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Statistical *In vitro* Model for Upscaling Biofilm of *Chroococcidiopsis cubana* by Media Optimization and its Protocol for DNA Extraction

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ABSTRACT

Manual process for the optimization of different salts for growth of bacteria is labour intensive work and has low precision. In this study, we had used design expert software for biofilm yield optimization of a strain of *Chroococcidiopsis cubana*. *C. cubana* was exposed on outer surface of the monuments due to adverse environmental conditions and formed the blackish biofilm on it. This biofilm was grown in culture media and their DNA was extracted for strain confirmation. Strain was confirmed by 16s rRNA gene sequence using sanger sequencing. The response surface method was used to optimize the concentration of two main components $NaNO_3$ and K_2HPO_4 among various salts of BG 11 media. RSM was studied by ANOVA coefficient estimation using F – test with very low probability value. The obtained goodness of fit was significant (R^2 = 0.99). Estimation of coefficient was used for calculation of t and p – values and decided their significancy. The model having p value less than 0.05 was considered for optimization. Desirability of optimization was resolved from contour plot having concentration of $NaNO_3$ = 13 and K_2HPO_4 = 6.50 for optimum biofilm yield. Using these parameters, the *in vitro* model of *Chroococcidiopsis cubana* resulted in a yield of 20 g/l biofilm in 10 days.

KEY WORDS: ANOVA, ENDOLITHIC BIOFILM, RESPONSE SURFACE METHOD, 16S RRNA GENE SEQUENCING.

INTRODUCTION

Biofilm is defined as sessile microorganisms growing on solid surface and embedded in matrix of extracellular polymeric substances (Garrett et al., 2008). These microorganisms include several bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* etc as well as some blue green algae like *Nostoc* sp., *Phormidium* sp., *Microspora* sp., *Chroococcidiopsis* sp. etc (Hancock 2013; Ljaljevic-Grbic et al., 2010; Miller et al., 2009). *Chroococcidiopsis* sp., a blue-green alga, has desiccation tolerance due to the thick polysaccharide sheath on the outer surface of the cells (Knowles and Castenholz, 2008). In some species such sheaths have the ability to self-recognise the surfaces. Due to this, cells auto-aggregate mostly at the bottom of the broth culture flask. Hence, exopolysaccharides are also known as auto-agglutinins. This is the first stage in the whole process of biofilm formation (Trunk et al., 2018).

The study of biofilm formation has importance in a broad scale of industrial application such as health and cosmetics products, food, water, paper mills, medical health and pharmaceutical (Molobela and Ilunga,

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ORIGINAL PAPER

Cyanobacteria and microalgae growing on monuments of UNESCO World Heritage site Champaner Pavagadh, India: biofilms and their exopolysaccharide composition

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Abstract

The present study investigated the biofilm organisms growing on selected monuments of the Champaner Pavagadh complex (Gujarat, India), which is a UNESCO World Heritage Site. The cyanobacteria and microalgae were isolated from biofilms collected through non-destructive methods. The identification of these biological organisms was done using micro-morphological characters and confirmed by 16S rRNA gene sequencing. The exopolysaccharide of each of the isolated strains was extracted, hydrolysed and analysed by the HPTLC. Six isolated strains representing five cyanobacteria and one microalga belong to the genera *Desmonostoc, Nostoc, Leptolyngbya, Chroococcidiopsis* and *Asterarcys*. The relationships between substrates' specificity of these isolated biofilm organisms and those identified globally were evaluated using maximum parsimony analysis to generate a consensus phylogenetic tree. The five strains of cyanobacteria isolated were closely clustered with cyanobacteria belonging to a tropical region. At the generic level, no relationship between the species and substratum specificity was recorded. The exopolysaccharide analysis of the isolated strains revealed the presence of seven monosaccharides. While glucose was present in all the analysed species, the concentration of either fucose or arabinose was high. The current study presents a novel HPTLC-based method for determination of monosaccharides composition from the extracellular polymeric substances.

Keywords Extracellular polymeric substances · HPTLC · 16S rRNA · Nostocales · Synechococcales

Introduction

Monuments are witnesses of the past and guardian pillars of the tangible cultural heritage of the area where they are found (Hassan 2014). India is known for its culture, ancient buildings, forts, temples and palaces, and these monuments are inspiration for future generations. (Dhotre and Joshi 2019). The state of Gujarat has a rich diversity of monuments which represent its glorious historical past. Three hundred and seventeen of these monuments have been protected as per the records of the Archaeological Survey of India, (ASI 2020). Due to a lack of active maintenance, several of these monuments show the presence of different stages of greenish and blackish biofilms. These biofilms cause damage to the monument wall structure and this process is termed as biodeterioration (Allsopp et al. 2004). While many biological organisms such as cyanobacteria, green algae, bryophyte, lichen, fungi and some allied vascular plants are responsible for biodeterioration, the cyanobacteria and the green algae are major pioneer organisms (Ortega Calvo et al. 1991; Tiano et al. 1995; Gaylarde and Gaylarde 2000; Crispim and Gaylarde 2005; Samad and Adhikary 2008). At the global level, the presence of cyanobacteria and microalgae biofilms has been reported from diverse monuments. These include monuments and buildings in South Eastern Spain (Uher et al. 2005), diverse substrate building facades in France (Barberousse et al. 2006), archaeological structures at El Palacio of Palenque, Mexico (Ramírez et al. 2010) and the Stucco Mask of North Acropolic, Tikal, Guatemala (Ortega-Morales et al. 2013). Similar studies have been conducted on a diversity of monuments like temples, caves and statues across India. The main temple at Thanjavur, the Sivan temple and the Venkatasaperumal temple all located

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