



ISOLATION AND IDENTIFICATION OF BIOLOGICAL STAINS AND DYES RESISTANT THERMOPHILIC BACTERIUM

Mayuri S. Sarsar and Anupama P. Pathak*

School of Life Sciences (DST, FIST & UGC- SAP Sponsored), Swami Ramanand Teerth Marathwada University, Nanded. 431606

Corresponding author*: anupama.micro@rediffmail.com

ABSTRACT

In the present study thermophilic organisms were isolated from coastal thermal spring Unhala of Rajapur (16° 35' N, 73° 30' E) in the district of Ratnagiri, Maharashtra, India. Of these, one rapidly growing gram positive rod shaped, motile bacteria was selected and identified as *Bacillus lentus* on the basis of cultural, microscopic and biochemical characters and whose resistance to different dyes and biological stains was confirmed by observing luxuriant growth in the medium containing respective dyes and biological stains.

Key Words: thermal spring, resistance, dyes and biological stains.

INTRODUCTION

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life. synthetic chemicals dyes with the greatest variety of colours, have been used extensively used in different industries, including textiles, cosmetics, paper, leather, pharmaceutical and food , with an annual consumption of about 0.7 million ton. Wastewaters from these industries pose a threat to the environment, as significant proportion of these dyes enter the environment via wastewater. These dyes cannot be easily degraded, while some are toxic to higher animals. During the dyeing process, approximately 10–15% of the used dye, depending on the structure is released into the wastewaters, causing serious environmental and health hazards . Presence of the dyes in aqueous ecosystems diminishes the photosynthesis by impeding the light penetration into deeper layers thereby deteriorating the water quality and lowering the gas solubility. Furthermore the dyes and/or their degradation products may be toxic to flora and fauna.(Annika et al 2012)

Thus, decolourization of textile wastewaters has been a major environmental concern for a long time. Numerous studies indicate that biodegradation by microorganisms is a promising method for the decomposition of dyes in sewage. Biological methods possess many advantages in comparison to other methods used to utilization of compounds presented in wastes, particularly in dyestuff wastes. This is relatively cheap method, very effective, and an availability of a biological material is its great asset. Present investigation is therefore aimed at isolation and identification of selected dye and biological stain resistant thermophiles from Unhala hot water spring, Ratnagiri, Maharashtra, India.(Karthik et.al 2015)

MATERIALS AND METHODS

A) Sample collection

The water sample was collected from Unhala hot water spring in pre sterilized polyethylene bottles by Grab sample collection method. Temperature and Ph were recorded at the time of sample collection by digital thermometer and Ph strips respectively. Samples were transported in to lab within 12 hours and kept in refrigerant and maintained at 4°C.(Pathak and Kambale 2015,Pathak and Rathod 2016)

B) Isolation of thermophiles

Isolation was carried out by spreading 0.1 ml water sample on nutrient agar plates. The plates were incubated for 24 hours at 50°C and morphologically different and rapidly growing colony was selected for further study.

(Pathak and Jethaliya 2015,Polkade 2015)

C) Identification and confirmation of isolate for resistance to biological Stains and dyes

Morphological and biochemical characteristics of the isolate were studied for the identification of the isolate. Further for study of resistance activity for dyes and biological stains, isolate was inoculated in to nutrient broth medium containing 0.1 % of respective dyes and biological stains i.e. acridine orange, Brilliant blue R-250, Crysatal violet, safranine, congo red, methylene blue, malachite green, phenol red. (Khairnar et.al2012, Pathak and Rathod 2016)

RESULTS AND DISCUSSION

One opaque white colony was selected and identified as *Bacillus lentus* by microscopic feature, cultural characteristics, biochemical tests and sugar profile as shown in table 1 and table 2 respectively and by comparing with Bergey's manual of systemic bacteriology. Further selected isolate was subjected to conformation of resistance to different dyes and biological stains and it showed remarkable growth in presence of selected dyes and biological stains i.e. acridine orange, Brilliant blue R-250, Crysatal violet, safranine, congo red, methylene blue, malachite green instead of phenol red.

Table no.1: Morphological Characteristic

Characters	Observed Character
Size	1mm
Shape	Circular
Color	White
Margin	Entire
Surface	Smooth
Elevation	Flat
Consistency	Sticky
Opacity	Opaque
Grams Nature	+ve, rod
Motility	Motile

Table no.2: Biochemical Characteristics

Test	Result	Enzyme profile	Result	Sugar profile	Result
Catalase	+	Amylase	+	Glucose	+
Indole production	-	Urease	+	Ribose	+
Methyl red	+	Gelatine	-	Maltose	+
VP	-	Cellulase	-	Salicin	+
Citrate utilization	+	H ₂ S production	-	Sucrose	+
Esculin Hydrolysis	+	Laccase	-	Xylose	-
Nitrate Reduction	+	Oxidase	-	Fructose	+

CONCLUSION

Isolated *Bacillus lentus* showed luxuriant growth in presence of different dyes and biological stains, therefore it can be useful for different industries.

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