OPEN ACCESS

Int. Res. J. of Science & Engineering, 2017; Vol. 5 (6): 119-125
ISSN: 2322-0015

http://www.irjse.in
UGC Approved Journal No. 63628

RESEARCH ARTICLE

Isolation and screening of antioxidant bacterial pigments from different ecological niche

Mali Devyani, Shraddha R and Nabar Bela

Department of Microbiology, Smt. C.H.M. College, Affiliated to University of Mumbai, Ulhasnagar-03, India
*Corresponding author E-mail ID: devyani.mali86@gmail.com

Manuscript Details
Received : 09.11.2017
Accepted: 25.12.2017
Published: 31.12.2017

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Mali Devyani, Shraddha R and Nabar Bela . Isolation and screening of antioxidant bacterial pigments from different ecological niche. *Int. Res. Journal of Science & Engineering,* 2017, 5 (6): 119-125.

ABSTRACT

Synthetic colorants are commonly used in the cosmetics, textiles, and paper and food industry. The problem associated with the synthetic colorants is they are non-biodegradable and harmful in nature. Now there is an increase in demand towards the use of the natural colorants in market. Microorganisms, plants and animals produce various pigments as secondary metabolites. Thus various water and soil samples were collected from the different ecological niche i.e. extreme environment and polluted water. Total eighty five pigmented bacteria were isolated from different ecological niche. Pigment was extracted from pigmented bacteria using different solvent such as Methanol, Ethanol, Hexane and Chloroform. Absorption maxima pattern for extracted pigments was studied and thin layer chromatography of extracted pigment was performed using different solvent system, out of which Ethyl acetate: chloroform: Methanol (1:1) was found to give better separation of constituents. Antioxidant activity was studied using DPPH assay and pigment giving highest scavenging activity were selected for the further studies. Pigment having antioxidant property are useful for application in cosmetic industry.

Keywords: Pigments, Antioxidant, Antidandruff, synthetic colorants, DPPH assay

INTRODUCTION

Colorants are used in a vast majority of products, ranging from food, cosmetic and textiles. The colorants are synthetic, and harmful to humans and the environment as they are non-biodegradable and contain toxic compounds.

Several synthetic dyes contain dioxins which can cause disorders in the immune, nervous, and digestive systems.

Disposed dioxin-containing pigment contaminates the environment and accumulates in living organisms, thus damaging the global ecosystem. There has been a growing demand for natural dyes, which are biodegradable and have a less harmful impact on the environment [1]. Microorganisms are associated with all the foods that we eat and are responsible for the formation of certain food products by the process of fermentation and can also be used as a source of food in the form of single cell proteins and food supplements in the form of pigments, amino acids, vitamins, organic acids, and enzymes. Colorants obtained from microorganism, animal, plants and algae are of great interest due to their natural properties and the pigments from microbial sources are a good alternative. Microorganisms are known to produce a variety of pigments; therefore they are promising source of natural colorants. Various carotenoids, flavins, chlorophyll, quinines, prodigiosins are the pigments produced by coloured microorganisms found in different environmental niche. These natural pigments are of interest in various commercial fields of pharmaceutical, nutraceuticals, foods animal feeds and cosmetics industry .

In the present study the potential orange pigment producing bacteria were screened and isolated for pigment production with good antioxidant property.

METHODOLOGY

Isolation of pigment producing bacteria

Various water and soil samples were collected from different ecological niche of Maharashtra state (Latitude: 20" 00' N′ Longitude: 76" 00' E). Soil water and air samples were collected and processed. Sample were serially diluted in sterile 0.85 % saline and plated on to the Tryptic soya agar and incubated at room temperature for 7 days. Prominent orange pigmented colonies were selected and purified on to the Tryptic soya agar. Orange pigment belongs to carotenoid group which are of great importance.

Primary screening:

Colony characteristics and biochemicals were studied as per the standard protocol. Biochemical identification using Methyl red, Voges-Prauskar, Citrate, Indole test, Urease, Triple sugar iron slant, Catalase, oxidase and XLD test were carried out.

The bacteria giving luxuriant pigment were selected for further studies. Colourless colonies were negatively selected. Pigment yield was monitored in presence of additives such as dextrose, lactose, sucrose and glycerol. Tryptic soya agar containing 1 % dextrose, lactose, sucrose and 4 % glycerol was used for the study of growth optimization of pigmented bacteria.

Pigment extraction and Absorption maxima study

The bacteria grown on Tryptic soya agar containing 4% glycerol for 72-96h till the maximum pigment production is achieved. Pigment extraction was done by using different solvents such as methanol, ethanol, chloroform and acetone. Standardisation of extraction procedure was determined using absorption maxima study.

Study of pigment constituents:

Thin layer chromatography was performed to analyse the pigment constituent. Methanolic extract of pigment was subjected to evaporation at 45^{o}C and thin layer chromatography was performed. TLC plates were impregnated with the pigment spot, dried and were placed in the pre saturated solvent system containing Ethyl acetate: chloroform: Methanol (2:2:0.5). The chromatogram was analysed visually for band pattern and spots were marked. Relative Rf values were calculated.

Antioxidant Potential:

The antioxidant activity of extracted pigment was determined by using the DPPH assay. 200µL of pigment extract was mixed with 2 ml of 0.02% DPPH incubated at dark for 30 min. The absorbance was measured at 517 nm using (Vidyut kanad 0392 instrument) using methanol as blank and controls were also maintained. The ability to scavenge DPPH radical was calculated by using equation: DPPH scavenging effect (%) = [(A0-A1)/A0] ×100; where A0: absorbance of control reaction and A1absorance of sample.

RESULTS AND DISCUSSION

Isolation of pigmented bacteria

The aim of the study was to screen the pigment producing bacteria isolated from different ecological niches. Various samples of air, soil, water and tree bark were collected from different ecological niche of Maharashtra.

Table1: Details of the sampling locations.

Sr. No.	Location	Sample	Altitude & Magnitude
1	Lonar	Soil and water	19⁰58'N & 76⁰30'E
2	Tadsar	Soil	17⁰27'N & 74⁰20'E
3	Titwala	Water	19⁰29'N & 73⁰22'E
4	Bhainder	Water	19⁰30'N & 72⁰84'E
5	Air count of Taloja industrial area	Air	19°30'N & 72⁰84'E
6	Taloja industrial water samples	Water	19°30'N & 72⁰84'E
7	Garden soil	Soil	19°17'N & 72⁰95'E
8	Ganeshpuri	Water	19°46'N & 72⁰97'E
9	Bhandup salt pan	Water	19°14'N & 72⁰933'E
10	Jagadia soil	Soil	21°72'N & 73⁰15'E
11	tarapur soil samples	Soil	19°85'N & 72⁰70'E
12	Tree bark sample	Tree bark	19°17'N & 72⁰95'E
13	Palava soil	Soil	19°22'N & 73⁰09'E
14	Sagareshwar	Water	17°8'46.42"N 74°22'59.44"E

The details of the samples are listed in table 1. Sampling locations are mentioned in image 1. Soil and water samples collected from alkaline soda lake like Lonar and salt pan which are extreme environment gives maximum number of pigmented organisms. Soil samples were collected from agriculture land from Tadsar, Tarapur and Jagadia having different types of fertilisers. Garden soil collected from different locations thane residential area and Palava city. Section of tree bark collected from garden plants. Water sample was collected from Sagareshwar which is a very old lake from wild life sanctuary area near Karad district.

Various coloured and colourless colonies were observed. Organisms not procuring pigments were negatively selected. 85 orange pigmented bacteria were isolated from 59 different ecological niches. The occurrence of pigmented bacteria varied as per the ecological conditions. The colony characteristics were studied for comparative analysis of bacterial colonies. The water sample collected from Sagareshwar water pond harbours high number of orange pigmented bacteria. Garden soil collected from different location also gives wide variety of bacterial isolate having distinct colony characters as well as pigment production.

16 orange pigment producing isolates were obtained from water sample collected from polluted environment, vessel cleaning, sanitisation water, effluent water and industrial waste water containing various chemical such as chlorine, sulphate and detergents. Organism produces various pigments as secondary metabolites in stressful environment.

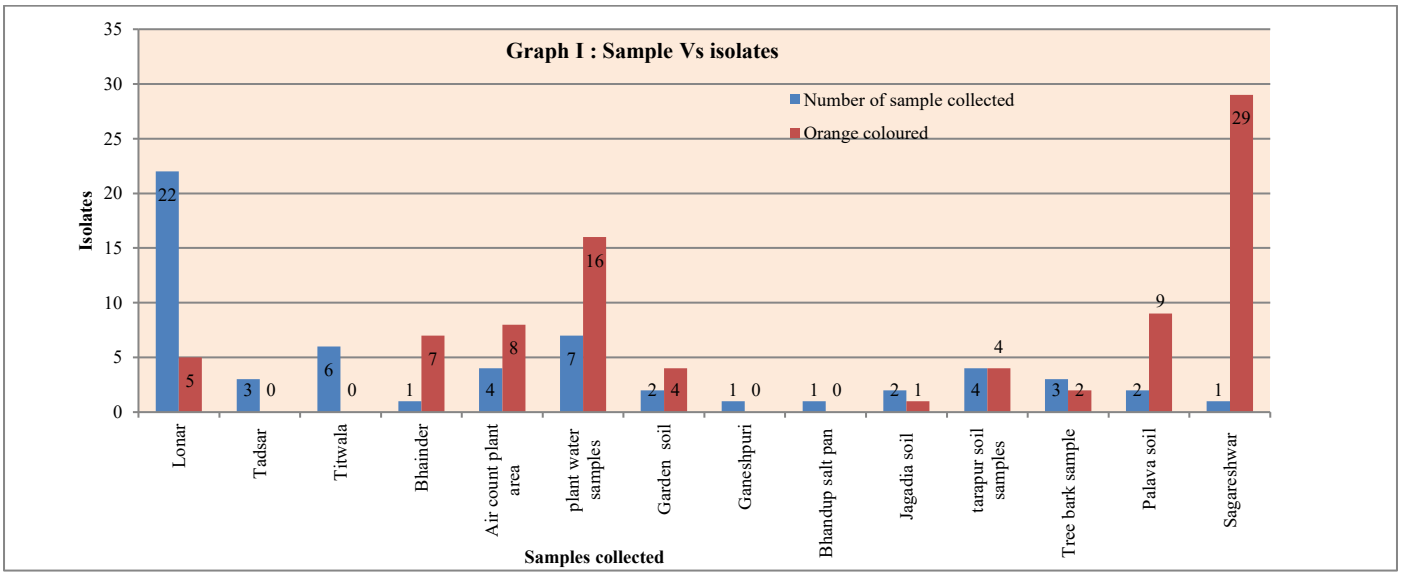

Figure 2: No. of bacteria and orange pigment producing colonies isolated from various samples

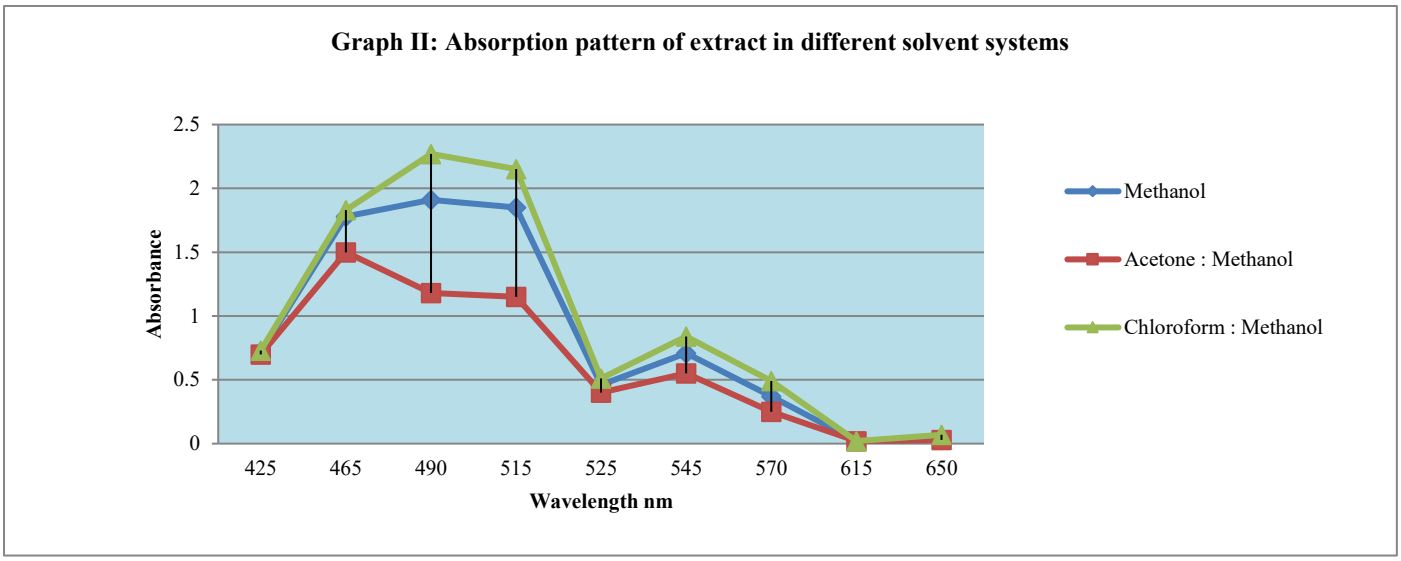

Figure 3: Absorption maxima of various extracted pigments in visible range

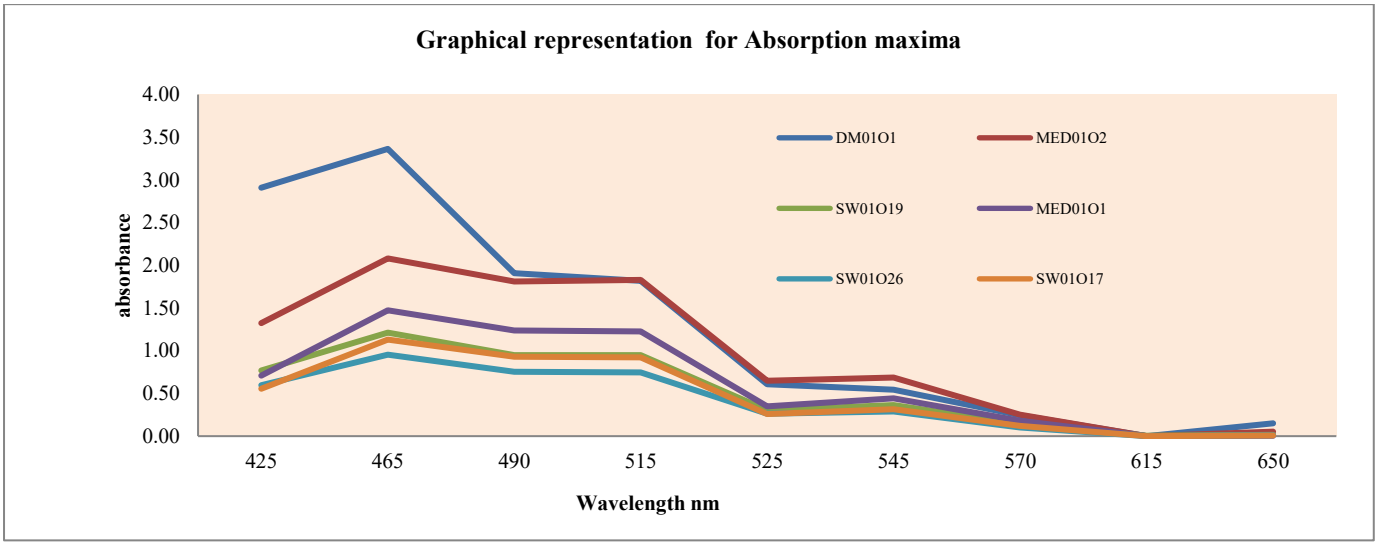

Figure 4: Absorption maxima of various extracted pigments in visible range

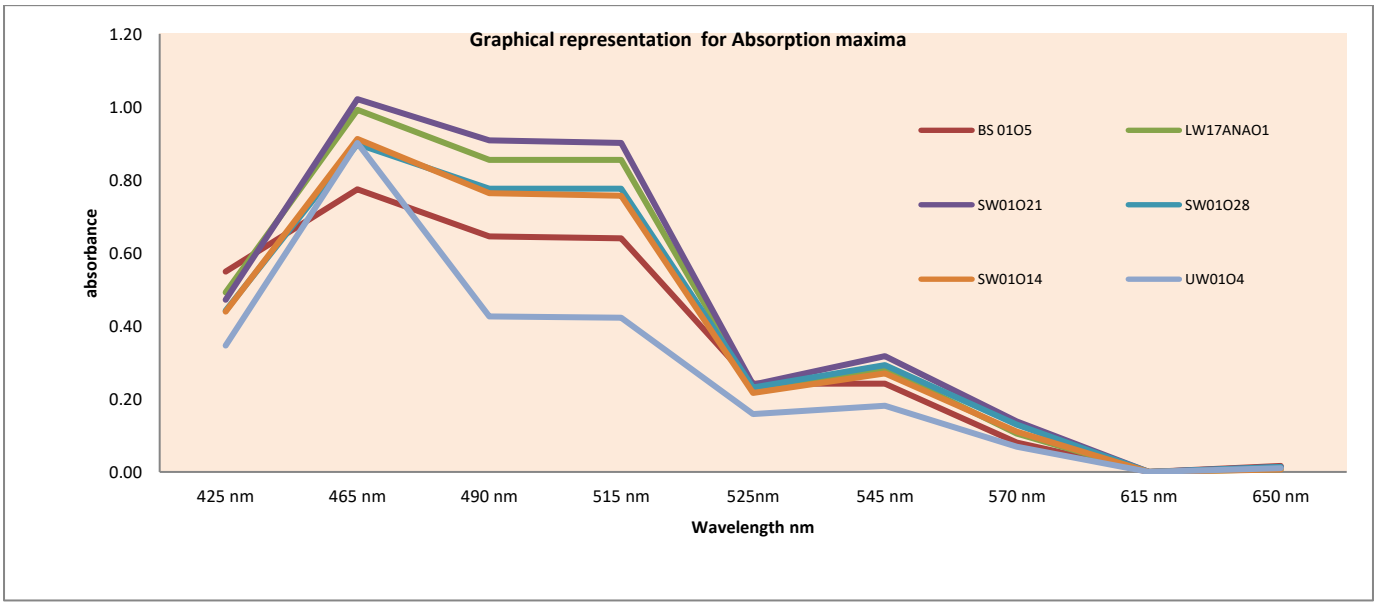

Figure 5: Absorption maxima of various extracted pigments in visible range

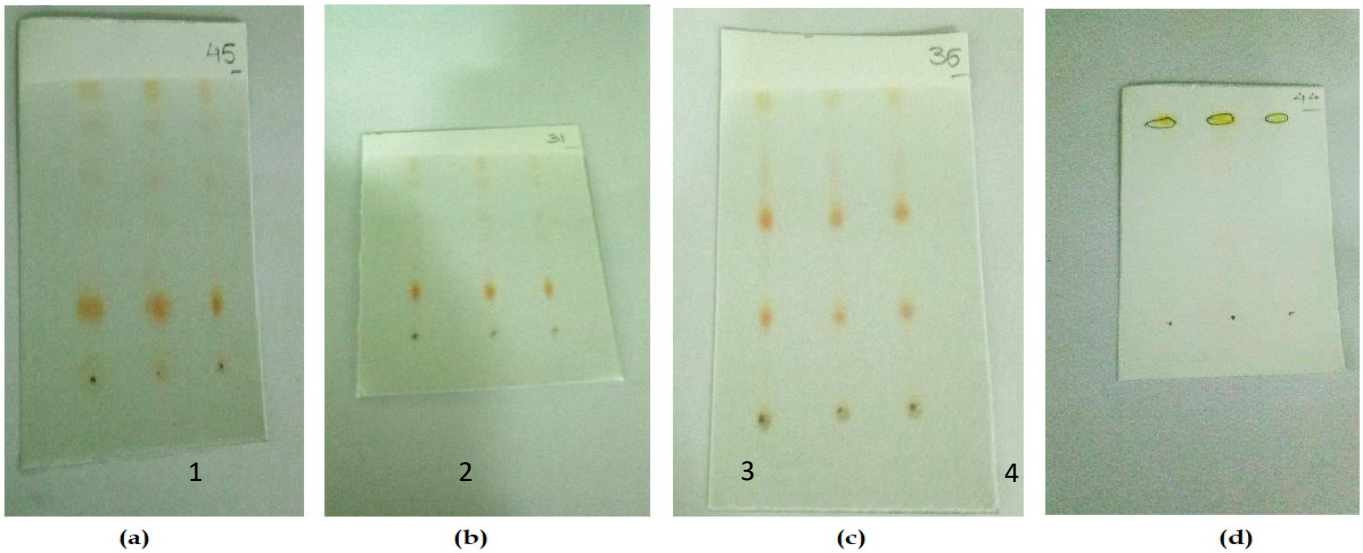

Figure 6: Thin layer chromatography plates. Presence of carotenes and Xanthophylls

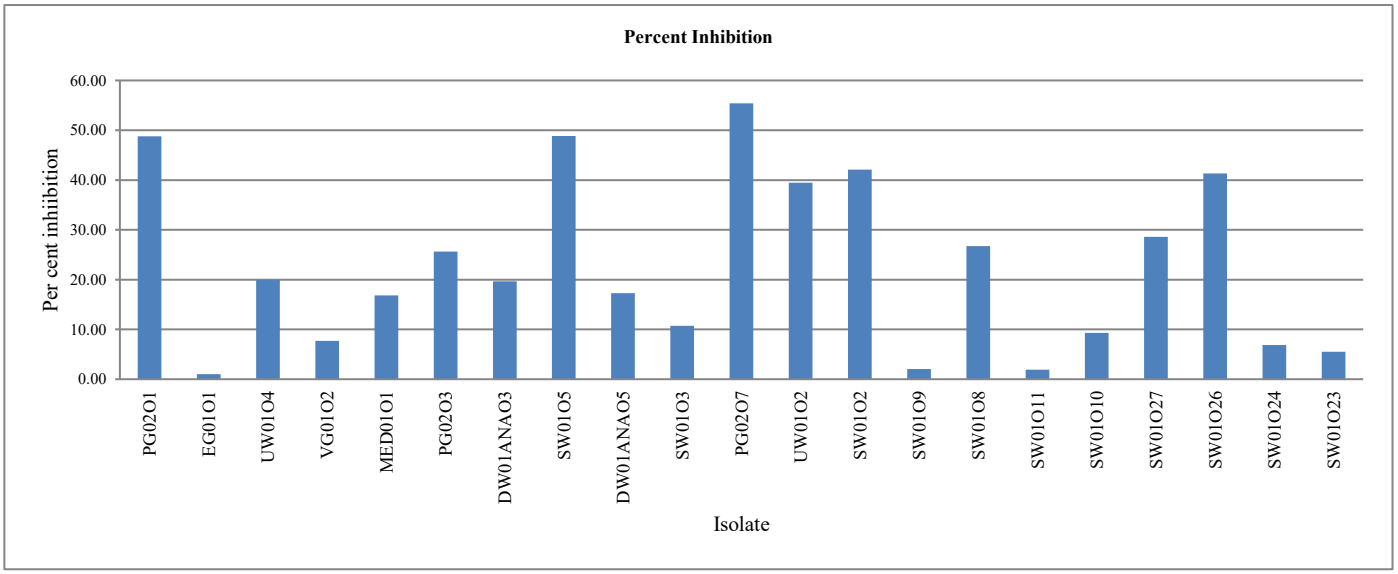

Figure 7: percent inhibition activity of orange pigment.

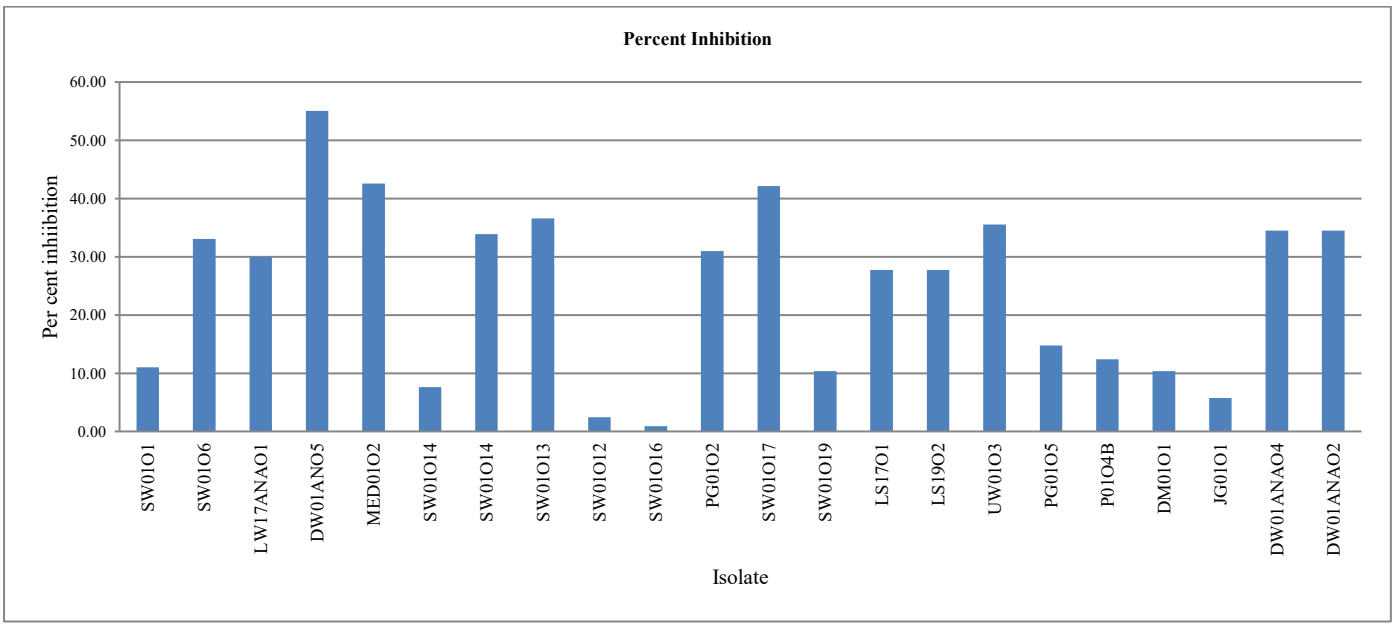

Figure 8. Percent inhibition activity of orange pigment

Total 8 isolates were obtained from air exposure plate method in different locations. Maximum numbers of isolates were obtained from Sagareshwar, Industrial water samples and Garden soil which are producing orange pigments. Samples collected from lonar harbours only 5 isolates which gave sustained orange pigment. Soil samples collected from agricultural land such as Tadsar showed no orange pigmented bacteria. The data of isolates obtained from collected samples is presented in figure 2.

Colony characteristics and Biochemical study:
Colony characteristic and Biochemical studies for the isolated strains were carried out.
The Tryptic soya agar containing 4% glycerol in tryptic soya agar was found to be effective compared to the Tryptic soya agar with different sugars like dextrose sucrose and lactose.

Pigment extraction and Absorption maxima study:
Extraction of pigment followed by various different solvent systems Methanol, Acetone: Methanol (1:1) and Chloroform: Methanol. Among various pigment extraction method Methanol: chloroform (1:1) is found to be more effective for complete extraction of pigment from bacterial cell. Figure 3 represents the Absorption pattern of pigment extracts in different solvents. Chloroform: Methanol (1:1) solvent system found to be best compared to Methanol and Acetone: methanol.

Absorption maxima of extracted pigment were determined. λmax is calculated for extracted pigments. Figure 4 and 5 represents the absorption pattern was analysed from 425 nm to 650 nm on Vidyut kanad Hans 0392. Maximum absorbance is observed in the wavelength 465nm and 490 nm. The pigment extracted from the strains isolated from Sagareshwar and polluted industrial water showed maximum absorbance compared to other isolates.

Absorption maxima for total 85 strains was analysed on colorimeter. The pigment extracted from strains isolated from Sagareshwar pond, Lonar salt water lake and polluted industrial water gives maximum absorbance at 465 nm. Lambda max. for the orange pigment is observed in the range of 465-490-515 nm indicate that this pigments are mainly from the group of carotenoids. Bridoux mentioned most carotenoids absorb light maximally at three wavelengths resulting in 3 peak spectra.

Out of 85 strains 42 strains were positively selected as per the absorption maxima. The strains were positively screened for the further studies based on consistency in pigment production, pigment intensity and maximum absorption at 465-490-515 nm.

Thin Layer Chromatography:
Thin layer chromatography was performed for the study of pigment constituents. The carotenoids contained two or more hydroxyl group. The crude Methanolic extract analysed by thin layer chromatography reveals the presence of one carotene and dihydroxylated xanthophylls. All the Methanolic extract of the pigment showing single carotene found to have Rf value 0.9-1.0. (Figure6) Two or more spots were observed for the monohydroxylated and dihydroxylated compounds. Monohydroxylated compounds were observed at the middle of the plate with Rf value 0.5-0.7 while dihydroxylated compounds remains close to the baseline of TLC plate with Rf value 0.1-0.2. (plate 2 and 3).

Antioxidant potential study by DPPH assay:
DPPH free radicals determines the free radical scavenging activity of the pigment extract, which shows its effectiveness by prevention, interception and repair mechanisms against injury in biological system Percent radical scavenging activity calculated for the Methanolic extract of pigment. Ascorbic acid was used as a standard ascorbic acid (figure 7)

Total 42 Methanolic extracts were analysed for percent scavenging activity using DPPH assay. 22 isolates observed with more than 25 % inhibition activity (figure 7 and 8). DW01ANA05, SW01O17 UW01O3, DW01ANA04 and DW01ANA02 are giving more than 30% DPPH radical scavenging activity. Strain isolated from Palava garden soil viz.PG0201, PG0207 are giving maximum percent inhibition of DPPH radical. Isolates obtained from Sagareshwar SW0105 SW01O26 SW0108 are having good antioxidant potential.

CONCLUSION

The orange pigment producing bacterial strains were isolated from Lonar salt water lake, Sagareshwar pond, Garden soil, Polluted industrial water sample and tree bark of chickoo and mango. 85 different orange pigment producing bacterial strains isolated from wide variety of environmental niche. Sagareshwar pond harbours wide

variety of orange pigmented bacteria. Total 29 isolates were obtained from Sagareshwar pond water sample.. The variation in colony characteristic and biochemical test is observed indicates the diversity of orange pigmented bacteria in environment. Luxuriant growth is observed in presence of 4% glycerol in tryptic soya agar. Bacterial isolates showing more than 25% inhibition in DPPH assay have potential for use in cosmetic and textile industry as an antioxidant natural colourant.

Future Studies:

Potential bacterial isolates will be studied for their application in cosmetic industry.

REFERENCES

1. Aberoumand A. A Review Article on Edible Pigments Properties and Sources as Natural Bio colorants in Foodstuff and Food Industry, *World J. Dairy Food Sci.*, 2011, 6 (1): 71-78.
2. Ahmad, WA, Ahmad, WYW, Zakaria ZA and Yusof NZ. Application of Bacterial Pigments as Colorant. *Springer Briefs in Molecular Science*, 2012, pp 57-74.
3. Bridoux MC (2008). Algal biomarkers and their metabolites in the lower food web of thegreat lakes, analysed by HPLC-PDS/FC, LC-MS and GC-MS. Albany state university of new York proquest USA.
4. Shatila F, Yusef H and Holail H. Pigment production by Exiguobacterium aurantiacum FH, a novel Lebanese strain.,*International journal of current microbiology and applied sciences*, 2013, ISSN:2319-7706 Volume 2 Number 12 , pp. 176-191.
5. Joshi, VK, Attri D, Bala A and Bhushan S. Microbial Pigments, *Indian J. Biotech.*,2003, 2: 362-369
6. Malik K., Tokkasl J and Goyal S. Microbial Pigments : A review, *International Journal of Microbial Resource Technology*,2012,ISSN 2278 – 3822.
7. Prasad MP(2015). Optimization of media parameters for pigment production in bacteria from effluent water samples. *Biolife*, 2015, 3(2),pp 428-433
8. Bhat MR and Thankamani M. Media optimization, extraction and partial characterization of an orange pigment from Salinicoccus sp. MKJ997975. *International journal of life sciences biotechnology and pharma research*,Vol. 4, No. 2, Aril 2015.
9. Manon MV, Keerthana G and Preethi K. Evaluation of antioxidant potential of bioactive colored metabolite isolated from Exiguobacterium profundum BC-11 and its bioactivities. *International journal of recent scientific research*, April, 2015, Vol.6,Issue 4, pp. 3612-3617.
10. Nagpal N., Munjal N. and Chatterjee, S. Microbial Pigments with Health Benefits - A Mini Review. Trends Biosci, 2011, 4: 157-160.
11. Varsha C and Arpana J.Screening of significant nutrient parameters for pigment production from newly isolated organism Planococcus ,aritimus AHJ_2 using Plackett-Burman design., *J. Microbiology Biotechnology. Res*, 2013,. 3: 7983