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A study to differentiate the nitrogen fixing and microcystin producing cyanobacteria by TLC profiling.

Biotechnology

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ABSTRACT

Lipid profiling by Thin Layer Chromatography is an effective method to differentiate between different species of same organism. Cyanobacteria are one of the most diverse groups of microbes that can be of numerous benefits to humans. It is important to identify or differentiate the species of interest from others. This study aims to differentiate between the nitrogen fixing and the toxin producing species of cyanobacteria by generating Thin Layer Chromatogram using different solvents.

KEYWORDS:

Cyanobacteria, Thin layer chromatography, mcy, nif, Cyanotoxin, Biological pigments.

Introduction

Cyanobacteria (Blue Green Algae) are photoautotrophic, prokaryotic microorganisms distributed in diverse habitats (Thajuddin *et al*, 2005). Cyanobacteria have thousands of different species, most common being *Anabena sp.*, *Microcystis sp.*, *Nostoc sp.* etc, (Ghosh *et al*, 2007). Many species are helpful in Nitrogen cycle (*Anabena sp.*, *Oscillatoria sp.* Etc), many are toxic producers and produce toxic compounds like microcystin, cylindrospermosin etc. called as cyanotoxins.

Exposure to cyanotoxins can cause skin allergy, nausea, vomiting, liver problems etc. Death of dialysis patients when exposed to cyanotoxin has also been reported (Wayne *et al.*, 2001). There are about 40 known analogs of microcystin molecules (Lambert *et al*., 1994). Transhumance mortality has been reported in Switzerland due to microcystin ingestion (Konstanze *et al*., 1997). Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil has also been reported (Elise *et al*., 1998).

Several investigations have been based on Thin Layer Chromato graphy technique for separation of various biological substances like lipid, pigments, amino acid, sugar and dyes (Skipski *et al*, 1963). TLC has been effectively used in obtaining and testing of the biomolecule against cancer cell lines (HT29 and A498). These studies have resulted in a possible source of anti-cancer molecule (Akanksha *et al*., 2015).

Water Reservoirs of Ranchi like Kanke Dam, Dhurwa Dam, Ranchi Lake etc. are the habitat of many species of cyanobacteria. Nitrogen fixing species and the microcystin producing species have been genetically verified in the waters of Dhurwa Dam {Rahul *et al*, 2016(a); Rahul *et al*, 2016(b)}. TLC proves to be an excellent method to identify them on the basis of their Lipid profile with a particular solvent. The study aims at TLC profiling of pigments produced and differentiate by the R_f value and evaluated for the capacity screening of cyanobacteria.

Material and Method

Sample Collection: Isolated and pre-screened cyanobacteria strains (*Microcystis aeruginosa*, *Microcystis floss-aquae*, *Anabena sp.* and *Chroococcus turgidus*) were provided by the laboratory {Rahul *et al*, 2016(a); Rahul *et al*, 2016(b)}.

Sample Processing: Cells of different strains were collected by centrifugation. The cells were crushed with different solvents namely, Petroleum Ether, Chloroform, Acetone, N-hexane, Diethyl Ether, Ethanol and Water.

Thin Layer Chromatography: The extracts were dried and approximately 5-10 mg of total extracted samples was used to spot on TLC plate (Aakriti Biotechnology). Sample spotted on TLC plate were separated for components using TLC Running Buffer (Aakriti Biotechnology).

R_f value were calculated using the formula below

$$Rf = \frac{Distance\ travelled\ by\ solute}{Diatance\ travelled\ by\ solvent}$$

Triplicates of R_f value were averaged for final R_f calculation.

Result and Discussion

The difference of R_f values by TLC profiling of Nitrogen fixing and microcystin producing cyanobacteria clearly shows the significant difference in the chromatogram (Figure no- 1). *Chroococcus turgidus* species of cyanobacteria obtained 15 different components on chromatogram; *Anabena sp*, *Microcystis aeruginosa* and *Microcystis floss-aquae* obtained 13, 13 and 11 different components respectively on the chromatogram.

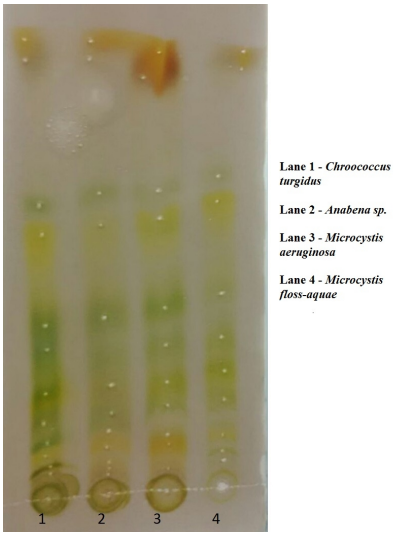

Figure 1: Arrows indicate the Polymorphism in TLC banding pattern.

Separation of banding pattern was observed and R_f values were calculated with following observations:

Table No.- 1: Details of banding patterns with reference to R_f values.

Similarity in Bands(Rf Values)		Difference in Bands(Rf Values)	
N2-fixing species	Microcystin producing species	N2-fixing species	Microcystin producing species
0.0375	0.0375	0.05	0.0312
0.0875	0.0875	0.175	
0.1125	0.1125	0.468	
0.2	0.2	0.0625	

0.7812	0.7687	0.143	
0.825	0.8125		
0.52	0.5062		
0.275	0.2687		

Five different R_f values obtained which create a valuable difference to identify cyanobacteria containing *nif* genes. Eight R_f values obtained which are commonly present among cyanobacteria screened under this study. Difference among R_f values of nitrogen fixing and microcystin producing strains are listed in (Table no-1) which provide sufficient data to differentiate the nitrogen fixing and the microcystin producing cyanobacteria by TLC profiling.

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