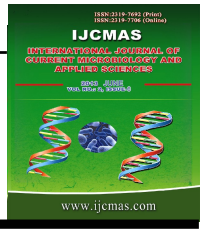
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 6 (2013) pp. 113-116**
http://www.ijcmas.com



Original Research Article

Screening of Actinomycetes from biodegraded buildings material and their antibacterial potential

Garima Arya, P. Kaushik and Padma Singh

Department of Botany and Microbiology, Gurukul Kangri Vishwavidyalaya, Haridwar, 249407 Uttarkhand, India
***Corresponding author e-mail:** purshotam.kaushik@gmail.com, garimaaryaphd@gmail.com*

Keywords

Actinomycetes; metabolites; antibacterial potential.

A B S T R A C T

In this work three isolates of actinomycetes, named A_{11}, A_{20} and A_{27}out of twenty nine screened for antibacterial activity were found to be more effective. All the three isolates were found Gram positive rods. These were subjected to secondary screening and the isolate A_{11} was found to be most effective against test bacteria *Escherichia coli* and *Staphylococcus aureus*. Production of antibacterial metabolites was carried out by submersed fermentation process and extracellular metabolite was extracted in solvent ethyl acetate. Their role as new antimicrobial drugs has been discussed.

Introduction

According to 'Bergey's Manual of Systematic Bacteriology' volume IV, the actinomycetes are Gram positive filamentous bacteria having G+C (<55%) content in their DNA. The name "actinomycete" was derived from Greek word 'aktis' (a ray) and 'mykes' (fungus). The actinomycetes are most widely distributed group of microbial consortia found in nature which primarily inhabit the soil (Manjula *et al*., 2009). These have been reported to be most common antibiotic producing microorganisms found in soil. Actinomycetes provided about two third part (more than 4,000) naturally occurring antibiotics discovered including many of those important in medicine such as aminoglycoside, actinomycin, anthracycline, β lactam, chloramphnicol, macroides, peptides and tetracycline (Wakesman, 1968). These bioactive compounds have highly commercial value and continue to be routinely screened for new bioactive compounds. Almost 80% of the world's antibiotics are known to come from actinomycetes mostly from the genera *Streptomyces*, and *Micromonospora* (Pandey *et al*., 2004). According to world health organization, regular uses of antibiotics have led to the generation of antibiotics resistance in many bacterial and fungal pathogens. Thus drug resistant strains of pathogen emerge quickly than the rate of discovery of new antibiotics. This study focussed on the screening of actinomycetes from soil and assessed their antibacterial potential against pathogenic bacteria.

Materials and Methods

Screening and identification

The isolates of actinomycetes were isolated from biodegraded building samples collected from Gurukul Kangri residence, Haridwar and industrial building (B.H.E.L) using starch nitrate agar and actinomycetes agar. The phenotypic identification of isolates were determined by the method described by Shirling and Gottlieb (1970). The screening of isolates was examined by disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*.

The screening procedure was adopted for the actinomycetes (Kon-Wendisch, *et al*., 1992). The soil sample was dried in incubator at 35˚C for 24 hours. 1.0 g soil sample was suspended in 10 ml distilled water and vortexed for 10 minutes and made the serial dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. 0.1 ml of aliquots were transferred from each dilutions into sterile seeded agar plates. Spread the inoculum with the help of spreader and incubated the cultured plates in an incubator at 28˚C for one week. After incubation plates were selected which contained colonies and made further pure form of isolates. The pure form of isolates was preserved at 4˚C in glycerol agar.

Characterization of actinomycetes

The isolates of actinomycetes were characterized by morphological and physio-biochemical methods. The morphological method consists of microscopic and macroscopic observations of isolates. The microscopic observations was done by Gram staining technique and cover slip method (Kawato and Sinobu, 1979). The mycelial structure, colour of colonies on media and arrangement of cells were observed. The observed results were compared with Bergey's Mannual of Systematic Bacteriology (**2000**) and isolates were identified. Various biochemical tests performed for the identification of isolates are as starch hydrolysis, catalase test, nitrate reduction, hippurate test, aesculin reduction, casein hydrolysis, and starch degradation.

Test organisms

Two bacterial species *Escherichia coli* and *Staphylococcus aureus* were used to determine the antibacterial activity of the isolates of actinomycetes. The said test bacteria were obtained from the laboratory of Kanya Gurukul Mahavidyalaya 2nd campus of Gurukul Kangri Vishwavidyalaya, Haridwar, India.

In vitro screening of isolates for antibacterial potential

The disc diffusion method was used for the determination of the level of antibacterial potential. Antibacterial potential of actinomycetes against test bacteria from a solidified agar layer in a Petridish to an extent such that the growth of added microorganisms present entirely with in a zone created around the disc containing extract of metabolites or antibiotics (Kaushik and Kishore, 1991; Kaushik and Chauhan, 2009; Kaushik and Goyal, 2011 and Slavica *et al.*, 2005).

Fermentation and extraction of metabolites

The extraction of metabolites of isolates of actinomycetes were grown in broth culture in 250 ml flask containing 50 ml of liquid medium (NaCl 0.8g, NH_4Cl 1g, KCl 0.1g, KH_2 PO_4 0.1g, $MgSO_4.7H_2O$ 0.2g,

$CaCl_2.7H_2O$ 0.04g, glucose 2g, yeast extract 3g, Distilled water 1000 ml, pH 7.3). The flasks were inoculated with 1ml of active isolates of actinomycetes and incubated at 28˚C for 120 h with shaking at 105t/ min. After growth, the contents of each flask were extracted twice with butanol (1-2.5 v/v). Filter paper disc (6 mm in diameter) were impregnated with extract broth, dried and placed on nutrient agar plates previously seeded with *E. coli* and *S. aureus*. The plates were incubated at 35 ˚C for 24 h and examined for zone of inhibition.

Results and Discussion

In this study isolates of actinomycetes were isolated from 1 g scratched material collected from sited mentioned earlier and inoculated on actinomycetes isolation agar and starch casein agar. The pure colonies were maintained in glycerol agar medium at 4˚C. According to morphological and biochemical characteristics, isolates belonged to three different groups (Table 1). Isolates grew well on actinomycetes isolation agar showing morphology of typical of actinomycetes colonies which were granular powdery to red, pink, orange, green, chocolaty in colour, rudimentary to extensively branched vegetative hyphae growing on or beneath agar surfaces. The hyphae fragment into rod shaped to coccoid elements and poorly to well developed conidia were seen on the aerial hyphae. The isolates A_{11}, A_{20}, and A_{27} resembled as *Streptomyces*, and *Nocardia* based on the basis of Bergey;s Mannual of Systematic Bacteriology (1989). Antibacterial potential was exhibited by 66% of isolates. Isolate A_{11} and A_{20} showed the highest antibacterial respectively i.e.17and 8 mm against *E. coli* and 12 and 9 mm against *S. aureus* respectively as shown in Table 2. Isolate A_{27} did not seen any activity against test bacteria. After observing the characteristic shown by isolates we conclude that the antibacterial potential of actinomycetes isolates may lead to the discovery of new antibacterial drugs. However, the maximum inhibition was noted against *E. coli*.

Kaushik and Chauhan (2009) recorded antibacterial potential of cyanobacterial species: *Anabaena variabilis, A. fertilissima, Nostoc muscorum, N. punctiforme, N. linckia, N. commune, Spirulina platensis* and *Hapalosiphon* against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus,* and Kaushik and Goyal (2011) noted diverse medicinal plants against a large number of pathogenic bacterial strains. They have cited further need of searching new antimicrobials because of fastly increasing drug resistance among pathogenic bacteria.

References

Kaushik, P., and Chauhan, A. 2009. Cyanobacteria: Antibacterial activity. 101 Vikas Surya Plaza, C U Block, Pitampura, New Delhi-110088. Pp XIII+1-198.

Kaushik, P., and Goyal, P. 2011. Medicinal plant:Antibacterial potential. Today and Tomorrow's Printers and Publishers, Ansari road, Darya Gang, New Delhi. pp. 4435-36.

Kaushik, P., and Kishore, N. 1991. Antibacterial potential of *Pholidota articulata* Lind: A study in *vitro*. J. Orchid Soc. 5(1,2): 93-96.

Kawato, M., and Shinobu, R., 1959. A simple technique for the microscopical observation. Liberal art. Education. 114.

Korn-Wendisch, F., and Kutzner, H. J. 1992. The Prokaryotes. A handbook on the biology of bacteria:Isolation, identification and application. Springer-Verlag, Berlin 921-995.

Manjula, C., P. Rajaguru and Muthuselvam, M. 2009. Screening for antibiotic sensitivity of free and immobilized actinomycetes isolated from India. Advan. Biol. Res. 3: 84-88.

Pandey, B., P. Ghimire and Aggrawl, V. P. 2004. Studies on the antibacterial activity of actinomycetes isolated from Khumbu region of Mt.Everest. A paper presented in the International conference on the great Himalayas: climate, ecology, management and conservation. Kathmandu. January 12-15. Organised by Kathmandu Uviversity and Ecosystem Health and Management Society, Canada.

Shirling, E. B., and Gottieb, D. 1970. Methods of characterization of *Streptomyces* species. *Inter. J.Systematic Bacteriol.*16: 316-340..

Slavica, B., S. Sandra and Zobran, B. 2005. Analysis and antimicrobial activity of *Streptomyces* isolates. Medi.Biol. 12(1): 44-46.

Waksman, S. A. 1968. Actinomycetes; nature, and activities. *Ap*pl. Microbiol. 5: 235-293.

Williams, S. T., M. Goodfellow and Alderson, G. Genus *Streptomyces* (Wakesman and Hanrici, 1943) *In*: Bergey;s Mannual of Systematic Bacteriology. Edited by Williams, S. T., Sharpe, M. E., Holt, J. G. 4:2452-2494