	UK-1001	+	+	+	++
	UK-1002	+	++	+	-
	UK-1003	+	+	+	-
	UK-1004	+	+	++	+
	UK-1005	+++	++	+	-
	UK-1006	-	-	++	+
	UK-1007	++	+	+	+
	UK-1008	-	-	-	-
	UK-1009	+	-	++	++
	UK-1010	+	+	-	-
	UK-1011	-	-	-	-
	UK-1012	+	-	-	+
	UK-1013	++	-	+	+
	UK-1014	++	++	++	+
	UK-1015	-	++	-	-
	UK-1016	++	+	-	-
	UK-1017	-	+++	+	+++
	UK-1019	++	-	+	++
	UK-1020	+	-	-	-
	UK-1021	+	+++	-	-
	UK-1022	+	-	+	+
	UK-1025	++	++	+	+
	UK-1026	+	-	+	-
	UK-1027	-	+++	+	-
	UK-1028	+	-	+	-
	UK-1029	++	-	+	-

	UK-1030	+	-	+	-
	UK-1031	+	+	+	-
	UK-1032	-	+	-	-
	UK-1033	+	+	-	+
	UK-1037	-	-	-	-
	UK-1038	+	+	+	-
	UK-1039	-	-	-	-
	UK-1040	+	+	+	+
	UK-1041	+	-	+	-
	UK-1042	-	+	-	-
	UK-1043	+	+	-	+
	UK-1045	-	-	+	+
GYE	UK-201	+++++	+++++	++	+++++
	UK-202	-	-	-	-
	UK-203	++	+++	++	++++
	UK-204	++++	++++	++	+++
	UK-205	++++	++++	+++	++++
	UK-206	+	-	-	+
	UK-207	+++	++++	++	++++
	UK-208	+++	++++	+++	+++
	UK-209	-	-	-	-
	UK-210	-	++	-	-
	UK-211	-	+	-	-
	UK-212	++	++++	+	+++
	UK-213	+	+	-	-
	UK-214	+	++++	+++	++
	UK-215	+	++	+	++
	UK-216	+	++++	++	++
	UK-217	++++	+++++	++	++++
	UK-218	+	+	++	+
	UK-220	+++	+++++	+++	+++
	UK-221	+	+++	+++	+++
	UK-222	+++++	+++++	++	++++
	UK-223	+++	++++	++	+++
	UK-224	-	-	-	-
	UK-225	-	++	-	+
	UK-228	+	+++	+	+++
	UK-229	+++	+++++	-	++
	UK-230	-	-	-	-

**Ranges of zones of inhibition (in cm)*

- indicates 0

+ indicates 0.5-1.0

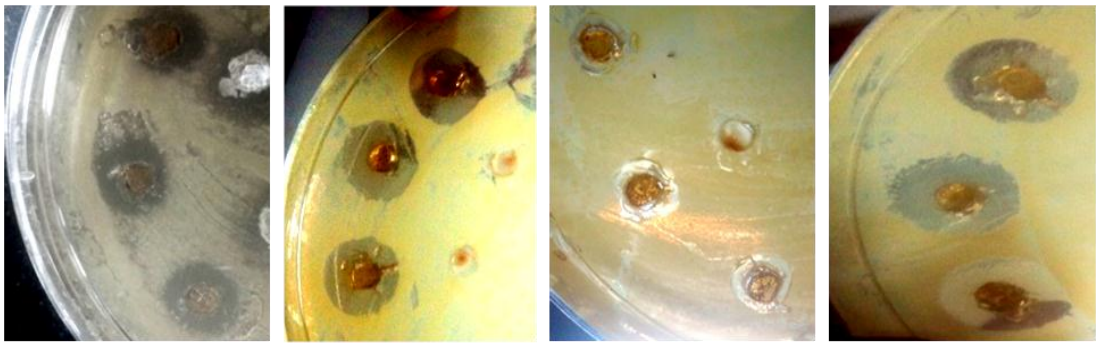
+ indicates 1.0-1.5

+++ indicates 1.5-2.0

++++ indicates 2.0-2.5

+++++ indicates 2.5-3.0

Fig. 3 Shows the antimicrobial zones of inhibition produced by isolate UK-201 against the selected panel of target organisms.



(a) (b) (c) (d)

Fig. 3. Antimicrobial activity of UK-201 against (a) *B. subtilis* (b) *S. epidermidis* (c) *E. coli* and (d) *S. cereviseae.*

Amongst the isolates obtained on NA, 85% isolates showed antimicrobial activity against Gram positive bacteria, 64% against Gram negative bacteria (*E. coli*) and 44% against yeast (Fig. 4). Broad spectrum antibacterial activity was observed in 59% isolates. Both antibacterial and antifungal activity was exhibited by 31% of the isolates (UK-1000, UK-1001, UK-1004, UK-1007, UK-1009, UK-1013, UK-1014, UK-1017, UK-1019, UK-1022, UK-1025 and UK-1040). Amongst the isolates obtained on GYE, 85% isolates showed antimicrobial activity against Gram positive bacteria, 63% against Gram negative bacteria, and 74% against yeast. Broad spectrum antibacterial activity and both antibacterial and antifungal activity was exhibited by 63% of the isolates. It is also clear from Fig. 4 that isolates regardless of whether isolated on NA or GYE media, exhibited similar levels of activity against Gram positive and negative target organisms. However more GYE isolates showed antifungal activity as compared to those from NA. A similar trend was observed with respect to isolates showing both antibacterial and antifungal activity (UK-201, UK-203, UK-204, UK-205, UK-207, UK-208, UK-217, UK-218, UK-220, UK-222, UK-223 and UK-228) obtained on GYE plates.

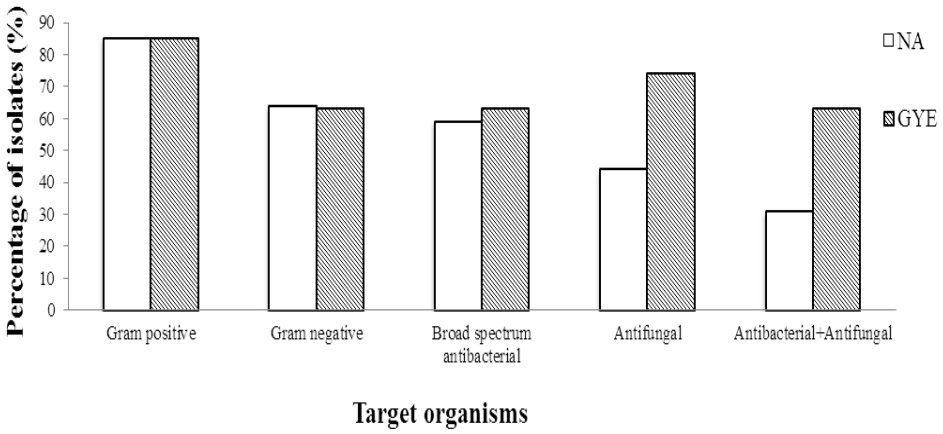


Fig. 4. Antimicrobial activity of isolates obtained on nutrient agar and glucose yeast extract from Lachhiwala Reserve Forest.

With respect to isolates obtained from NA, minimum range of zone of inhibition (0.5-1.0 cm) was exhibited by most of the isolates against *E. coli* (Fig. 5). Intermediate range of zone of inhibition (1-1.5 cm) was exhibited by most of the isolates against *B. subtilis* and a maximum range of zone of inhibition (1.5 to 2.0) was observed by most of the isolates against *S. epidermidis.* None of the isolates showed zones greater than 2.0 cm.

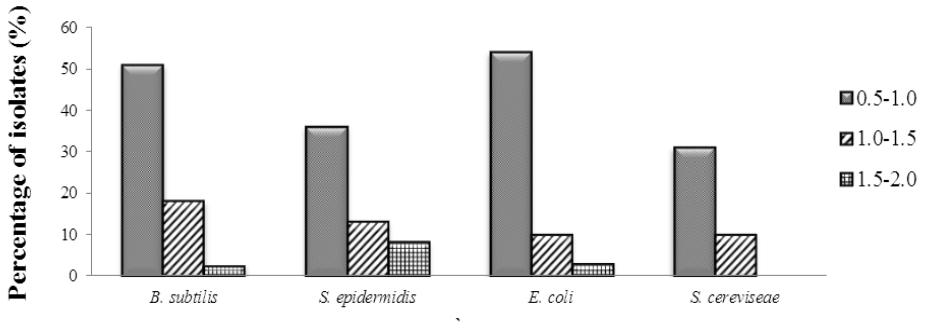


Fig. 5. Antimicrobial activity of isolates from Lachhiwala reserve forest, obtained on nutrient agar.

Of the isolates obtained on GYE plates (Fig. 6), it was observed that minimum range of zone of inhibition (0.5-1.0 cm) was exhibited by most of the isolates against *B. subtilis.* While intermediate range of zone of inhibitions (1-1.5, 1.5-2.0, 2.0-2.5 cm) was exhibited by most of the isolates against *E. coli, S. cereviseae* and *S. epidermidis* respectively and maximum ranges of zone of inhibition (2.5-3.0) was observed by most of the isolates against *S.*

epidermidis. Organisms isolated on GYE showed more trends towards production of larger zone of inhibition (2-3 cm). Zones of 2-2.5 cm were produced by minimum 11% of isolates against *B. subtilis* and maximum 30% of isolates against *S. epidermidis*, while zone of 2.5-3.0 cm were produced by minimum 4% of isolates against B. *subtilis* and maximum 19% of isolates against *S. epidermidis*. In this regard GYE was better media for isolation. The isolates exhibited larger inhibitory zones overall (UK-201, UK-203, UK-204, UK-205, UK-207, UK-208, UK-217, UK-220, UK-222 and UK-223) indicating their promise for further studies.

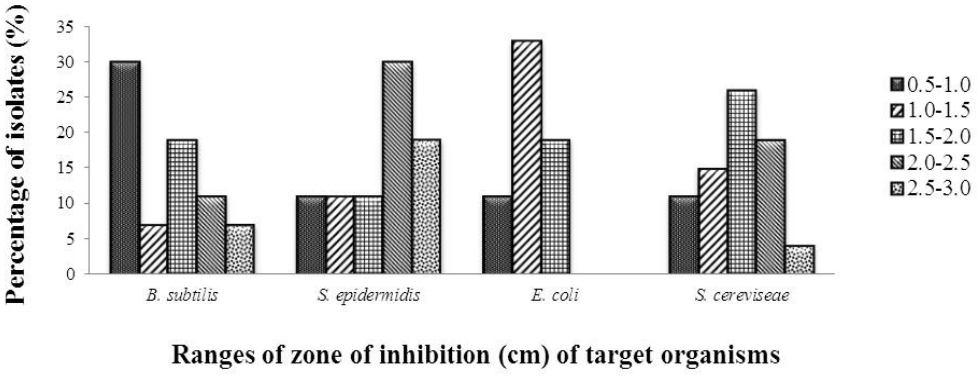


Fig. 6. Antimicrobial activity of isolates from Lachhiwala reserve forest, obtained on glucose yeast extract agar.

Enzyme production

The isolates were screened for production of enzymes (protease, lipase, amylase and cellulase) (Fig. 7).

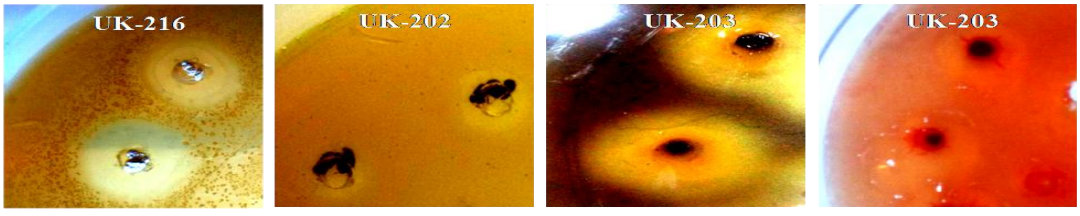


Fig. 7. Enzyme production (a) protease (b) lipase (c) amylase (d) cellulose.

Table 6 shows the zones of clearance produced on the respective substrates, as a measure of enzyme production. The number of isolates secreting different enzymes varied amongst isolates, depending on whether they were obtained from NA or GYE. Majority of isolates produced protease. Lipase was secreted by lowest number of NA isolates and amylase by GYE isolates. UK-1005 and 1021 produced maximum protease, UK-1009, UK-1012, UK-

1019, UK-204, UK-205, UK-214, UK-216 and UK-224 produced maximum Lipase. UK-1017 and UK-1045 produced maximum amylase and cellulase respectively. UK-1026, UK-1033 and UK-1039 did not produce any enzyme.

Table 6: Production of enzymes by isolates obtained on nutrient agar and glucose yeast extract agar from Lachhiwala Reserve Forest.

Media	Isolates	Range of clearance zones (cm)*			
		Protease	Lipase	Amylase	Cellulase
Nutrient Agar	UK-1000	+++	-	+	++
	UK-1001	++++	-	+	+
	UK-1002	++++	-	-	+
	UK-1003	++++	-	++	+
	UK-1004	+++	-	++	-
	UK-1005	+++++	+	-	++
	UK-1006	++++	+	++	-
	UK-1007	++++	+	-	+
	UK-1008	+++	++	+	+
	UK-1009	++++	+++	+	+
	UK-1010	++	-	+	++++
	UK-1011	++++	+	++	++++
	UK-1012	++	+++	-	+
	UK-1013	++	++	+	+
	UK-1014	++	-	-	+
	UK-1015	+++	-	+	-
	UK-1016	++	-	+	++
	UK-1017	++	-	++++	+++
	UK-1019	-	+++	+	-
	UK-1020	-	-	+++	+++++
	UK-1021	+++++	-	-	++
	UK-1022	-	-	+++	+
	UK-1025	+	+	-	-
	UK-1026	-	-	-	-
	UK-1027	+++	-	+++	+
	UK-1028	++++	-	-	+
	UK-1029	-	+	+	-
	UK-1030	+++	+	-	-
	UK-1031	++	-	+	+
	UK-1032	+	-	-	-
	UK-1033	-	-	-	-
	UK-1037	++	-	-	-
	UK-1038	+++	-	+	+
	UK-1039	-	-	-	-
	UK-1040	++	-	+	+
	UK-1041	++	-	-	++++
	UK-1042	++	-	-	-
	UK-1043	+++	-	-	++

	UK-1045	-	-	+	+++++
GYE	UK-201	++	+	+	++
	UK-202	++	++	-	++
	UK-203	++	++	++++	+++
	UK-204	+++	+++	++	++++
	UK-205	+++	+++	++	+++
	UK-206	+	+	+	-
	UK-207	+	-	-	-
	UK-208	+	-	-	+
	UK-209	+	-	+	-
	UK-210	-	-	+	-
	UK-211	+++	+	-	-
	UK-212	++++	++	-	++
	UK-213	+	+	-	-
	UK-214	++	+++	-	-
	UK-215	+	+	-	-
	UK-216	++++	+++	+	-
	UK-217	++++	+	+	-
	UK-218	++	++	-	++
	UK-220	+++	+	+	++
	UK-221	+	+	-	-
	UK-222	-	++	-	-
	UK-223	+++	+	-	++
	UK-224	+	+++	-	-
	UK-225	++	+	+	+
	UK-228	-	+	-	+
	UK-229	++	++	-	-
	UK-230	+	-	-	+

**Ranges of clearance zones indicating enzyme activity (in cm)*

- indicates 0

+ indicates 0.5-1.0

++ indicates 1.0-1.5

+++ indicates 1.5-2.0

++++ indicates 2.0-2.5

+++++ indicates 2.5-3.0

DISCUSSION

Many infectious diseases can be treated by antibiotics, but because of the high burden of multidrug resistance pathogens worldwide, challenges exist for effective treatment and hence, there has been increased interest in searching for effective antibiotics.[25]

Forests are reported to harbour rich microbial diversity in many studies.[9, 26] Lachhiwala forest is a biodiversity rich region and was investigated for preliminary screening of bacteria

and fungi for antimicrobial compounds. To our knowledge this forest has not been investigated for microbial diversity. Tropical ecosystems have been found to be less studied areas in terms of soils and their microbial diversity as compared to grasslands, agriculture lands, Boreal and temperate forests.[27]

The physicochemical data of our study indicated that the soil sample was near neutral in nature while it was earlier reported to be slightly acidic (6.36).[28] Difference in sampling locations and differences within micro-environments can explain this variability. Electrical conductivity of approximately 167 µs indicates that it is a nutrient poor soil.[29] The organic carbon content obtained in the present study (1.40%) was as in previous reports (1.45%) by Mukesh et al. 2011.[28]

We report sixty eight isolates (bacteria, fungi and actinomycetes) from Lachhiwala Reserve Forest, Dehradun. The initial results are based on distinct colony characteristics. Saravanan et al. (2012) reported twenty five bacteria from Anaimalai and Parambikulam tiger reserve forest, Western Ghats of Tamil Nadu,[30] which also constitute biodiversity-rich areas. Amongst these, 48% isolates showed antibacterial activity. In our results 60% unicellular bacteria showed broad-spectrum antibacterial activity, this is higher than that of the other reports. Velayudham and Murugan (2012) reported thirty six actinomycetes from the Eastern Ghats of India of which only one isolate showed antimicrobial activity against both bacteria and fungi.[31] Other reports from tropical forests have also shown lower percentage of isolates showing such broad-spectrum antimicrobial activity. Rachdiati et al. (2016) reported ten actinomycete isolates from the forest soil of Manong, Malaysia of which only three isolates showed broad spectrum antibacterial activity.[32] Chanthasena and Nantapong (2016) reported one hundred twenty three isolates from a dry dipterocarp forest soil of Thailand of which only two isolates showed broad spectrum antibacterial activity and activity against fungi.[33] In our study 13 isolates showed antimicrobial activity against both bacteria and fungi. Further, many isolates (95%) also have shown production of industrially important enzymes, to various degrees. These results indicate that the habitat under study harbours rich microbial diversity.

Using selective media for isolation of various classes of bacteria and fungi have resulted in better cultivable microbial profile. In turn, this has resulted in enhanced microbial diversity in terms of antimicrobial activity.

CONCLUSION

Screening for microorganisms from the Lachhiwala Reserve Forest has shown the presence of many distinct unicellular bacteria, actinomycetes and fungi. This initial screening on the basis of antimicrobial activity provides a platform for further studies focusing on promising isolates (such as UK-201, UK-203, UK-204, UK-205, UK-207, UK-208, UK-217, UK-220, UK-222, UK-223) for identifying compounds of pharmaceutical and other industrial interest.

CONFLICTS OF INTEREST

Authors declare no conflict of interest.

COMPLIANCE WITH ETHICS REQUIREMENTS

This article does not contain any studies with human or animal subjects.

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