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Bioprospecting studies of pigmenting *Pseudomonas aeruginosa* SU-1, *Microvirga aerilata* SU14 and *Bacillus megaterium* SU15 isolated from garden soil

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ABSTRACT

Microbes that live in soil face more adverse environments, to adapt to the conditions they produce certain biomolecules or enzymes, where these metabolites or enzymes have industrial or biomedical applications. Some microorganisms produce carotenoid pigments for protecting themselves from photo-oxidative damage, whereas the pyocyanin pigment produced by *Pseudomonas aeruginosa* increases its pathogenicity. These pigments have much more applications too. In this present study, three pigment producing organisms like were isolated from soil. Pigment production by these organisms was optimized and the produced pigments were analyzed for their bioactivity. The pigment from *Bacillus megaterium* SU 15 was found to be with antibacterial and antioxidant property. All the isolated organisms were also checked for their ability to produce enzymes. *Microvirga aerilata* SU 14 and *Bacillus megaterium* SU 15 were found to produce amylase, thus optimization and characterization of amylase was done.

1. Introduction

It is well known that microorganisms adapt to environment much better and faster than any other higher organisms and they do produce various metabolites or enzymes which helps them live better (Kamat and Kerkar, 2011; Proksch, 1994; Meiying and Zhicheng, 1998). These metabolites or enzymes are having several benefits to the human population. Bioprospecting is the process of discovering new compounds from the organisms as the biodiversity is proven to be the sources of most bioactive compounds. Bioprospecting for new medicines from natural products has a long history; in fact most of the drugs available in the market are either natural products or derived from natural products (Zotchev et al., 2012). Today, more than 50,000 microbes derived natural products are discovered and being used as drug, few more are helping in drug discovery and most of these products are derived from soil microbes (Newman and Cragg, 2007; Berdy, 2005; Xiong et al., 2015). Emergence of new diseases and multidrug resistance made us to find new drugs from natural resources especially from microorganisms. Soil microorganisms produce wide range of products which includes hydrolytic enzymes such as DNases, lipases, amylases, chitinases, gelatinases and proteases etc (Gohel et al., 2005). Most of these enzymes are being used by the food industries and cosmetics industries (Shahidi and Kamil, 2001; Lods et al., 2000; Britton et al., 2004). As a part of adaptation, bacteria tend to produce carotenoids, which prevents them from oxidative damage (Frank and Cogdell, 1993). Till date, over 700 structures of carotenoids have been reported from plants, fungi and bacteria (Britton et al., 2004). Many carotenoids have been reported to have health promoting properties. The dietary intake of carotenoids is proven to protect against cancer, heart disease and age-related macular degeneration (AMD) (Britton, 1995; Bernstein et al., 2002; Snodderly, 1995). Carotenoids of bacterial origin have gained interest in the past years due to their ability to function as virulence factors in some pathogenic bacteria (Liu and Nizet, 2009) as well as a suitable biosource with medicinal value (Liu et al., 2005; Romero-Martinez et al., 2000; Baron and Rowe, 1981). *Pseudomonas aeruginosa* produces a bluish-green pigment called Pyocyanin, a redox-active Phenazine compound has been found to exhibit antibacterial activity (Baron and Rowe, 1981). Having gained the basic knowledge about the microbes derived products; this study was done with an intention to tap out the potency of some pigmenting microorganisms isolated from soil.

2. Materials and methods

2.1. Isolation and identification of organisms

Garden soil was collected in a sterile container from Sathyabama

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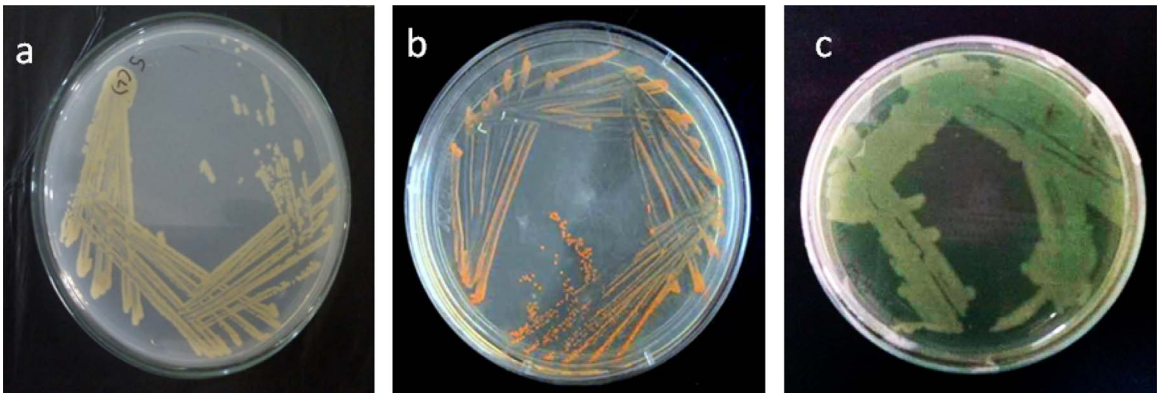

Fig. 1. Cultural characteristics of isolates a) *Pseudomonas aeruginosa* on cetrimide Agar, b) *Bacillus megaterium* on Nutrient Agar, c) *Microvirga aerilata* on Nutrient Agar.

Table 1
Identification of organism by biochemical tests.

Test	*Microvirga aerilata*	*Bacillus megaterium*	*Pseudomonas aeruginosa*
Citrate utilization	+	+	+
Lactose Fermentation	–	–	–
Catalase	+	+	–
Methyl Red	+	+	+
Voge's Proskauer	–	–	–
Oxidase	–	–	+
Mannitol	–	–	–

University, Chennai, Tamil Nadu, India. 1 g soil was serially diluted and pour plate was performed on nutrient agar (HiMedia, India). The plates were incubated at 37^{0}C for 72–96 h. Plates were checked for pigmenting organisms. Pigmented organisms were streaked on a sterile nutrient agar. Plates were incubated for 48–72 h. After incubation, isolated colonies were taken and stored in a sterile nutrient agar slants. Identification of the organisms was done by Gram's staining, biochemical tests and 16SrRNA sequencing (Weisburg et al., 1991; Pitcher et al., 1989).

2.2. Optimization of growth parameters

Organisms were tested for their optimal growth on different media like nutrient agar, nutrient broth, potato dextrose agar, minimal media etc. All the optimization parameter was done on either solid media or liquid media which was depended on the growth of microorganisms. Growth on solid media was confirmed by colony formation, colonies were scraped and transferred to saline. Absorbance was taken at 600 nm. Growth in liquid media was confirmed by reading absorbance at 600 nm. Highest absorbance was confirmed as maximal growth.

2.3. Extraction of extracellular pigment

Pyocyanin pigment extraction was performed as described by El-Shouny et al. (2011), with certain modifications. The overnight culture of *Pseudomonas aeruginosa* was standardized to OD value at 600 nm to 1.0 and inoculated into 20 ml nutrient broth. After inoculation, culture was incubated at 37^{0}C for 24–48 h. The culture was centrifuged at 10,000 rpm for 10 min. 20 ml of the supernatant was mixed with 4.5 ml chloroform and vortexed. The above mixture was spun again at 10,000 rpm for 10 min which resulted in two phases (chloroform along with pyocyanin sinks to the bottom). 3 ml of the lower phase was mixed with 0.2 N HCL till the color changes from blue-green to pink and centrifuged at 10,000 rpm for 2 min. The resulting pink layer containing pyocyanin was collected, dried and weighed.

Table 2
Growth characteristics of organisms on different growth media.

Growth media	*Bacillus megaterium*	*Microvirga aerilata*	*Pseudomonas aeruginosa*
Minimal media	+	N/G	+ +
Minimal media with yeast extract	+ +	+ +	+ +
Nutrient broth	+ (very less pigment)	+ (very less pigment)	+ + +
Nutrient Agar	+ + +	+ +	+ + +
Potato dextrose media	N/G	+	N/G

N/G – no growth + - growth + + - moderate growth + + + - abundant growth.

Table 3
Growth characteristics of organisms in nutrient agar/ broth at various incubation time.

Incubation time (hours)	*Bacillus megaterium* on nutrient agar	*Microvirga aerilata* on nutrient agar	*Pseudomonas aeruginosa* in nutrient broth
24	+	+	+
48	+ + +	+ + +	+ + +
72	+ + +	+ + +	+ + +
96	+ + +	+ + +	+ + +
120	+ + +	+ + +	+ + +

N/G – no growth + + - moderate growth + + + - abundant growth.

Table 4
Growth of organisms in/on nutrient broth / agar at various NaCl concentration.

NaCl Concentration	*Bacillus megaterium*	*Microvirga aerilata*	*Pseudomonas aeruginosa*
0.1%	N/G	N/G	N/G
0.2%	N/G	N/G	N/G
0.3%	+	N/G	+ +
0.4%	+ +	+	+ +
0.5%	+ + +	+ +	+ + +
0.6%	+	+	+ +
0.7%	N/G	+	+ +
0.8%	N/G	N/G	+ +
0.9%	N/G	N/G	+ +
1.0%	N/G	N/G	+ +

N/G – no growth + + - moderate growth + + + - abundant growth.

2.4. Extraction of intracellular pigment

2.4.1. Separation of biomass

Some of the organisms were producing much pigment on nutrient agar alone. Thus, they were streaked on nutrient agar and incubated for