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ISOLATION AND DETECTION OF BIOACTIVE COMPOUND PRODUCING ABILITY OF STREPTOMYCES ISOLATED FROM SOIL SAMPLE

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Abstract :

The present investigation was undertaken to find out the production of bioactive compounds from Actinomycetes. Actinomycetes are Gram positive spore forming, filamentous bacteria, with high G + C content (69-80%) in their Genome. About 70% secondary metabolites are reported to produce by Actinomycetes, out of these 2/3rd are contributed by the genus *Streptomyces* spp. The *streptomyces* are distributes in soils, compost, water and in ocean. The key feature of *streptomyces* is to produce secondary metabolites such as neomycin, streptomycin, chloramphenicol, rifamycin, etc. These compounds are naturally produce in soil by these microorganism as a antagonistic process.

This study was undertaken to isolate the potential antibiotics producer from the soil. In this work, soil sample was collected from the Nanded region, (M.S) India. Starch casein agar was used for the isolation of antibiotic producer. The isolate was characterised on the basis of morphological and biochemical properties. The isolates were allowed to produce bioactive compounds under optimized conditions. The bioactive compound was extracted and purified. The bioassay of bioactive compound was performed on human pathogens such as *S.typhi, E.coli, K.pneumoniae* and *P.aeruginosa.* The result showed that the bioactive compound shows a marginalised antagonistic effect against the test pathogens. From the above study it is proven that the isolate *Streptomyces* spp. is a potent bioactive compound producer.

Key words:

Actinomycetes, characteristics, bioactive compounds, test organism, antimicrobial activity.

1.0 Introduction:

For decades, microbial products have gain more attention because naturally obtained products are chief and affordable to human beings. Since the discovery of Penicillin. Research in bioactive compound production are expands and researcher have exploited their knowledge to produce different kinds of antibiotics from microorganisms. Near about 70% of antimicrobial compounds reported from soil microbes specially Actinomycetes group. Actinomycetes have been exploited for production of antibiotics. Recently multidrug resistance strain of pathogenic bacteria reported by many clinical researcher. To overcome on this menance contineous finding of novel bioactive compound from microbes is the need of the time. Actinomycetes are physiologically diverse group of microbes which produce numerous extracellular enzymes and other metabolic products (Kekuda et al., 2010) due to their metabolic diversity, Actinomycetes have biotechnological potential for the production of pharmaceuticals as well as a bio-remediators (Berdy, 2005).

There are about 22000 known secondary metabolites reported from microorganism such as antibacterial, antifungal, antiviral, antitumor, anti-protozoal, anticancer, immune-suppressor and several enzymes. About 70% secondary metabolites are reported from Actinomycetes and two thirds of them are contributed by the genus *Streptomyces* (Subramani and Aalbersberg, 2012). *Streptomyces* belongs to genus of Actinobacteria and the family Streptomycetaceae (Hong et al., 2009). Over 500 species of *Streptomyces* have been described (Lee, Jung & Hwang, 2005). *Streptomyces* species are chemo-organotrophic, filamentous Gram-positive bacteria (Ikeda et al., 2003). They have genome with high G+C content 69-78% (Kavitha, et al. 2010). The filaments and spores are usually 1μm or lesser in diameter (Willemse, et al. 2011). The spores are formed by the fragmentation of the filaments and borne in straight, wavy or helical chains. The colonies are slow growing and often have earthy odour due to production of a volatile metabolite like geosmin (Jüttner & Watson, 2007). Appearently colonies are relatively smooth surfaced and later develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety (Ambarwati, et al. 2012). *Streptomyces* produce a

wide varieties of pigments responsible for the colour of the vegetative and aerial mycelia (Flärdh & Buttner, 2009).

1.2 *Streptomycetes* and antibiotics :

Streptomycetes are potential to produce secondary metabolites such as antibiotics (MC Intyre, 2002), anthelminthic enzymes, herbicides (Kariminik & Baniasadi, 2010), anti- cancer drugs (Berdy, 2005), growth factors like vitamin B-12 (Bibb, 2005) and immune-modulators (Mann, 2001). Louis Pasteur was one of the pioneer of modern antibiotics in 19th century (Gray & Jacobs 2003). He reported that, some micro-organisms are able to kill other micro-organisms. Penicillin was the first antibiotic was discovered by Alexander Fleming from *Penicillium notatum* in 1929 (Silva & Anne, 2004). The history of antibiotics obtained from *Streptomyces* spp. began with the discovery of Streptothricin in 1942 (Sanglier et al., 1993), and the discovery of Streptomycin in 1943 (Schats, Bugie, & Waksman, 1994), and then scientists intensified the search for other antibiotics within the same genus (Watve et al. 2001). The golden age of antibiotic discovery (1945- 1960) (Watve et al., 2001), Today 80% of the antibiotics are derived from *Streptomyces* spp.(Kharat et al., 2009).

The antibiotics potential of extracellular metabolites of *Streptomyces* strains against some bacteria was previously reported from different locations of the world. However, there is no any report on Actinomycetes study from Nanded region are not documented. Therefore, the present study was undertaken to determine the potential of antibiotics from *Streptomyces* on some human pathogen.

2.0 Materials and Methods

2.1 Collection of soil sample: Soil samples were collected in sterile plastic bags from different places of Nanded region, (MS) India. Samples were transported to Microbiology laboratory A.C.S College, Gangakhed, labeled and stored in refrigerator for further investigation.

2.2 Isolation and cultural conditions: One gram of the soil sample were weighed and dissolved in 10ml of distilled water and 10 fold dilution were performed. 0.1 ml of dilution were spread on starch casein agar medium and incubated at 28-30□ for 4-5 days. The grown colonies of Actinomycetes were sub-cultured and maintained for further use.

2.3 Screening for bioactive compound producing Actinomycetes: The isolates were re-used for the production of bioactive compounds. The isolates were inoculated in starch casein broth medium. The inoculated flask was kept for incubation at 28-30□ for 4-5 days on rotatory shaker. After incubation, the broth was extracted and purified for secondary metabolite.

2.4 Morphological and Taxonomical identification of isolate: The Actinomycetes isolate were proceed for morphological characters and microscopic observation to check mycelial growth and spore chain structure by cover slip method fig.2. The taxonomic identification of isolate Was based on Bergey's Mannual of systematic bacteriology 9th edition.

2.5 Antimicrobial activity: 24hrs old active culture of *S.typhi, K.pneumonia, E.coli,* and *P.aeruginosa* were spread on Muller hinton agar. The wells (6 mm diameter) were cut using a sterile cork borer on Muller hinton agar. The partially purified metabolite was loaded into each well of agar plate and left for 20 min until the metabolite was diffuse. Then after plates were incubated at 37□ for 24-48hrs. after incubation the zone of inhibition were measured and recorded. (Table 4.0)

3.0 Result and discussion :

Antibiotics are the most important bioactive compounds for the treatment of infectious diseases. But now, because of the emergencies of multi-drug resistant pathogens, there are basic challenges for effective treatment of infectious diseases. Thus, due to the burden for high frequency of multidrug resistant pathogens in the world, there

has been increasing interest for searching effective antibiotics from Actinomycetes in diversified ecological niches. Actinomycetes are one of the most efficient groups of secondary metabolite producers and are very important from an industrial prospectives. Among the various genera, *Streptomyces* is the major producers of commercially important bioactive compounds. Many species have been isolated and screened from the soil in the past decades.

Consequently the chance of isolating a novel *Streptomyces* strain from novel habitat, which would produce new biologically active metabolites, has reduced. The most relevant reason for discovering novel secondary metabolites is to overcome the problem of multidrug resistant pathogens, which are no longer susceptible to the currently used antibiotics. Existence of *Streptomyces* is efficient producers of secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, and insecticidal and enzyme inhibition. *Streptomyces* have been proven as a potential source of bioactive compounds and richest source of secondary metabolites. Bioactive compounds from *Streptomyces* possess distinct chemical structures that may be form the basis for synthesis of new drugs that could be used to combat multidrug resistant pathogens.

Now a days, severe diseases such as Typhoid, Pneumonia and Shigella like diseases causes due to pathogenic bacteria viz. *S.typhi, K.pneumoniae, E.coli,* and *P.aeruginosa.* The present antibiotics available in the market are resistant to such organism. So it is necessary to find out novel potent antibiotics, which is effective against pathogens. The isolate was identified according to the criteria of Bergey's manual of bacteriology. The result of morphological and biochemical properties (Table no.1) reveals that the isolate was found to be the spp. of *streptomyces.* Antibiotic production in starch casein media occoured after 7-8 days in different extract shown in table3.0.

The result obtained in the study showed that antibacterial activities against pathogenic bacteria. In this method, the obatain results revealed that the isolates, exhibited broad spectrum activities against tested pathogens. After production,

metabolites was extracted by solvent. Crude extract of Ethyl acetate, Chloroform and n-butanol of *Streptomyces* exhibited the antibacterial effect against different pathogens. This study reveals that n-butanol extracted metabolite shows good results against human pathogen, moreover this extract shows effective against *P.aeruginosa.* when a novel metabolite compared with standard streptomycin, the novel metabolite shows better results.

Masna Rai, et. al., 2016 had reported that the genus *Streptomyces.* isolated colonies largely inhibited the growth of *E. coli* and *Staphylococcus aureus* with zone of inhibition more than 20mm for both.

Prakasham Reddy Shetty, et. Al., 2014, reported that the antibacterial activity of pure compound was performed by cup plate method against some pathogenic bacteria including of streptomycin resistant bacteria like (*Pseudomonas mirabilis*, *Pseudomonas putida and Bacillus cereus*).

Abebe Bizuye et. al., 2013, reported that isolation and screening of Actinomycetes from such areas in optimum condition may contribute the discovery of new antibiotics. Potent antibiotics from these Actinomycetes could contribute a lot to fight against antibiotic resistant pathogens. Selected isolates have been shown strong antimicrobial activity against resistant pathogens. Further purification, structural elucidation and characterization are recommended to know the quality, novelty and commercial value of these antibiotics.

P.Ashok Kumar et. al., 2016, has reported 32 strains of Actinomycetes were isolated and subjected to primary screening by giant colony method against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Aeromonas hydrophilia.*

Nooshin Khandan et. al., 2015, had reported that the crude n-butanol extract of the ACTK2 strain of *S. flavogriseus* showed a broad spectrum of antimicrobial activities against the test organisms and this opened further research investigations on purification and structural characterization of the active compounds from the crude extract.

Basavaraj K Nanjwade et. al., 2010 had reported the production of antibiotic from Actinomycetes isolated from soil and evaluate its antimicrobial activities. Some Actinomycetes strains showed promising antimicrobial activity against different strains of bacteria and fungi. Out of the six strains selected, one strain, designated A-4, showed maximum antimicrobial property against Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram negative strains *(Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia*) as well as against various fungi (*Candida albicans, Saccharomyces cerevisiae*).

Our results are in accordance with the reported study and found to be useful for controlling the multidrug resistant pathogens. The reported production of Actinomycetes metabolites and our results are matching 95% similarity hence this study is useful in the future.

Table 1.0 Morphological characteristic of sub-cultured Actinomycetes.

Colony Character	C-1	C-2
Size	3 mm	2 mm
Shape	Circular	Circular
Colony Color (Surface)	White	Brown
Colony Color (Submerged)	Pale Yellow	Dark Maroon
Margin	Entire	Irregular
Surface	Chalky	Powdery
Elevation	Convex	Convex
Opacity	Opaque	Opaque
Consistency	Sticky	Mucus
Gram's Nature	Positive	Positive
Mycelia arrangement	Areal filamentous	Areal Filamentous

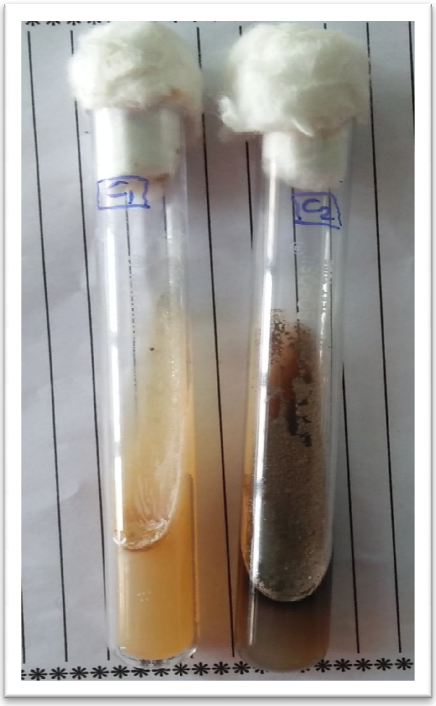

Fig. 1.0 Sub-cultured Actinomycetes on Starch Casein Agar slant

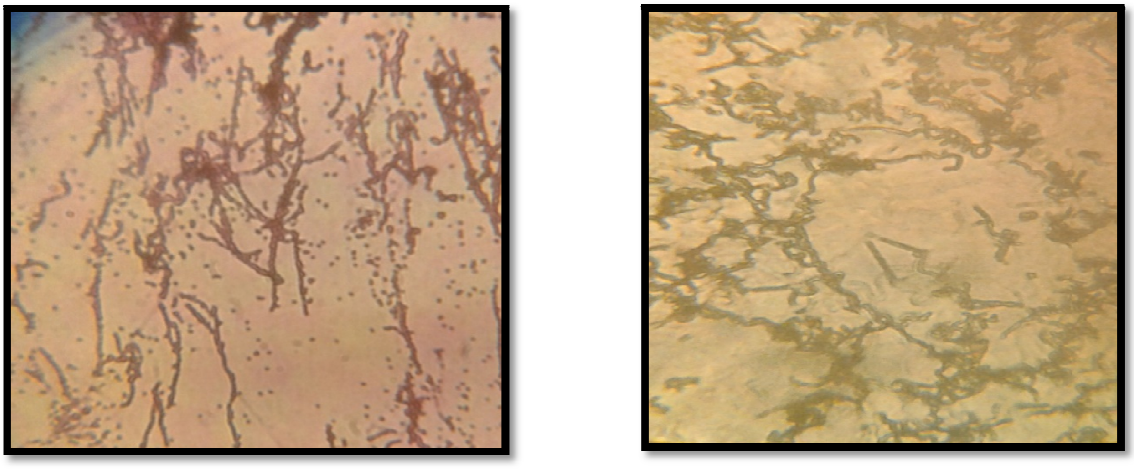

Fig. 2.0 Microscopic view of Actinomycetes

Table 2.0 Biochemical analysis of Actinomycetes

Biochemical Test	C-1	C-2
Melanin production	-	-
Starch Hydrolysis	+	+
Carbon Source Utilization	+	+
Urease Teat	+	-
Pigmentation	-	+
Catalase Test	+	-
Gram's Nature	+	+
Oxidase Test	+	+
Mycelial Arrangement	Filamentous	Filamentous
Gelatin Hydrolysis	+	-
Nitrate Reduction Test	-	-

3.1 Identification of Isolates of Actinomycetes : From the biochemical analysis and Bergey's manual of systemic bacteriology, genus was identified as ***Streptomyces*** **spp**

Fig. 3.0 Production Of metabolites in Starch Casein Broth

3.2 Extraction of Bioactive Compounds from Fermented Broth

Table 3.0 Extraction of metabolite by solvents

Solvent	Weight of empty ependrop tube (g)	Weight of ependrop tube after drying (g) **C-1**	Weight of ependrop tube after drying (g) **C-2**
Ethyl acetate	1.12	1.17	1.16
Chloroform	1.12	1.18	1.14
n-butanol	1.12	1.14	1.14

3.3 Screening of *Streptomyces* for antibacterial activity:

The result obtained in the study showed that antibacterial activities against pathogenic bacteria. In antagonistic activity method, results revealed that the isolates, exhibited broad spectrum activities against tested pathogens. After production, metabolites was extracted by solvent. Crude extract of Ethyl acetate, Chloroform and n-butanol of *Streptomyces* exhibited the antibacterial effect against different pathogens is shown in table 4.0. This study reveals that n-butanol extracted metabolite shows good results against human pathogen, moreover this extract shows effective against P.aeruginosa.

Table 4.0 Secondary screening showing zone of inhibition of n-butanol extract.

Actinomycetes isolate	Organism	zone of Inhibition in (mm) shown by **n-butanol**
C-1	*S.typhi*	10mm
C-2	*S.typhi*	9mm
C-1	*E.coli*	9mm
C-2	*E.coli*	7mm
C-1	*K.pneumoniae*	8mm
C-2	*K.pneumoniae*	0mm
C-1	*P.aeruginosa*	**11mm**
C-2	*P.aeruginosa*	4mm

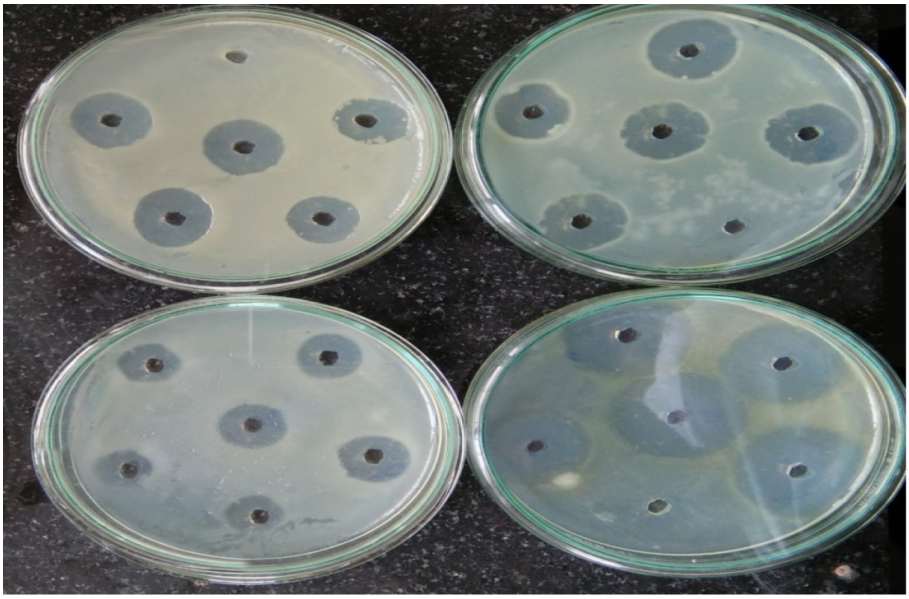

Fig. 4.0 Zone of inhibition due to n-butanol extract of metabolites

4.0 Conclusion:

The present study concludes that the isolated strain is identified as Streptomyces spp. and was referred with the Bergey's manual of systematic bacteriology. The product of novel spp. produce bioactive compound in 50ml starch casein media about 1.18gm in chloroform extract. The isolate have proven to be effective against human pathogen like *E.coli, S.typhi, K.pneumoniae* and *P.aeruginosa.*. but the most potent activity showed against *P.aeruginosa.* this novel strain produce bioactive compound which is crude one that need to be further purification for better results.

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