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ISOLATION AND PRELIMINARY CHARACTERIZATION OF ASSOCIATED MICROOORGANISMS FROM *SPIRULINA* PRODUCTS AND THEIR SILVER MEDIATED NANOPARTICLE SYNTHESIS

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ABSTRACT

Cyanobacteria are known to be colonized by various heterotrophic bacteria. With a view to understand the associated organisms from cyanobacterial products especially 6 different *Spirulina* products such as two forms of *Spirulina* powder, one crunchy form and three different tablets were selected. A total of 30 bacterial strains were isolated and their biochemical and cultural characteristics were studied. The isolates were subjected for silver nanoparticle production. Microscopic results revealed that most of them were gram positive and non-motile. Among the 30 isolates most of them can able to synthesize nanoparticles with silver. It indicates that the colonial characteristics alone cannot serve as a tool to characterize the bacterial isolates. Hence these isolates were carefully examined and selected for further analysis.

Keywords: Associated bacteria,Cyanobacteria- *Spirulina*,Silver nanoparticles

INTRODUCTION

Cyanobacteria are the oldest oxygenic photosynthetic organisms and they also serve as a rich source of novel bio active metabolites, including many cytotoxic, antifungal, and antiviral compounds. Among this, *Spirulina* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates and alpha- linoleic acid (Ramadan, *et al.* 2008). It is gaining attention not only for the food aspects but also for the development of potential pharmaceuticals. Recent works have indicated that this species has immuno promoting effects also. It act as a nutritional food source on powerful immune stimulant and effective anti oxidant and a supplement

that helps to reduce LDL (bad cholesterol), increase HDL (good cholesterol) and control blood sugar levels. It can be served as a supplementary cure for many diseases, used as a food for human and animals. *Spirulina* capsules have effective action in lowering blood lipid levels, decreasing white blood corpuscles, chemotherapy and also used as an inducer of immune system (Orio Ciferri, 1983). Cyanobacteria are often found to be associated with other group of micro organisms. The micro flora associated with *Spirulina* is generally non pathogenic (Praveen Kumar, *et al.* 2009). The bacteria are found to be associated with the extra cellular mucus zone of the phototrophic cells and attached more tightly with their cell surface. In general, the epiphytes are heterotrophic and are found to interact positively and improve their growth rate. Thus the role of these epiphytes leads in maintaining the health of their host has received the attention of the researchers (Thajuddin and Subramanian, 2005).

Silver nanoparticles are emerging as one of the fastest growing product categories in the nanotechnology industry. Many organisms, both unicellular and multicellular are known to produce inorganic materials either intracellularly or extracellularly. Sowtham and Beveridge, 1994 found these inorganic materials as possible eco friendly nanoparticles. Not only bacteria and fungi but also actinomycetes as well as algae are involved in the biosynthesis of metal nanoparticles. Among the algae, cyanobacteria especially *Spirulina* have received the most attention in the area of biosynthesis of nanoparticles (Mandal, *et al*. 2005).

The main objective of the study is to isolate and characterize the associated microbes from *Spirulina* products and the production of silver mediated nanoparticles. The conventional culturing methods were used for the isolation and preliminary characterization (Praveen, *et al*. 2009).

MATERIALS AND METHODS

COLLECTION OF SPIRULINA PRODUCTS

Six different *Spirulina* products were collected from pharmaceutical markets. Among this, three tablets Fruslac, Fruslac- DS, Lacilactone were

obtained from KMCH pharmacy, Coimbatore. One type of *Spirulina* powder was obtained from OFERR Nalhayan Research Centre, Chennai. Crunchy forms and the other powder forms were obtained from Aarovil, Pondichery.

ISOLATION OF ASSOCIATED BACTERIA

The *Spirulina* products were serially diluted. 1gm of sample was mixed with 100 ml of sterile distilled water and suspension was allowed to stand for 30 mins. Among this the dilutions 10^{-2}, 10^{-3}, 10^{-4} were subjected for spread plate technique in nutrient agar and was incubated at 37^0C for 24 hrs (Anderson and Heffernan, 1965).

PRELIMINARY CHARACTERIZATION OF ASSOCIATED BACTERIA

The macroscopic observations of the isolated colonies were carried out. Sub culturing from the colonies were done to isolate the bacterial strains in pure form. Then the preliminary characterization of the isolates was carried out by carefully recording cultural characteristics. They were also tested for their Gram staining and motility behaviour. The biochemical characterization was also carried out.

SILVER NANOPARTICLE SYNTHESIS

10^{-3} molar concentration of aqueous silver nitrate solution was prepared and the culture filtrate was allowed to contact with it. For the confirmation of silver nanoparticle synthesis, the p^H, OD and colour change was noted in regular intervals (Daniel, *et al.* 2004).

RESULT AND DISSCUSION

SPIRULINA PRODUCTS

A total of 6 different products from *Spirulina* were taken for the study.30 bacterial isolates were obtained upon repeated sub culturing from these products.

ISOLATION AND PRELIMINARY CHARACTERIZATION OF ASSOCITAED BACTERIA

The number of associated bacterial isolates varied from 2 to 10 from each sample, in which *Spirulina* powder from Chennai was found to

harbour maximum number of cultivable bacteria(10 isolates.). The cultural and microscopical characteristics of these isolates were also showing the diversity (Salmen, *et al*. 2003). The *Spirulina* tablets were found to harbour minimum number of isolates (2, 3, 3 isolates for Lacilactone, Fruslac and Fruslac- DS respectively). *Spirulina* crunchy form contains five bacterial isolates and the Aarovil *Spirulina* powder form harbour seven cultivable bacteria. The variable number suggests that not all *Spirulina* products had same numbers of bacterial association.

Of the total 30 isolates 14 were found to be pigmented and the remaining 16 were either white or colourless. Two of the bacterial isolate from the tablet Fruslac DS produces yellow pigmentation. Non pigmented colonies were produced by the tablet Fruslac and Lacilactone, the isolates from Aarovil *Spirulina* powder showed lemon yellow and golden yellow colonies and non pigmented ones. Where as the bacterial isolates from Chennai *Spirulina* powder showed different types of pigmentation. Apart from pigmentation, all their colonial parameters were recorded and isolates were found to differ significantly in their colonial morphology (Hube, *et al*. 2009)

Many of the isolates from the *Spirulina* powder formed smooth mucoid colonies. The isolate from the tablet Lacilactone was glistening with irregular margins. The bacterial isolates from the Fruslac were smooth mucoid in nature. The Fruslac-DS bacterial isolates were rough and non sticky.

Microscopically, the isolates were cocci (single, clusters and chains), bacilli (short, blunt, single, and long chains), and coccobacilli in shape. Among which cocci type cell were found to dominate the population. The gram staining of these isolates revealed that most of them were Gram positive and few of them only Gram negative. In general, two different cultures showing same cultural morphology need not to be the same always. The hanging drop technique of these isolates showed as non motile. Biochemical analyses were also done in the case of bacilli. The isolates showed mostly negative results.

The results showed that the colonial characteristics alone can not serve as a tool to characterize the bacterial isolates. With this in mind

considering both cultural & microscopic morphology, the isolates were carefully examined and selected for further analysis. The results of the preliminary characterisation of these isolates were summarised in Table 1. The classification was done with primary weightage to their colonial characteristics followed by their microscopic morphology and Gram's reaction (Worm and Sondergaard, 1998).

SILVER NANOPARTICLE SYNTHESIS

Among the 30 bacterial isolate, 18 of them are having the ability to produce silver nanoparticle. The isolates from Lacilactone, Fruslac- DS and Spirulina powder and crunchy forms produced nanoparticles were confirmed by the increase in the values of pH and OD. The colour change from pale yellow to brownish colour also indicated the silver nanoparticle synthesis (Gleiter, 2000).

30 bacterial isolates were obtained from the six *Spirulina* products and their characterization was carried out by the conventional culturing techniques. The characterisation was done with primary weightage to their colonial characteristics followed by their microscopic morphology and Gram's reaction and the results revealed the diversity among these isolates. Many of the isolates were synthesising silver nano particles.

Table 1:- Cultural and morphological characteristics of the associated bacteria isolated from *Spirulina* products.

***Spirulina* products**	**Representative bacterial isolates**	**Cultural characteristics**	**Gram reaction &Microscopic morphology**
Aarovil *Spirulina* powder	SSGPB1	Pin pointed lemon yellow smooth colonies	Gram positive bacilli
	SSGPC1	Minute, yellowish, irregular colonies	Gram negative cocci in chains.
	SMGNB1	Large dull white mucoid creamy colonies	Gram negative bacilli

	SSGPC2	Minute white irregular edged colonies	Gram positive cocci in pairs.
	SSGPC3	Very minute, spherical, colourless colonies	Gram positive cocci
	SMGNC1	Minute, yellowish, irregular, oval shaped with bulged edges	Gram negative cocci in chains.
	SSGPB2	Small whitish oval shaped colonies	Gram positive bacilli with bulged edges
Spirulina powder from Chennai	SSGPB3	Large, dark, yellowish, creamy & mucoid colonies	Gram positive bacilli
	SSGPC4	Minute, dull yellowish irregular colonies	Gram positive cocci in clusters
	SMGNB2	Dull white, circular, mucoid colonies	Gram negative bacilli
	SMGNB3	Minute lemon yellow colonies	Gram negative bacilli
	SSGPC5	Small, white, non sticky colonies	Gram positive cocci in clusters
	SKGNCB1	Orange yellow minute mucoid colonies	Gram negative coccobacilli
	SMGNC2	Dark yellow, creamy, mucoid colonies.	Gram negative cocci in pairs.
	SSGPC6	Large yellowish rough irregular edged colonies	Gram positive cocci in clusters
	SSGPC7	Minute lemon yellow, mucoid colonies	Gram positive cocci in chains
	SSGPC8	Pin pointed smooth golden yellow colonies	Gram positive cocci in pairs
Spirulina crunchy forms from Aarovil	SKGPCB1	White, round, tiny, glistening irregular colonies	Gram positive coccobacilli
	SMGNC3	Minute lemon yellow, mucoid colonies	Gram negative cocci in pairs.
	SKGNCB2	Large, dark, yellowish, creamy & mucoid colonies	Gram negative coccobacilli
	SMGNB4	Small, white, non sticky colonies	Gram negative bacilli
	SSGPC9	Dull white, circular, mucoid colonies	Gram positive cocci
Lacilactone	SMGNC4	White, round, tiny, glistening irregular colonies	Gram negative cocci in pairs.

	SSGPC10	Small, white, creamy irregular colonies	Gram positive cocci in clusters
Fruslac	SSGPC11	Small, white, smooth, mucoid colonies	Gram positive cocci
	SSGPC12	Transparent dirty white colonies	Gram positive cocci in clusters
	SMGNB5	Minute, dull whitish colonies	Gram negative bacilli
Fruslac- DS	SKGPCB2	Large, rough, raised, white, irregular colonies	Gram positive cocco bacilli
	SSGPC13	Pin pointed yellow colonies	Gram positive cocci in chains
	SKGPCB3	Large, round, circular yellowish colonies.	Gram positive coccobacilli

SSGPB → Sudha Soumya Gram Positive Bacilli
SSGPC → SudhaSoumyaGramPositiveCocci
SMGNB → SudhaMebinGramNegativeBacilli
SMGNC → SudhaMebinGramNegativeCocci
SKGNCB → SudhaKarthicGramNegativeCoccoBacilli
SKGPCB → SudhaKarthicGramPositiveCoccoBacilli

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