International Research Journal of Human Resource and Social Sciences
ISSN(O): (2349-4085) ISSN(P): (2394-4218)
Impact Factor 6.924 Volume 9, Issue 12, December 2022
Website- www.aarf.asia, **Email** : editoraarf@gmail.com

ASSESSING ISOLATION OF ACTINOMYCETES STRAINS AND ITS DIFFERENT ACTIVITIES

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ABSTRACT

Since actinomycetes have the potential to produce antibiotics, anticancer agents, immunosuppressive agents, and enzymes, they are an economically and biotechnologically valuable group of prokaryotes. Most antibiotics (about 80%) come from two Actinomycetes species, Streptomyces and Micromonospora. Over the course of the last century, scientists have created close to a hundred unique antibiotics for use across multiple species. Streptomyces bacteria account for almost 60% of all antibiotic production. Researchers have examined the antimicrobial efficacy of actinomycetes isolated from the ecosystems of Jeddah and Al-Madinah Al-Munawwrah. Growth and antibiotic production by the selected Actinomycetes isolates were studied, as well as the effects of the culture filtrate on cell shape, cell growth, and cell wall composition of the microorganisms used in the experiment. The Madinah isolate D8 was shown to be the most potent antibacterial agent. This sample was identified as a Streptomyces flavogriseus strain using 16s rRNA. Since actinomycetes have the potential to produce antibiotics, anticancer agents, immunosuppressive agents, and enzymes, they are an economically and biotechnologically valuable group of prokaryotes. Most antibiotics (about 80%) come from two Actinomycetes species, Streptomyces and Micromonospora. Over the course of the last century, scientists have created close to a hundred unique antibiotics for use across multiple species. About 60 percent of all antibiotics are made by the bacterium Streptomyces.

Keywords: -Antimicrobial, Streptomyces, Isolation, Actinomycetes, Bacteria

I. INTRODUCTION

Gram-positive, prokaryotic bacteria are known as actinomycetes. Most are classified as members of the order Actinomycetes and the subclass Actinobacteria. A high percentage of G+C (>55 mol%) may be found in the DNA of all members of this group. Akitino means "ray" and "mykes" is the Greek word for "fungus," hence these bacteria are classified as fungi despite their bacterial origins. In actinomycetes taxonomy, the shape and arrangement of spores and cell chemistry (cell wall and total cell composition, type of lipid, isoprenoid Quinone's) play crucial roles in classifying these bacteria. The Actinomycetes are divided into seven groups in Bergey's Manual of Systematic Bacteriology, 1st edition, mostly based on cell wall type, conidia arrangement, and sporangium presence or absence. The use of 16S rRNA sequences to consolidate the phylum Actinobacteria into a single super phylum was included in the second edition. There was a single class, five subclasses, six orders, fourteen suborders, and forty families in this phylum.

Low-molecular-weight molecules that aren't the end result of an organism's core metabolic pathway are considered secondary metabolites. It was previously believed that these products had no part in the fundamental cellular processes that sustain microbial life. Therefore, it was assumed that the host microbe gained nothing from the production of secondary metabolites. Secondary metabolites come in a wide range of structural complexity, some of which include enzyme inhibitors, anticancer drugs, immunosuppressants, and anti-parasitic medicines. These chemicals have extensive industrial, agricultural, and forestry uses in addition to their medicinal significance. The actinomycetes, and particularly the genus Streptomyces, are a significant resource for the production of physiologically active chemicals that have significant economic value and widespread medical and agricultural uses. A wide variety of antibiotics, immunosuppressant’s, extracellular hydrolytic enzymes, plant growth stimulants, and siderophores are generated by Actinomycetes. Although over 5,000 antibiotics have been discovered through the cultivation of Gram-positive and Gram-negative bacteria and filamentous fungi, only about 100 have been successfully introduced into clinical practice for the treatment of human, animal, and plant diseases.

II. ISOLATION OF ACTINOMYCETES

For a week, the samples were allowed to air dry at room temperature. Serial dilution and spread plate counting was used to isolate the Actinomycetes [8]. One gram of soil was diluted in nine milliliters of double-distilled sterile water. Aliquots (0.1 ml) of 10-2, 10-3, 10-4, and 10-5 Gram staining and lacto phenol blue staining were spread over the agar used to isolate actinomycetes, and the cellular and spore chain morphology was evaluated using a coverslip culture technique and a light microscope. A combination of nalidixic acid (100 mg/l) and actidione (20 mg/l) was effective in inhibiting bacterial and fungal growth. Ten days later, the plates were still at 30 degrees Celsius. The plates were periodically inspected throughout incubation to ensure the actinomycetes were expanding. After incubation, colonies with distinct morphologies on actinomycetes isolation agar plates were selected for further purification by the repeated streak plate method. After isolating pure colonies, scientists analyzed them to identify which species

they belonged to by smelling them (for earthiness or sweetness), seeing their colony shape and color, and checking for signs of aerial and substrate mycelium. Actinomycetes colonies were isolated, identified, and then cultured on starch casein agar slants at 27 degrees Celsius. After incubation, the pure actinomycetes isolates were stored on slants in the refrigerator at 4 degrees Celsius.

III. MATERIALS AND METHODS

Chemicals and media

Starch Casein agar (SCA), Actinomycetes isolation agar (AIA), Mueller Hilton agar, nutrient broth, starch casein broth, glucose phosphate broth, peptone water, Simmons citrate agar, triple sugar iron agar, dimethyl sulfoxide, nalidixic acid, actidione, and ciprofloxacin were all used as culture media and chemicals in this investigation.

Sample collection

Soil samples were collected at 19 sites around Ad-Dawadmi, Saudi Arabia, including the Applied Medical Science College campus, AdDawadmi Park, and the Ad-Dawadmi old market. At the Applied Medical Science College in Ad-Dawadmi, a sample of soil was taken from a depth of 5 to 25 centimeters, sealed in sterile plastic, and delivered to the Microbiology lab. When soil samples are tagged, they are immediately placed into polypropylene bags to prevent significant moisture loss. We collected clinical strains of bacteria such Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus from the Ad-Dawadmi General Hospital in Saudi Arabia.

Statistical analysis

The experiment has been conducted three times. The mean and standard deviation were calculated. The mean values of inhibitory zones formed by crude extracts and the control antibiotic tested against test organisms were compared, and the statistical significance of the differences between the two groups was determined using a Students t-test (based on Primer of statistics).

IV. RESULTS AND DISCUSSION

Isolation of actinomycetes

From 19 soil samples tested at the Applied Medical Science College Microbiology lab in Ad-Dawadmi, Saudi Arabia, 9 actinomycetes isolates were found. Table 1 demonstrates that four (44%) of the nine isolates were discovered in Ad-Dawadmi Park, three (33%) in the Ad-Dawadmi old market, and two (22%) on the Applied Medical Science College campus. Actinomycetes were isolated and counted using an agar medium for isolating the organisms, followed by a serial

dilution and spread plate technique. All nine isolates displayed typical actinomycetes morphology when cultured on actinomycetes isolation agar. Colonies grew slowly; oxygen was present; mycelia in the air and on the substrate were different colors; and the colonies were aerobic.

Table 1: Actinomycetes isolates from soil samples collected from different sites of Dawadmi

Collection sites	No. of soil samples	No. of isolates	Isolate number
Applied Medical College campus	6	2	AMC1, AMC2
Dawadmi old market	6	3	DOM1,DOM2, DOM3
Dawadmi park	7	4	DP1, DP2,DP3,DP4

Characterization and identification of actinomycetes

The actinomycetes with the highest antibacterial activity were determined by their morphological and biochemical characteristics (tables 2 and 3). Bergey"s manual of determinative bacteriology [23] was used in order to cross-reference the observed structure. With the exception of DP3, none of the isolates were able to generate indole, however they all passed the methyl red and vogus-proskauer tests and generated citrate. Although none of the isolates passed the TSI test, they all reduced nitrate, hydrolyzed starch, and produced catalase (with the exception of DOM3). Mannitol was used successfully by both DP3 and DP4. Every isolate tested positive for sucrose intake except DP3. Culture, morphology, physiology, and biochemistry data were used to determine that these isolates belonged to the genus Streptomyces. Antibacterial effects with a broad spectrum of activities have been shown for Streptomyces sp., which produces a favorable bioactive metabolite [13, 17-22].

Table 2: Morphological characteristics of potential isolate

Organism identified	Nature of mycelium	Color of the colony	Spore surface	Gram stain
Streptomyces species (DOM1)	extensively branched, aerial and substrate mycelium	white yellow	wrinkled	+
Streptomyces species (DOM3)	branched, aerial and substrate mycelium	white pink	wrinkled	+
Streptomyces species (DP3)	granular, aerial and substrate mycelium	yellowish brown	smooth	+
Streptomyces species (DP4)	branched, aerial and substrate mycelium	creamy	wrinkled	+

+= Positive

Table 3: Biochemical characteristics of potential isolates of actinomycetes

Biochemical test	DOM1	DOM3	DP3	DP4
Indole	-	-	-	-
MR	-	+	+	+
VP	-	-	-	-
Citrate	+	+	-	+
TSI	-	-	-	-
Nitrate reduction	+	-	+	+
Starch hydrolysis	+	+	+	+
Catalase test	+	+	+	+
Mannitol utilization	-	-	+	+
Sucrose utilization	+	+	-	+

V. CONCLUSION

Based on these findings, actinomycetes present in soil samples from Ad-Dawadmi are capable of inhibiting bacterial pathogens via the use of metabolites. We showed that the crude metabolites from four different Streptomyces isolates had significant antibacterial action against four different clinical infections. Purifying bioactive metabolites from these Streptomyces sp. and carrying out molecular characterization of the isolates is crucial for discovering novel compounds with commercial importance. This topic is still being investigated. Actinomycetes were tested against harmful bacteria and fungi isolated from diverse sites in Jeddah and Al-Madinah Al-Munawwrah. According to the findings, the Madinah isolate D8 is the most potent antibacterial agent. Using 16s rDNA, this isolate was later determined to be Streptomyces flavogriseus (KF235416).

REFERENCES:-

1. Adegboye MF, Babalola OO. Taxonomy and ecology of antibiotic-producing actinomycetes. Afr J Agric Res 2012;7:2255-61.

2. Yuan L, Zhang Y, Yu L, Sun C, Wei Y, Liu H, et al. Actinopolymorphacephalotaxi sp. nov., a novel actinomycete isolated from rhizosphere soil of the plant Cephalotaxus fortune. Int J SystEvolMicrobiol 2010;60:51-4.

3. Berdy J. Bioactive microbial metabolites. J Antibiot 2005;58:1-26

4. Poopal AC, Laxman RS. Studies on the biological reduction of chromate by Streptomyces griseous. J Haz Mat 2009;169:539-45.

5. Elardo PSM, Scheuermayer M, Kozytska S, Hentschel U. Streptomyces axinellae sp. isolated from the Mediterranean sponge. Axinellapolypoides (Porifera). Int J SystEvolMicrobiol 2009;59:1433-7.

6. Aghamirian RM, Ghiasian AS. Isolation and characterization of medically important aerobic actinomycetes in the soil of Iran. Open Microbiol J 2009;3:53-7.

7. Pandey, B., Ghimire, P. and Agrawal, V. P. 2004. Studies on antibacterial activity of soil from Khumbu Region of Mount Everest. International Conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Kathmandu. Turk. J. Biol. 27:79-84.

8. Bharti, A., Kumar, V., Gusain, O. and Bisht, G. S. 2010. Antifungal activity of actinomycetes isolated from Garhwal region. Journal of Sci. Engg. & Tech. Mgt. 2(2):3-9.

9. Rahman, M., Islam, M. Z., Islam, M. and Ul, A. 2011. Antibacterial activities of Actinomycete isolates collected from soils of Rajshahi, Bangladesh. Biotechnol. Res. Int. Articel ID 857925, 6 p.

10. Arasu, M. V., Duraipandiyan, V. and Ignacimuthu, S. 2013. Antibacterial and antifungal activities of polypeptide metabolite from marine Streptomyces sp. AP-123 and its cytotoxic effect. Chemosphere 90:479- 487.

11. Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

12. Raja, A., Prabakaran, P., Gajalakshmi, P. and Rahman, A. 2010. A population study of psychrophilic actinomycetes isolated from Rothang hill-Manali soil sample. J. Pure Applied Microbiol. 4:847- 851.

13. Al-Zahrani, S. 2007. Studies on the antimicrobial activity of Streptomyces sp. isolated from Jazan. JKAU Sci. 19:127-138.

14. Abo-Shadi, M. A. A. R., Sidkey, N. M and Al-Mutrafy, A. M. 2010. Antimicrobial agent producing microbes from some soils' rhizosphere in Al-Madinah Al-Munawwarah, KSA. Journal of American Science 6(10):915-925.

15. Valanarasu, M., Kannan, P., Ezhilvendan, S., Ganesan, G., Ignacimuthu, S. and Agastian, P. 2010. Antifungal and antifeedant activities of extracellular product of Streptomyces spp. ERI-04 isolated from Western Ghats of Tamil Nadu. Journal de MycologieMédicale 20:290-297.