Int.J.Curr.Microbiol.App.Sci (2019) *8*(8): 2930-2943

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.808.337

Studied on the Antibacterial Potential of Actinomycetes Isolated from the Haridwar Region of Uttarakhand, India

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Keywords

Actinomycetes, Antibacterial activity, Bacteria, Soil

Article Info

Accepted:
22 July 2019
Available Online:
10 August 2019

A B S T R A C T

The search for new antibiotics continues in a rather overlooked hunting ground. In this study screening for new antibiotic-producing microorganisms, isolates showing antimicrobial activity were isolated from soil samples of various habitats in the coastal region of Ganga, Neeldhara river bank, and K.G.M.campus, Haridwar, Uttarakhand, India. 29 isolates of actinomycetes were isolated from soil samples collected in the area of various localities of Haridwar region. These isolates were tested for their antagonistic properties against test bacteria *Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermis, Pseudomonas aeruginosa, Serratia marcesens,* and *Escherichia coli.* Pure culture of isolates were identified by morphological, cultural, physiological and biochemical studies. Thirteen of 29 were identified as members belonging to the genus *Streptomycetes,* nine belong to genus *Nocardia* and the remaining seven belong to the genus *Micromonospora*. The study indicated that 'Haridwar' soil had diverse group of actinomycetes and isolates which have relatively high antibacterial activities among these isolates underlined their potential as a source of novel antibiotics of pharmaceutical interest.

Introduction

Actinomycetes are the most widely distributed group of Gram positive bacteria in nature which primarily dwell in the soil (Oskay *et al.*, 2004) and usually grow by filament formation. They belong to the order Actinomycetales (Superkingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae) (Okami and Hotta 1988).These are aerobic, Gram-positive bacteria. They are one of the major groups of soil population and are very widely distributed (Kuster, 1968). The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. Actinomycetes populations are relatively lower than other soil microbes and contain a predominance of *Streptomyces* that are tolerant to acid conditions (Davis and Williams, 1970). The role of microorganisms, especially soil

microbes as degradation and biocontrol agents, has been widely known and studied. They are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents, extra cellular enzymes (Ravikumar *et al.,* 2011), cosmetics, vitamins, aminoacids, nutritional materials, herbicides, pesticides (Ogunmwonyi *et al.,* 2010) and also well known as a rich source of antibiotics and bioactive molecules (Sateesh *et al.,* 2011).

Among about 23,000 bioactive secondary metabolites by microorganisms have been reported and over 10000 of these compounds are produced by Actinomycetes (Vimal *et al.,* 2009). There are around eighty diverse group and comprise 63 genera of actinomycetes in the soil (Sateesh *et al.,* 2011). However, Arid soils of alkaline pH tend to contain fewer *Streptomyces* and more of the rare genera such as *Actinoplanes* and *Streptosporangium*. However, alkaliphilic actionmycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents (Mitsuiki *et al.,* 2002; Tsujibo *et al.,* 2003).

As biodegradative agents, microorganisms are important in the degradation of soil organic materials into humus (Stach and Bull,2005). But some actinomycetes secrete a range of enzymes that can completely degrade all the components of lignocellulose (lignin, hemicellulose and cellulose), while others may secrete a narrower range of enzymes that can only partially achieve such type of degradation (Masoon *et al.,* 2001). With their ability to secrete these enzymes, they are effective at attacking tough raw plant tissues and softening them for other microbes. The use of chemicals to control plant disease pathogens may be harmful for both human and environment. Gu (2003) expressed that since pathogenic bacterial strains are gaining drug resistance. There is need to discover novel sources of antimicrobials. Many researchers are working towards isolating actinomycetes which have the ability to degrade harmful chemicals and also those with ability to act as biocontrol agents.

The present study was undertaken to isolate actinomycetes from the soil samples of river bank and garden of Kanya Gurukul Mahavidyalaya and to assess their anti-bacterial potential. The resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. So we need to screen new actinomycetes from tested habitats for antimicrobial activity in hope of getting new actinomycete strains that produce new antibiotic that may be effective against drug resistant pathogens.

Materials and Methods

Soil Sample collection and isolation

A total of nine soil samples (5-6g for each) were collected from different sites of Ganga and Neeldhara river coastal area and Kanya Gurukul Mahavidyalaya, Haridwar district, Uttarakhand, India at a depth of 4-5 cm from surfaces from November 2008 to January 2009. All samples were pre-treated by heating at 55°C for 10minutes to minimize the bacterial and fungal contamination (Saadoun and Gharaibeh, 2003). The soil samples were dried separately at 37^{o}C for 1 hour in hot air oven (Williams *et al.,* 1972). Then the soil samples were cooled at room temperature. 1.0 gm of each soil sample was added to a conical flask containing 10 ml of sterile water and few drops of Tween-80 solution. All flasks were shaken for 30 minutes in orbital shaker incubator at 27^{0}C. These flasks were considered as stock cultures.

Isolation of actinomycetes and maintenance

Isolatiaon and quatification of actinomycetes were done by serial dilution method from collected samples (Porter *et al.,* 1960). 1.0g of each sample was suspended in 10ml of sterile distilled water and mixed properly. Serial dilutions were done up to 10^{-5} using sterile distilled water and agitated with the vortex at maximum speed. An aliquot amount of 0.1 ml of each dilution from 10^{-2} to 10^{-5} was taken and spread evenly over the surface of starch casein nitrate agar plates using glass L-rod. Plates were incubated at 28^{0}C for 7-10 days (Narendra Kumar *et al.,* 2010). After incubation, the individual actinobacterial colonies were picked out and subcultured into freshly prepared yeast extract malt agar plates. Then the pure colonies were maintained in yeast extract malt extract agar slant and kept at 4°C until further use.

Test bacteria

The test bacteria used in this study were the three Gram positive bacteria *Staphylococcus aureus, Bacillis subtilis, Micrococcus luteus* and three Gram negative bacteria *Serratia marcecans, Escherichia coli* and *Pseudomonas aeruginosa.*

Morphological characterization

For the morphological characterization different media were used. These media were Starch-nitrate agar medium; Glycerol-asparagine agar medium; Inorganic salt-starch agar medium; Yeast extract-malt extract agar medium and Oatmeal agar medium (Gordon, 1966).

Actinomycetes were streaked onto actinomycetes isolation agar, starch case. Cover slip and Gram staining techniques (Khan and Williams, 1975) were employed for microscopic observation where the cover slip was stabbed onto the agar at an angle of 45° and incubated at 30 °C for 6 days. After 6 days of growth, the actinomycetes were examined. Cover slips were then taken out from the agar and put onto the prepared slides. The mycelium structure, arrangement of conidiospore and arthrospore on the mycelium was observed through the oil immersion (1000×). The observed structure was compared with the Manual and the organism was identified. Crystal Violet staining dye was used for this purpose (Sahilah, 1991). Slides were then viewed using a research microscope. Identification of actinomycetes to genus level was then carried out based on 'Bergey's Manual of Determinative Bacteriology', 9th edition (Zenova *et al.,* 2004).

Characterization and identification of potential actinobacteria

To identify the actinomycetes, it was characterized by standard those methods described by Shirling and Gottileb (1996) and Holt *et al.,* (2000). Cultural morphology, Microscopic appearance, Utilization of carbon, Physiology and biochemical characters was studied. Based on the expressed phenotypic characters (Gordon,1967), the potential actinobacteria strains were tentatively identified with the help of the actinobase database (Ugawa *et al.,* 1989).

Physiological characterization

These tests were performed as described by Gordon (1966, 1967). Physiological tests included decomposition of Casein, Tyrosine, Xanthine, Hypoxanthine, Urea and Esculin, evaluation of lysozyme resistance and the ability to produce acid from various carbohydrates such as arabinose, fructose, galactose, inositol, lactose, mannitol, mannose, rhamnose, sorbitol and xylose.

Screening for antimicrobial potential of actinomycetes isolates

Primary screening

Antimicrobial activities of the isolates of actinomycetes were tested preliminarily by single streak method (Arifuzzaman, 2011) with some modification. In this method a loop full of inoculum was streaked in the middle of the petridish containing modified nutrient agar medium. After inoculation, petridishes were incubated at 28˚C for 7days for the growth of actinomycetes and then 24hrs old bacterial cultures were inoculated near the growth line of actinomycetes in the same petridish. The single streaked plates were incubated at 28˚C for 24 hrs. The inhibition zone produced between the actinomycetes and the bacteria were measured.

Secondary screening

Based on the zone of inhibition, secondary antimicrobial screening and further analysis of promising isolates were done under submerged fermentation conditions by agar well diffusion assay. The selected isolates were further tested in the secondary screening by shake flask studies to confirm their antimicrobial activity. The spore suspension of the selected isolates were inoculated into the soya bean medium and kept in the shaker. After 96hrs, the culture broth was separated from the mycelium by centrifugation at 5000rpm and tested for antimicrobial activity.

Agar well diffusion method

100ml of sterilized starch casein nitrate agar in 250ml conical flask was seeded with 50µl of standardized test bacteria, swirled gently and aseptically poured into Petri dishes and allowed to solidify. Sterile cork borer (6 mm diameter) was used to make wells in the plate. About 100 µl of the sample was carefully dispensed into wells. The experiment was repeated for three times (Pandey, 2004). Extracts were allowed to diffuse for about 2h before incubating. Plates were incubated at 37°C for 24h. The diameter of the inhibition zone for each strain was recorded. Among the selected strains the most potent strain was selected for further analysis. Negative control contain only liquid broth media. Each experiments was repeated three times and mean of inhibitory zone recorded.

Colour determination of actinomycetes isolates

The aerial mass colour on (oatmeal agar) ISP3 and (inorganic salt starch agar) ISP4, substrate mycelium colour and diffusible soluble pigments on (glycerol asparagine agar) ISP5, melanin production on (peptone yeast extract iron agar) ISP6 were observed at 27°C after 15 days using a reference colour key (Kuster and Williams, 1959).

Results and Discussion

Actinomycetes isolation

Actinomycetes were isolated and the morphological appearance of isolates is shown in Figure 1. A total of 29 morphologically different actinomycetes colonies were selected from nine soil samples and made pure culture.

Morphological and cultural characteristics of selected isolates

Isolation plates developed various types of bacterial actinomycete colonies. Fifty to sixty colonies were found per plate. Colonies selected from each plate were 5 to 20 based on colony appearance. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and colour ranging from white, gray to pinkish and yellowish were selected. Colonies

observed at 5th and 7th day were eliminated because actinomycetes are considered as slow grower (Currie *et al.,* 2006). Furthermore, bacterial configuration same as actionmycetes were accepted from Gram staining. Twenty nine selected isolates were examined microscopically and identified by their morphological and culture characteristics. These isolates placed under three genera such as *Streptomyces, Nocardia and Micromonospora* (Table 1) on the basis of morphological physiological and taxonomic characteristics.

Physiological and biochemical characteristics of isolates of Actinomycetes

Physiological and biochemical characteristics result indicates that all isolates showed the ability of starch and urea hydrolysis. The isolates A_{11}, A_{20} and A_{27} were able to hydrolysis celatin; A_{11}, A_{20} and A_{27} were able to hydrolysis casein. The positive utilization of citrate was recorded in A_{20} and A_{27} and A_{11}. The tested actinomycetes isolates showed resistance capacity to grow in 3 and 5% concentration of sodium chloride. The optimum temperature for the growth of two isolates (A_{11}, A_{20}) was between 25-35 °C and isolates A_{27} exceed up to 35 °C (Table 2).

The prevalence % of the isolates of actinomycetes

Percentage of isolates of *Streptomyces* species in the K.G.M. College Campus, Ganga river canal bank and neel dhara river bank, were 10.23%, 12.48% and 22.12% respectively, and *Nocardia* species were 4.80%, 7.49% and 18.75% respectively. The prevalence percentage of *Micromonospora* species in the College Campus was not found but in Ganga river and neeldhara river bank percentage were 7.20% and 10.56%. Thus the total percentages of *Streptomyces, Nocardia* and *Micromonospora* species were 44.83%, 31.04% and 24.13 in the respective locations (Table 3). The graph of cumulative frequencies of the isolates of actinomycetes in the soil showed that the frequencies of isolates of actinomycetes in neel dhara river site has more comparatively to Ganga river bank and Kanya Gurukul Campus (Figure 2).

Antimicrobial sensitivity assay of purified metabolites of isolates

For antibacterial sensitivity assay agar wall diffusion methods were followed (Hayakawa *et al.,* 2004; Cheah, 2001) The metabolites were extracted with the solvents chloroform and EtOAc as shown in Figure 3.

Primary screening

Among 29 isolates of actinomycetes isolated from coastal area of Ganga and Neeldhara river and Kanya gurukula mahavidyalaya campus, Haridwar, Uttarakhand. 18 isolates showed antibacterial potential against at least three or more (4 to6) of the tested bacteria. In single streak plate method, results revealed that isolates A_3, A_5, A_7 to 9, A_{13} to A_{19}, A_{23} to A_{25} and A_{29} exhibited broad spectrum activities against test bacteria. A_2, A_{11}, A_{20} and A_{27} have shown a wide range zone of inhibition against *B.subtilis, M. luteus, S. epidermis, P. aeruginosa, S. marcecans,* and *E.coli.* The isolates A_1, A_4, A_{24} and A_{28} were active against only one or two test bacteria and isolates A_9 and A_{16} not produced any antibacterial potential (Table 4). Among these isolates, three isolate (A_{11}, A_{20} and A_{27}) showed significant antimicrobial activity against selected test bacteria and ware characterized by polyphasic taxonomy.

Secondary screening of crude extracts

The crude extracts prepared from 29 isolates of actinomycetes by using solid state and submerged state fermentation methods was

subjected to secondary screening by agar well diffusion methods. The crude extracts prepared from culture filtrates were analyzed for their antimicrobial activity by wall diffusion method. In this study, the chloroform extract showed good activity against all the test pathogens shown in Table 3.

Isolation of an antibiotic from culture filtrate is largely determined by its chemical nature. Solvent extraction is usually employed for the extraction of antibiotics from the culture filtrates.

Organic solvents with different polarities have been used by many researchers for the extraction of antimicrobial compounds from actinomycetes (Selvameenal *et al.*, 2009). This result clearly indicated that the antimicrobial activity of potential strain is due to the production of extracellular bioactive compounds. The previously published literature stated that most of the antibiotics from actinomycetes are extracellular in nature (Valan arasu *et al.*, 2008).

The ability of actinomycetes to produce antibiotic is often associated with its ability to be a biocontrol agent (Crawford *et al.*,1993). In this study, only two strains of actinomycetes were observed to show antimicrobial activity against pathogenic bacterial species. Isolates $A_{11,}$ A_{20} and A_{27} produced enzyme activities against cellulose, mannan and xylan and mannan and xylan respectively. Study done previously (Pandey *et al.*, 2004; Valois *et al.*,1996) stated that there are no correlation between the ability to secrete hydrolytic enzymes and the ability of actinomycetes as biocontrol agent. All the test strains that did not produce positive result in this study might give positive results if other pathogens were used. These actinomycetes were kept and preserved for future use

Table.1 Identification of actinomycetal isolates based on morphological and cultural characteristics

Colony characteristics on starch-casein agar (after 7 days)	Microscopic characteristics (on 5th day)	Actinomycetal isolate	Total number of Isolates
Light yellow-orange to orange-red colonies, occasionally brown maroon or blue green. The dark brown to black colonies surface darken with spores.	Fine substrate mycelium with spores as cluster of grape, no aerial mycelium.	*Micromonospora* (Suarez and Hardisson, 1985)	7
Colony appears waxy, shiny; several millimeters in diameter; aerial filaments are formed, the colony surface become dull and fuzzy.	Gram positive, non-acid fast, pleomorphic cells ranging from bacillary to coccoid structure; occasionally limited mycelium found which fragments produce rod shape or coccoid cell.	*Nocardia* (Good fellow and Lechevalier, 1989)	9
Powdery colony appears convex, concave or flat surface; white, gray to pinkish color colony.	Filaments long highly branched and non fragment; arial filament with spirali, coils, or multiple branching and long chains spores.	*Streptomyces* (Anderson and Wellington,2001; Williams *et al.*, 1989)	13

Table.2 Physiological characteristics of actinomycete isolates

Physiological Tests	A_{11}	A_{20}	A_{27}
Degradation of:Xanthin	+	+	+
Degradation of:Aesculin	-	+	+
H_2S Production	-	+	+
Nitrate reduction	±	+	+
Citrate utilization	+	+	+
Celatin hydrolysis		+	+
Urea test	-	+	+
Coagulation of milk	+	+	±
Utilization of: different con sources			
D-Xylose	+	+	+
D- Mannose	+	+	+
D- Glucose	+	+	+
D- Galactose	+	+	+
Sucrose	±	+	+
Rhamnose	±	+	+
Raffinose	+	-	+
Mannitol	+	+	+
L- Arabinose	+	+	+
meso-Inositol	-	-	-
Lactose	+	+	+
Maltose	-	±	+
Trehalose	+	+	+
L-Melizitose	+	-	+
D-fructose	-	+	+
Sodium citrate	+	+	+
Utilization of different amino acids			
L-Cycteine	-	+	+
L-Valine	-	+	-
L-Histidine	-	+	+
L-Phenylalanine	-	+	+
L-Arginine	+	+	+
L-Lysine and L-Hydroxproline	-	-	-
L-Glutamic acid	±	+	+
Growth inhibitors:			
Thallous acetate -0.001	-	-	-
Sodium azide -0.01	-	+	+
Phenol -0.01	-	+	+
Growth at different temperatures (˚C):			
10	±	+	-
20	+	+	+
25- 35	+	+	+
50	-	-	±
Growth at different pH values:			
4	-	±	±
5-9	+	+	±
10	-		
Growth at different concentrations of NaCl (%)			
3	+	+	+
5	+	+	+
7	-	-	-

Table.3 The prevalence (% present in the samples) of the isolates genera

Isolates	K.G.M.College Campus (%)	Ganga river bank (%)	Neel Dhara river bank (%)	Total (%)
Streptomyces	10.23	12.48	22.12	44.83
Nocardia	4.80	7.49	18.75	31.04
Micromonospora	-	7.20	10.56	24.13

Table.4 Zone of inhibition (mm in diameter)of isolates of actinomycetes against test bacteria using single streak plate method

Isolates	Test Bacteria Zone of inhibition (mm)					
	Bacillus subtilis	***Micrococcus luteus***	***Staphylococcus epidermis***	***Pseudomonas aeruginosa***	***Serratia marcescens***	***Escherichia coli***
A_1	+	-	-	-	+	++
A_2	+++	+	+++	++	+	+++
A_3	+	-	-	+	-	+
A_4	-	++	-	-	+	++
A_5	++	-	++	+	-	++
A_6	++	-	+	-	+	-
A_7	+	++	-	+	-	++
A_8	-	+	++	-	+	+
A_9	+	-	-	++	-	-
A_{10}	-	+	-	+	-	-
A_{11}	+	++	+++	+	++	+++
A_{12}	+	+	-	+	-	+
A_{13}	+	-	-	-	+	+
A_{14}	-	+	+	+	-	-
A_{15}	+	+	-	-	+	++
A_{16}	-	-	+	-	-	-
A_{17}	++	+	-	-	++	++
A_{18}	+	-	+	+	-	+
A_{19}	+	+	+	-	+	-
A_{20}	+++	++	+++	+	++	++
A_{21}	-	+	++	-	+	+
A_{22}	++	-	-	++	-	-
A_{23}	+	++	+	-	-	+
A_{24}	-	++	-	+	-	-
A_{25}	+	-	+	-	+	+
A_{26}	-	-	-	-	-	+
A_{27}	++	+	+	++	+	++
A_{28}	-	-	+	-	+	+
A_{29}	+	+	-	-	-	+

+++ = Better inhibition, ++ = Good inhibition, + = Moderate inhibition, - = No inhibition

Table.3 Zone of inhibition (mm) in secondary screening of crude extracts (10 mg/mL) produced from solid state fermentation by using disc diffusion method

Isolates	Test Bacteria Zone of inhibition (mm)					
	Bacillus subtilis	***Micrococcus luteus***	***Staphylococcus epidermis***	***Pseudomonas aeruginosa***	***Serratia marcescens***	***Escherichia coli***
A_1	-	-	-	-	8	12
A_2	**26**	**17**	**25**	**19**	**14**	**20**
A_3	9	-	-	10	-	8
A_4	-	12	-	-	-	10
A_5	20	-	16	8	-	14
A_6	14	-	-	-	10	-
A_7	12	16	-	10	-	14
A_8	-	-	12	-	9	12
A_9	-	-	-	-	-	-
A_{10}	-	9	-	10	-	12
A_{11}	**10**	**22**	**16**	**11**	**18**	**20**
A_{12}	10	12	-	8	-	16
A_{13}	14	-	-	-	8	11
A_{14}	-	10	9	10	-	-
A_{15}	8	7	-	-	7	14
A_{16}	-	-	-	-	-	-
A_{17}	16	14	-	-	14	20
A_{18}	10	-	9	6	-	12
A_{19}	8	7	9	-	8	-
A_{20}	**24**	**20**	**18**	**16**	**19**	**25**
A_{21}	-	9	14	-	12	15
A_{22}	19	-	-	12	-	-
A_{23}	9	12	10	-	-	16
A_{24}	-	14	-	12	-	-
A_{25}	12	-	10	-	14	10
A_{26}	-	-	-	-	-	15
A_{27}	**18**	**12**	**9**	**10**	**12**	**16**
A_{28}	-	-	-	-	-	10
A_{29}	7	9	-	-	-	12

Values are mean +SD of three replications; -: No zone of inhibition.

Table.5 Number of actinomycetes isolates that were able to hydrolyse cellulose, mannan and xylan

Area of Collection	Number of actinomycetes that are able to hydrolyse		
	Cellulose	**Mannose**	**Xylane**
College Campus	12	3	7
Jwalapur River Bank	7	6	9
Neel Dhara Bank	15	9	5

Figure.1 Plates show the pure form of isolates of actinomycetes

Figure.2 Shows the cumulative frequencies distribution of total concentration of viable actinomycetes in moisture soil

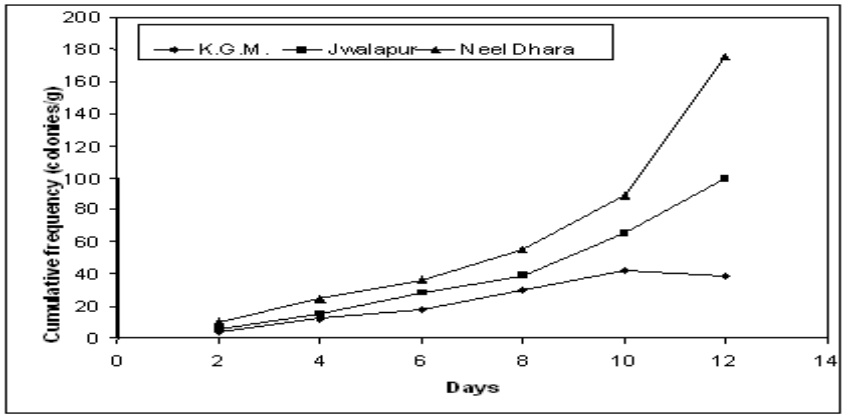

Figure.3 Extract of isolates of actinomycetes on glycerol broth media

The actinomycetes have wide distribution and they show variation in their population dynamics. Actinomycetes play an important role in the production of bioactive and antimicrobial agents. Perhaps, the incidence of multidrug resistant organisms is increasing day by day and compromising the treatment of a growing number of infectious diseases. As a result, there is an urgent need for developing new drugs which are effective against current antibiotic resistant pathogens. Actinomycetes have been proven as a potential source of bioactive compounds and richest source of secondary metabolites (Suthindhiran and Kannabiran,2009). In this study 29 actinomycete soil isolates were evaluated for their antimicrobial potential.

Out of 29 actinomycete isolates, only three isolates, $A_{11,}$ A_{20} and A_{27} exhibited a wide spectrum antimicrobial agent against Gram positive and Gram negative pathogenic test bacteria. Those three isolates were isolated from soil samples collected from Ganga river and Neeldhara river bank and allowed to grow on starch nitrate agar medium. Identification process had been carried out according to the Key's given in Bergey's Manual of Determinative Bacteriology 8th edition (Buchanan and Gibbsons, 1974), Bergey's Manual of Systematic Bacteriology, vol. 4 (Williams, 1989) and Bergey's Manual of Determinative Bacteriology, 9th edition (Hensyl, 1994). In the secondary screening crude extract was produced through solid state fermentation method compared to submerged state fermentation method when extracted with methanol and ethyl acetate solvents. The reason for the increased production of yield in solid state fermentation was due to lack of water and completely miscible in organic solvents (ethyl acetate and methanol) with the fermented biomass. The higher yields obtained by the solid state fermentation method was agreed with the previous research (Naggaret *et al.,* 2009; Tabaraie *et al.,* 2012). The lower yields obtained from submerged state fermentation method was attributed to the use of water immiscible solvent such as ethyl acetate during extraction. Similar findings were earlier reported by Subramaniyam and Vimala (2012).

The aerial mycelium, substrate mycelium growth and pigmentation showed distinct variation based on the culture media in which the isolates were grown. Among the four culture media used, most of the isolates growth was excellent in starch casein nitrate agar and this may be due to sufficient amount of nutrient included in this media. Researchers also observed leathery, white powdery, creamy, pinpoint and powder colonies of actinomycetes (Valli *et al.,* 2012). All the potential isolates in this study have the ability to hydrolysis starch and urea. Most of the isolates can tolerate at 5% concentration of sodium chloride and the optimum temperature for the growth of isolates was ranged from 25 to 30 °C. Therefore, these results indicate that the isolates obtained from river bank sites were grouped under the genera of *Streptomyces*, *Nocardia* and *Micromonospora*. Findings of the present study conclude that Haridwar river bank is the potential ecosystem for antagonistic actinomycetes which deserves for bioprospecting. In this study, twenty nine isolates of actinomycetes were studied. Actinomycetes isolated from river areas have more potential in hydrolysing cellulose and xylan than mannan. Three isolates (A_{11}, A_{20} and A_{27}) showed highest antibacterial potential. These isolates were identified as *Streptomyces, Nocardia* and *Micromonospora* using Bergey's Manual of Systematic Bacteriology based on their spores arrangement. They have the ability to produce potent, distinctive, adapted, exceptional bioactive secondary metabolites. The research work has established that there is rich

actinomycetes diversity in the region in general especially in the various microbial niche of Haridwar, river which can be exploited to develop the bio- industry.

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How to cite this article:

Garima Arya and Jugmendra Singh. 2019. Studied on the Antibacterial Potential of Actinomycetes Isolated from the Haridwar Region of Uttarakhand, India. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 2930-2943. doi: https://doi.org/10.20546/ijcmas.2019.808.337