



## RESEARCH ARTICLE

### ISOLATION OF PIGMENT PRODUCING ACTINOMYCETES FROM SOIL AND SCREENING THEIR ANTIBACTERIAL ACTIVITIES AGAINST DIFFERENT MICROBIAL ISOLATES

**\*Anjali Tandale, Mayuri Khandagale, Rutuja Palaskar and Snehal Kulkarni**

Department of Biotechnology, Shivchhtrapati College, Aurangabad, M.S., India

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#### ABSTRACT

The present study is focused on the extraction of intracellular pigment from actinobacteria isolated from different areas of Aurangabad, Maharashtra, India. A total 7 Actinomycetes strains were isolated, out of a total seven isolates, five actinobacterial isolates showed pigmented growth. The biochemical characterization of isolates was also studied. Different production media were used for pigment production and MGYP was selected for large scale production. Further the intracellular pigment was extracted by cell disruption technique using ultrasonication and partially purified using ethyl acetate as the solvent. Partial characterizations of the pigments were carried out using UV-Visible spectrophotometry. The extracted pigment showed significant antibacterial activity against Gram negative and Gram positive bacteria. The obtained results showed broad spectrum antibacterial activity.

**Key words:** Actinomycetes, Pigments, Agar well diffusion method, Antibacterial activity, Pathogenic microorganisms.

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#### INTRODUCTION

Actinomycetes are filamentous Gram-positive bacteria, are a large group of aerobic, high G-C percentage bacteria that form branching filaments or hyphae and asexual spores. These bacteria closely resemble fungi in overall morphology presumably this resemblance results partly from adaptation to the same habitat. The composition of cell wall in actinomycetes varies greatly among different groups and is of considerable taxonomic significance. Four major cell wall types are distinguished in these filamentous bacteria on the basis of the three features of peptidoglycan composition and structure. These features are (i) diaminopimelic acid isomer on tetrapeptide side chain position 3, (ii) sugar content of peptidoglycan, and (iii) the presence of glycine in interpeptide bridges. As is evident in, characteristic sugar patterns are present only in cell wall types II-IV of those actinomycetes with meso-diaminopimelic acid. Currently, the whole world is looking towards the usage of natural pigments over synthetic colorants due to the potential health hazard effects. Natural pigments obtained from plants are usually limited, unstable, highly priced, and require more complex and tedious process for production. In comparison, pigment obtained from microbes can be easily produced in sufficient amount, are cost effective and have a simpler extraction and purification process.

The range of shades that can be possible obtained is more varied. Moreover, these are safer and serve better as an economical substitute for the commercially available colorants. People are more interested in natural food, herbal medicines and traditional practices for healthy life. Higher demands were seen for the natural products from biological substances such as, plants and microorganisms. Recently, bio-cosmetics are pushing through the cosmetics industry in the world and the demand for the bio-cosmetics is rising and quite significant. Actinomycetes are potential source of many bioactive compounds, which have diverse clinical effects and important applications in human medicine. It has been estimated that approximately one-third of the thousands of naturally occurring antibiotics have been obtained from actinomycetes. According to the World Health Organization over prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens (Oskey M., et al., 2004). *Streptococcus aureus*, *E.Coli*, *Staphylococcus aureus* and *Bacillus* strains are virulent pathogen that is responsible for a wide range of infections and has developed resistance of most classes of antibiotics. Hence there is need to rediscover new drugs active against these drugs resistance pathogens. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi. The present study was under taken to isolate *Actinomycetes* from the soil samples of Aurangabad and to assess their antibacterial properties. The resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria

\*Corresponding author: Anjali Tandale,  
Department of Biotechnology, Shivchhtrapati College, Aurangabad, M.S.



Fig. 1. Isolates of pigment producing Actinomycetes



Fig. 2. Microscopic view of isolate under 100x observation

So we need to screen more and more actinomycetes from different habitat antibacterial activity in hope of getting some actinomycetes strains that produce antibiotics that have not been discovered yet and active against drugs resistant pathogens.

## MATERIALS AND METHODS

**Sample collection and Isolation of Actinomycetes:** 11 different soil samples were collected from different areas of Aurangabad, Maharashtra, India. Isolation of actinomycetes was performed by serial dilution and spread plate technique using Actinomycetes isolation agar (AIA) and Starch casein agar medium. Plates were incubated at 37°C for 4-8 days.

### Biochemical Characterisation and Identification of Actinomycetes:

- 1. Grams staining:** Grams staining was performed and Isolates of actinomycetes were observed under a high power magnifying lens.
- 2. Morphological characterization:** Isolates of actinomycetes colonies were observed and colony morphology was noted with respect to size, shape, margin, elevation, color, aerial and substrate mycelium, branching, and the nature of colony.
- 3. Biochemical characterization:** Actinomycetes isolates were biochemically characterized by IMViC test, catalase, nitrate reduction, starch hydrolysis test, Triple Sugar Iron test, urease test, gelatinase test, sugar fermentation test.

### Fermentation process of pigment producing Actinomycetes

The pigmented actinobacterial isolate was inoculated into production media MGYP, Luria-Bertani (LB broth) and actinomycetes isolation broth separately and incubated at 37°C for 7 days on a rotary shaker (REMI) at 100 rpm. MGYP produced more amount of actinobacterial pigment. Large scale production was done by using MGYP broth.

### Extraction and partial purification of pigment from Actinomycetes

After incubation, the production media was harvested and centrifuged at 10,000 rpm for 10 min in a cooling centrifuge (REMI). The cell pellet was collected to extract intracellular pigments. Further, cell pellet was subjected to ultrasonication and the intracellular pigments were extracted by using polarity

### Partial characterization of pigment

**UV-Visible Spectrophotometry:** The max of the isolated compound was observed by UV-Visible spectrum analysis which is useful to detect the absorbance range of the extracted pigment. The extracted pigment was dissolved in ethyl acetate solvent, subjected to UV analysis in the range level of 200-900 nm with the help of UV-visible spectrometer (Systronic)

### Applications of Bio Pigment

**Antimicrobial activity:** Actinomycetes pigments were selected for antibacterial activity screening against the pathogenic test organism by agar well diffusion method on agar medium. Antibacterial activity was tested in agar well diffusion technique against Gram positive and Gram negative bacteria like *Bacillus subtilis*, *S. aureus*, *E.coli*, *S. typhi*.

## RESULTS AND DISCUSSION

**Sample collection and Isolation of Actinomycetes:** 11 Soil samples were collected from different areas of Aurangabad, Maharashtra, India. Among them, seven isolates showed different colored growth and an aerial whitish mass. (Fig. 1) Actinomycetes isolation agar and starch casein agar were used for the isolation of actinobacteria, among these two media Actinomycetes isolation agar enhanced more number of actinobacterial isolate. They were studied for culture characteristics.

### Biochemical Characterisation and Identification of Actinomycetes:

- 1. Grams staining:** All actinomycetes isolates were Gram's stain positive (Fig.2).
- 2. Morphological characterization:** Isolates of actinomycetes colonies were observed and colony morphology was noted as in Table 1.
- 3. Biochemical characterization:** After preliminary studies, the isolates which were found to be positive were selected for biochemical studies. Biochemical tests generally carried as in Table 2, 3.

### Fermentation, Extraction and partial purification of pigment from Actinomycetes:

The pigmented actinobacterial isolate was inoculated into 3 different production media MGYP, LB broth and actinomycetes isolation broth separately. MGYP produced more amount of actinobacterial pigment. Large scale production was done by using MGYP broth. Extraction and partial purification of pigment was done by

**Table 1. Morphological characteristics of Isolates of actinomycetes**

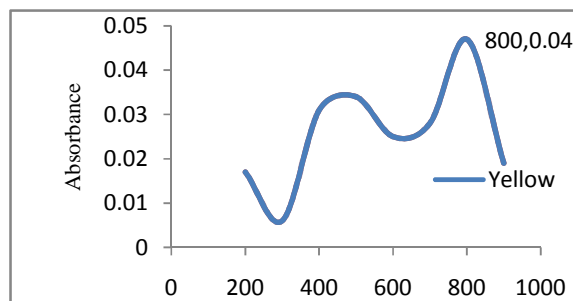
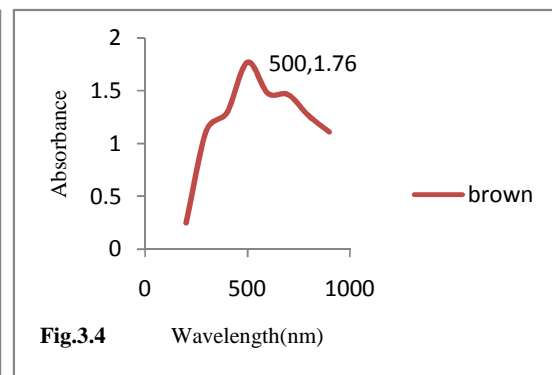
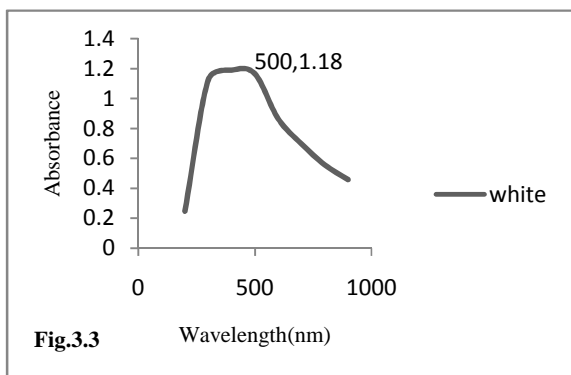
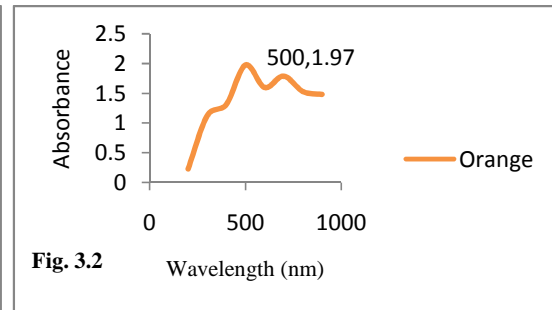
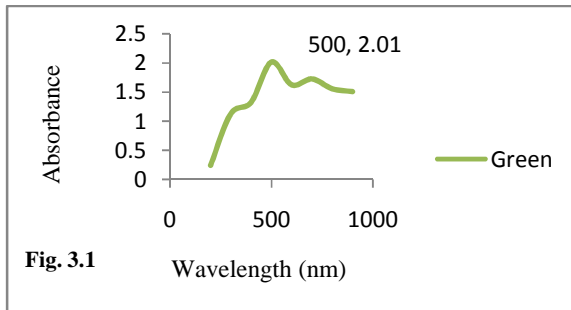
Colony characteristics	Colony I	Colony II	Colony III	Colony IV	Colony V	Colony VI	Colony VII
Size	9mm	4mm	4mm	8mm	3mm	9mm	3mm
Shape	Irregular	Circular	Circular	Irregular	Circular	Irregular	Circular
Color	Green	Orange	Deep Orange	Brown	Yellow	Grey	White
Margin	Entire	Convex	Convex	Undulate	Entire	Entire	Entire
Elevation	Flat	Umbonate	Flat	Umbonate	Umbonate	Flat	Umbonate
Consistency	Sticky	Sticky	Sticky	Dry	Sticky	Dry	Dry
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gram's Nature	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Motility	Motile	Motile	Motile	Non-motile	Non-motile	Non-motile	Non-motile

**Table 2. Biochemical characterization of actinomycetes**

Biochemical tests	Green	Orange	Deep Orange	Brown	Yellow	Grey	White
Indole Test	Positive	Positive	Positive	Positive	Negative	Negative	Positive
MR Test	Positive	Negative	Negative	Negative	Negative	Negative	Negative
VP Test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Citrate utilization Test	Positive	Positive	Positive	Positive	Negative	Positive	Positive
Catalase Test	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Starch Test	Positive	Positive	Positive	Positive	Negative	Positive	Positive
Urease Test	Negative	Negative	Negative	Negative	Negative	Positive	Positive
Gelatinase Test	Positive	Negative	Positive	Negative	Negative	Positive	Negative
Nitrate Reduction Test	Positive	Positive	Positive	Negative	Positive	Positive	Positive
TSI Test	Negative	Positive	Positive	Positive	Negative	Negative	Positive

**Table 3. Utilization of different carbon sources of Actinomycetes**

Carbon sources	Green	Orange	Deep Orange	Brown	Yellow	Grey	White
Glucose	Positive	Negative	Negative	Negative	Positive	Negative	Positive
Fructose	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Lactose	Negative	Positive	Negative	Negative	Positive	Positive	Negative



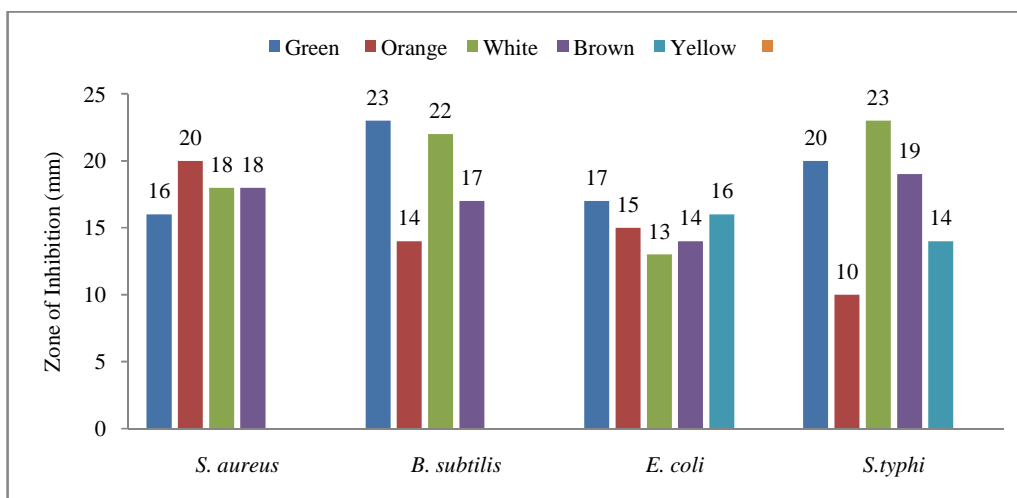


Fig. 4.1. Antibacterial activity for extracted pigments

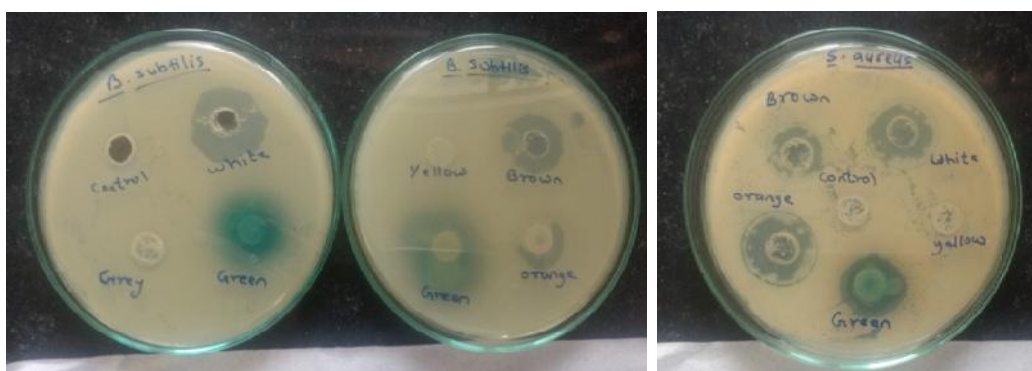


Fig. 4.2. Antibacterial activity for extracted pigments against *B. subtilis*, *S. aureus*.

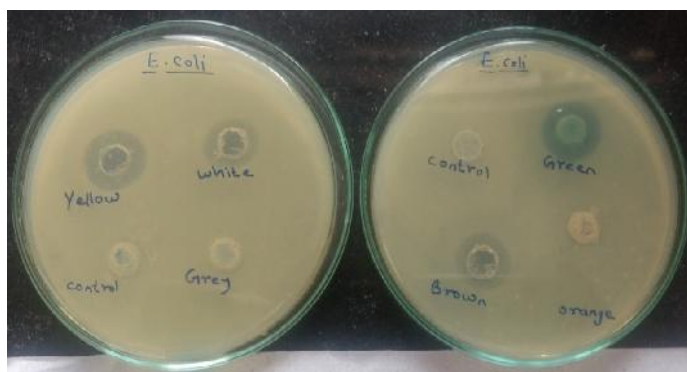
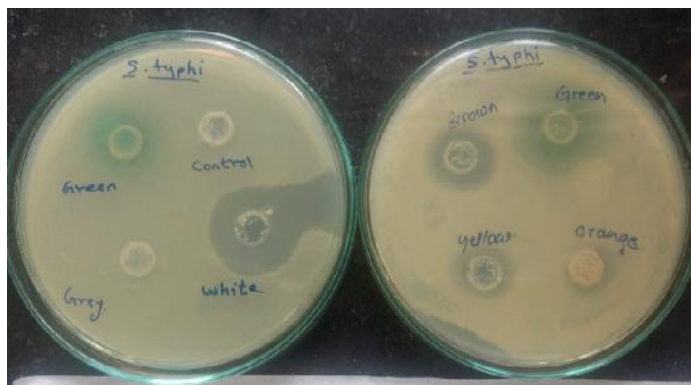


Fig. 4.3. Antibacterial activity for extracted pigments against *E. coli*.



**Partial characterization of pigment:** Out of 7 isolates 5 pigments were selected for partial characterization. The absorption spectrum of all pigments were observed at 500nm (Fig. 3.1-3.4) except yellow pigment which showed maximum absorption at 800nm that showed the maximum optical density as in figure (fig. 3.5)

### Applications of Bio pigment

**Antimicrobial activity:** Antimicrobial activity was tested in agar well diffusion technique against Gram positive and Gram negative bacteria. These isolates were selected for their broad spectrum of activity and zone of inhibition in mm as in antibiogram (Fig. 4.1) (Fig. 4.2, 4.3 & 4.4).

### DISCUSSION

In present work, seven isolates showed different colored growth and two isolates were motile as well as biochemical characterization was done in which many of the isolates were positive for Indole and nitrate reduction test. Antimicrobial activity was tested in agar well diffusion technique against Gram positive and Gram negative bacteria. The results indicated that extracted Actinomycetes pigments were highly active against *E.Coli*, *Staphylococcus aureus* and *Bacillus subtilis* and *Salmonella typhi*. Extracted pigments were highly active with an inhibition zone more than 18 mm in diameter and highest zone of inhibition was 23mm diameter.

### Conclusion

Extracted pigments of actinomycetes showed best inhibitory activity against broad spectrum of bacteria, i.e. gram positive and gram negative. The present study demonstrated that soil samples collected from different areas of Aurangabad, Maharashtra, India is congruous for the isolation of pigment producing actinomycetes. In this present study, from 11 different soil samples 7 pigment producing isolates were collected. From them 5 different pigments like green, orange, white, yellow and brown were extracted and purified successfully. Furthermore pigments were tested for antimicrobial activity which showed that all extracted pigments inhibited growth of gram positive and gram negative bacteria, except yellow pigment which showed inhibitory effect against only gram negative bacteria. Therefore the study concludes that actinomycetes have excellent ability to produce natural pigment and has antimicrobial activity which could be very useful for pharmaceuticals and other industries.

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### REFERENCES

Alanis A. J. 2005. Archives Med Res, 36, pp 697-705.

Baltz R. H. 2005. SIM News, 55, pp186-196.

Baltz R. H. 2007. Microbe, 2, pp125-131.

Butler M. S. and Buss A. D. 2006. *Biochem Pharmacol*, 71, pp919-929.

Doumbou, CL., V. Akimov, and C. Beaulieu, 1998. Selection and characterization of microorganisms utilizing thaxtomin A, a phytotoxin produced by *Streptomyces scabies*. *Appl. Environ. Microbiol.* 44: 4313-4316.

Doumbou, CL., V. Akimov, M. Côté, P.M. Charest, and C. Beaulieu, 2001. Taxonomic study on nonpathogenic streptomycetes isolated from common scab lesions on potato tubers. *Syst. Appl. Microbiol.* 24: 451-456.

Duripandiyani V., Sasi A. H., Islam V.I.H., Valanarasu M. and Ignacimuthu S. 2010. *J Mycologie Medicale*, 20 (1), pp15-20.

El-Abyad, M.S., M.A. El-Sayed, A.R. El-Shanshoury, and S.M. El-Sabbagh, 1993. Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. *Plant Soil* 149: 185-195.

El-Tarabily KA, M.H. Soliman, A.H. Nassar, H.A. Al-Hassani, K. Sivasithamparan, F. McKenna and G.E.St.J. Hardy, 2000. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathol.*, 49: 573-83.

El-Tarabily, K.A. 2003. An endophytic chitinase-producing isolate of *Actinoplanes missouriensis*, with potential for biological control of root rot of lupin caused by *Plectosporium tabacinum*. *Australian J. Botany*, 51: 257-66.

Endo, A., and T. Misato, 1969. Polyoxin D, a competitive inhibitor of UDP-N-acetylglucosaminyltransferase in *Neurospora crassa*. *Biochem. Biophys. Res. Commun.*, 37:718-722. Euzéby, J.P., 2008.

Enright M.C. 2003. *Curr Opin Pharmacol*, 3 (5), 474-479.

Fayad, K., A.M. Simao-Beauvoir, A. Gauthier, C. Leclerc, H. Mamady, C. Beaulieu, and R. Brzézinski. 2001. Purification and properties of a b-1, 6-glucanase from *Streptomyces* sp. EF-14, an actinomycete antagonistic to *Phytophthora* spp. *Appl. Microbiol. Biotechnol.*, 57 : 117-123.

Feduchi, E., M. Cosin, and L. Carrasco. 1985. Mieldiomycin: a nucleoside antibiotic that inhibits protein synthesis. *J. Antibiot.* 38: 415-419.

Feio, M.J., V.Rainha, M. A. Reis, A.R. Lino, and I.T.E. Fenical, W.; Sethna, K.M.; Lloyd, G.K. Marine microorganisms as a developing resource for drug discovery. *Pharm. News*, 2002, 9, 489-494.

Fenton, A.M., P.M. Stephens, J. Crowley, M. O'Callaghan, and F. O'Gara. 1992. Exploiting gene(s) involved in 2, 4-diacetylphloroglucinol biosynthesis in order to improve the biocontrol ability of a pseudomonad strain. *Appl. Environ. Microbiol.*, 58: 3873-3878.

Flardh, K. 2003. "Growth polarity and cell division in *Streptomyces*". *Curr. Opin. Microbiol.*, 6: 561-571.

Fonseca, 2000. "The influence of the *Desulfovibrio desulfuricans* 14 ATCC 27774 on the corrosion of mild steel," *Materials and Corrosion—Werkstoffe und Korrosion*, vol. 51, no. 10, pp. 691-697.

Genus *Streptomyces*. List of Prokaryotic names with Standing in Nomenclature [http://www.bacterio.cict.fr/s/streptomyc\\_esa.html](http://www.bacterio.cict.fr/s/streptomyc_esa.html). Everse, J., K. E. Everse, and M. B. Grisham. 1990. Peroxidases in chemistry and biology, vol. 1. CRC Press, Boca Raton, Fla

Goodfellow M. and Haynes J.A. 1984. Editors, Ortiz T; Bojalil L. F. and Yakoleff V. Academic Press, London, pp453-472.

Goodfellow, M. and S.T. Williams, 1983. Ecology of actinomycetes. *Annual Review of Microbiol.* 37: 189-216.

Goodfellow, M.; Williams, S.T. Ecology of actinomycetes. *Annu. Rev. Microbiol.*, 1983, 37, 189-216.

Ishita Chakraborty, Priyanka Redkar, Minki Munjal, S. R. Sathish Kumar and K. V. Bhaskara Rao, 2015. "Isolation And Characterization Of Pigment Producing Marine

- Actinobacteria From Mangrove Soil And Applications Of Bio-Pigments”.
- Jiang C. L. and Xu L. H. 1996. *Appl Environ Microbiol*, 62, pp. 249-253.
- Jyotityagi, Dr. Tripti Bhatnagar, Dr. Fanish Kumar Pandey School Of Sciences, Noida International University, Gautam Buddh Nagar, Greater Noida (U.P), India, October - December 2014, “Isolation And Characterization Of Actinomycetes From Soil And Screening Their Antibacterial Activities Against Different Microbial Isolates”.
- Lemriss S., Laurent F., Couble A., Casoil E., Lancelin J.M. and Saintpierre-Bonaccio D. 2003. *Can J Microbiol*, 49, pp669-674.
- Mangamuri, U. K., Muvva, V., Poda, S., Kamma, S., “Isolation, Identification And Molecular Characterization Of Rare Actinomycetes From Mangrove Ecosystem Of Nizampatnam”. *Malaysian. J. Microbiol.*, 2, 83-91(2012)
- Mary Ann Gerencser and John m. Slack, Department of Microbiology, Medical Center, West Virginia University, Morgantown, West Virginia 26506
- Mohanshrinivasan, V., Sriramkalyan, P., Ipsita N., Subhadra Devi, C, Selvaranjan E, Sugandhi, V And Jeminah NS. 2013. “Fermentative Production Of Extracellular Pigment From *Streptomyces Coelicolor*”. *Res. J. Biotechnol.* 8,1-8.
- Mukesh Sharma, Pinki Dangi and Meenakshi Choudhary Department of Biotechnology, Jaipur Institute of Biotechnology, Maharaj Vinayak Global University, Jaipur (Rajasthan) India “Actinomycetes: Source, Identification, and Their Applications”
- Ramendra Singh Parmar, Charu Singh, Pragya Jadon, Gaurav Bhadauriya and Ajay Kumar Dept. of Life Sciences, ITM University, Gwalior , “Exploration of Pigment Producing Actinomycetes, Isolated From Madhya Pradesh Region of India”, *IJHER*, Vol., 6(2), 2016, 132-140.

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