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STUDY OF MICROBIAL PIGMENTS AS ANTIDANDRUFF AGENTS

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ABSTRACT Dandruff is common problem affecting human beings and thus hair care industries are focusing on development of antidandruff agents. *Malassezia* species, lipophilic yeasts are the main causative agents of dandruff. Currently Zinc pyrithione, Ketoconazole are widely used as antidandruff agents. As there is an increase in demand towards the use of the natural antimicrobial agents, microorganisms can be one such source to be tapped. Microorganisms produce pigments which have antimicrobial property and can be assessed for antidandruff activity. The current study focusses on isolation of pigment producing bacteria from soil and water samples from different ecological niche. Pigments were extracted from the pigment producing organisms. The pigments were screened for their antimicrobial activity against *Malassezia* sp. and *Candida albicans* which are dandruff causing organisms. The pigments showed effective antimicrobial property in control of dandruff causing organisms and can be further used as antidandruff agents in shampoo or cream.

KEYWORDS : Pigments, Antidandruff, Malassezia Sp., Candida Albicans,

INTRODUCTION:

Dandruff is a skin problem mainly affecting the scalp, the symptoms are mainly flaking, itchiness, red and greasy patches of skin with tingly feeling. In severe cases, it may result in skin inflammation called as seborrheic dermatitis it is may be due to genetic or external environment factors.

In case of dandruff infection, it causes overgrowth of scalp cells and the skin layer continually replaces itself and the cells are pushed outwards where they die and flake formation occurs. In some conditions the flake formation occurs very rapidly and the dead cell accumulation leads to growth of microorganism such as *Malassezia* yeast. Various antidandruff agents are available in the market in the form of shampoo, gel cream and conditioner which are mainly antifungal drugs for the treatment purpose (7).

Currently ketoconazole, Zinc pyrithione and Selenium disulfide are mainly used for antidandruff treatment. Ketoconazole gives long duration protective effect and it is a broad spectrum antifungal agent active against *Malassezia* and *Candida* spp. But these synthetic agents have adverse effect associated with various allergic reactions.

Thus now there is an increasing trend towards the use of the natural compounds. Various natural compounds are also available in the market for antidandruff treatment. Microorganisms produce several antimicrobial compounds such as pigments which possess the antimicrobial potential. In the present study, we have isolated pigment producing microorganism and screened for their antidandruff activity

METHODOLOGY:

1) ISOLATION OF PIGMENTS PRODUCING BACTERIA:

Various soil and water samples were collected from different ecological niche of Maharashtra (Latitude: 20" 00' N' Longitude: 76" 00' E). Samples were processed for isolation of pigment producing microorganism using spread plate method. Samples were serially diluted in sterile 0.85 % saline and plated on to the sterile tryptic soya agar plates. The plates were incubated at 32 deg. C. for 7 days. Prominent red/ pink colonies were selected and purified on the Tryptic soya agar media. Deep red pigment belongs to the Prodigiosin alkaloid family of natural products. It is characterized by a pyrrolylpyrr omethene core and a deep, blood-red color. Some of the red pigments belong to carotenoid family (1).

2) SCREENING OF PIGMENT PRODUCING BACTERIA:

Identification of the pigment producing bacteria was done on the basis of microscopy, colony characteristics and biochemical studies. For biochemical screening tests such as IMViC, Nitrate, urease, triple sugar iron slant, oxidase catalase and carbohydrate assimilation were performed (8).

The bacteria showing prominent pigment were selected for the further studies. The maximum pigment production was observed for 1% dextrose and 4% glycerol in the Tryptic soya medium (9) (10).

PIGMENT EXTRACTION FROM THE ISOLATES:

The extraction of the pigment was carried out by using different solvents such as methanol, ethanol acetone and chloroform. (8) Each pigment has characteristic absorption pattern. Therefore, The lambda max was determined by using absorption maxima study in visible range 400-700 nm using colorimeter (Vidyut Kanad 0392).

3) QUANTITATIVE ASSESSMENT OF THE ANTIDANDRUFF ACTIVITY:

The antidandruff activity of the extracted pigment (1mg/ml in DMSO) was evaluated by initially determination of its antibacterial potential by agar cup method, MIC of the effective isolate and its activity with respect to time by Time kill study.

A) AGAR CUP METHOD:

The extracted pigment was screened for the antidandruff activity by agar cup method. Dandruff causing *Malassezia furfur* MTCC 1374 and *Candida albicans* ATCC 10231 were used for study as indicator organism (8). Cultures of *Malassezia furfur* and *Candida albicans* were surface spread on the Sabarouds agar as well as Muller Hinton agar. 100 µL of test pigment prepared in DMSO was added to the wells and the plates were incubated at 25 deg. C for 48 hrs. The plates were observed for the zone of clearance around the well indicating the antidandruff activity.(1)

The same set of extracted pigments were studied in shampoo formulation. The shampoo was prepared using 14% SLES 2 % CAPB and 1 % pigment. (15)

B) MINIMUM INHIBITORY CONCENTRATION STUDY:

The Minimum inhibitory concentration study of the extracted pigment was done by using tube method. 100, 250, 500, 750 and 1000 µg/ml concentration of the pigments were used for study and tryptic soya broth was used as the diluent. MIC study was performed against the *Malassezia furfur MTCC 1374* and *Candida albicans ATCC 10231*. Culture were inoculated in the different test dilutions and incubated at 25 deg. C for 48 hrs. The minimum concentration of the pigment exhibiting inhibition was considered as MIC.

C) TIME KILLASSAY :

The 48 hrs. old culture of *Malassezia furfur* and 24 hrs. old culture of *Candida albicans* were used for the study. 100 µL of respective cultures were added in to 10 ml of the shampoo with pigment. The aliquots were removed at the interval of 0 min, 30 secs, 2 min and 5 min. The aliquots were suspended immediately in neutralizer media. The serial dilutions were performed and plated on the Sabarouds dextrose agar by pour plate method. Plates were incubated at 25^{0}C for 48 hrs. (13). Reduction in initial microbial count with respect to time

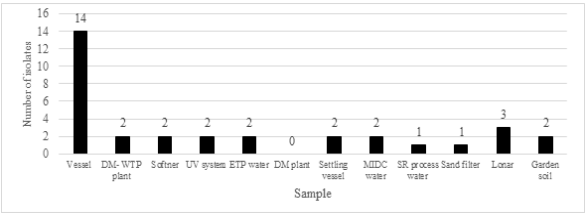
was monitored.

RESULTS & DISCUSSION:

ISOLATION OF PIGMENTS PRODUCING BACTERIA:

Pigment producing microorganism were isolated from different ecological niche of Maharashtra state. Water and soil samples were collected from different sites of industrial area and Lonar lake of Maharashtra which is alkaline soda lake. Water sample was collected from Lonar Lake and 9 different water samples were collected from water treatment plant and industrially contaminated water. Soil sample was also collected from garden soil. The results are presented in Figure 1.

Figure 1: Comparative data of the pigment producing isolates isolated from different collected samples

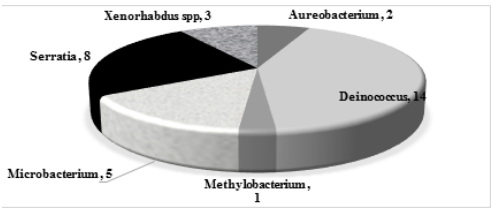


After isolation, 33 red pigment producing isolates were obtained. 3 were from soils of Lonar Lake. 28 pigment producing isolates were obtained from different Industrial effluent water and soil samples. Industrial effluent water contains sulfate, chlorine and other chemicals which creates stress conditions to the bacteria which results in the production of pigments (5). 2 isolates were from the garden soil. The 23 isolates producing prominent pigment after repeated sub culturing were selected for the study.

SCREENING OF PIGMENT PRODUCING BACTERIA:

Isolates were observed for size, colour, margin, elevation, opacity and Gram nature. Yellow pigment producing isolates are showing translucent colonies whereas as red and orange pigment producing isolates are opaque in nature. Depending on the colony characteristics and Biochemical results the isolates are grouped in the various genera such as and *Serratia* described in Bergeys manual. Graphical representation is shown in Figure II.

Figure II: Graphical representation of different pigment producing isolates *Microbacterium, Deinococcus, Acetobacter, Xenorhabdus* up to genus level



EXTRACTION OF THE PIGMENT:

Extraction was done using various solvents. Depending on lambda max and complete extraction of the pigment from the bacterial cell, suitable solvent system was finalised (8). Methanol and chloroform in 1:1 proportion was found to be suitable for the extraction of the pigment from the bacterial cell

ANTIDANDRUFF ACTIVITY:

Pigments were tested against *Malassezia furfur* MTCC 1374 and *Candida albicans* ATCC 10231 using agar cup method. Eight isolates viz. Isolate no 1, 10, 16, 19, 20, 21, 22 and isolate no 23 showed higher zone of inhibition compared to other pigments. These isolates are mainly obtained from industrially contaminated water and water treatment plant. Isolate 23 showed highest zone of inhibition i.e. 39.50±1.0mm for *Malassezia furfur* MTCC 1374 which was isolated from industrial water sample. Isolate 1 obtained from Sand filter sediments showed highest activity against *Candida albicans* i.e. 39.33 ±0.58 mm zone of inhibition. ZPTO showed zone of inhibition 43.00±1.00 mm for *Malassezia furfur* and 43.66±0.57 mm was for *Candida albicans*. Isolate 10, 16, 19, 20, 21 and 22 also showed the zone of inhibition ranging from 25 mm to 39 mm described in Table no 1.

Performance of the pigment in shampoo formulation was also studied using agar cup method. ZPTO in shampoo was studied as positive control. The results showed that isolate no 1, 10, 16, 19, 20, 21, 22 and isolate no 23 are effective in shampoo formulation at 1.0 % active concentration. Isolate 1 and isolate 21 showed 22.66 mm ±1.53 and 22.66 mm ± 0.29 respectively against *Malassezia furfur* MTCC 1374. Isolate 1 also showed 22.66±0.76 mm zone of inhibition activity against *Candida albicans*. The activity of other pigment are represented in table no 1.

Table no I: Antidandruff activity of isolates by agar cup method

Sr. No	Isolate ID	Zone of inhibition in mm		Zone of inhibition in shampoo (mm)	
		Malassezia furfur MTCC 1374	*Candida albicans ATCC 10231*	*Malassezia furfur MTCC 1374*	*Candida albicans ATCC 10231*
1	Isolate 1	33.67 ± 1.61	39.33 ± 0.58	22.66±1.53	22.66 ± 0.76
2	Isolate 2	12.33 ± 0.58	13.50 ± 0.50	20.50 ± 0.50	10.83 ± 0.29
3	Isolate 3	19.00 ± 0.0	18.33 ± 0.29	20.17 ± 0.76	11.5 ± 1.50
4	Isolate 4	17.50 ± 0.50	18.67 ± 0.29	21.67 ± 1.26	10.67 ± 1.04
5	Isolate 5	26.00 ± 0.50	19.33 ± 0.58	16.84 ± 0.76	15.17 ± 0.29
6	Isolate 6	21.17 ± 0.76	20.17 ± 1.04	15.00 ± 0.87	15.83 ± 0.76
7	Isolate 7	18.33 ± 0.29	18.83 ± 0.29	13.50 ± 0.50	17.83 ± 1.04
8	Isolate 8	21.50 ± 0.50	20.33 ± 0.29	21.00 ± 1.32	16.00 ± 0.00
9	Isolate 9	17.67 ± 0.29	16.50 ± 0.50	16.50 ± 0.50	12.17 ± 1.04
10	Isolate 10	38.00 ± 3.77	38.17 ± 0.29	21.5±0.5	21±0
11	Isolate 11	12.83 ± 0.76	14.33 ± 0.29	12.67 ± 0.29	16.17 ± 0.76
12	Isolate 12	21.67 ± 1.04	23.67 ± 0.29	21.50 ± 0.50	12.17 ± 0.29
13	Isolate 13	31.67 ± 0.29	33.33 ± 0.76	22.00 ± 0.87	15.33 ± 1.76
14	Isolate 14	23.00 ± 1.32	20.67 ± 0.29	18.17 ± 0.76	17.50 ± 0.50
15	Isolate 15	13.50 ± 0.50	18.33 ± 0.29	14.67 ± 0.29	15.17 ± 0.58
16	**Isolate 16**	**25.00 ± 0.50**	**25.83 ± 0.29**	**20.33±0.58**	**19.5±0.5**
17	Isolate 17	22.50 ± 0.87	22.83 ± 0.29	19.00 ± 0.87	17.33 ± 1.26
18	Isolate 18	14.67 ± 0.76	13.33 ± 0.29	14.00 ± 0.50	13.17 ± 0.58
19	Isolate 19	31.83 ± 1.89	34.33 ± 0.29	20.83±0.58	21±1
20	**Isolate 20**	**24.17 ± 0.29**	**33.33 ± 0.58**	**21.16±0.58**	**20.66±0.76**
21	Isolate 21	32.50 ± 0.87	31.50 ± 0.87	22.66±0.29	20.83±0.76
22	**Isolate 22**	**32.50 ± 0.50**	**38.50 ± 0.87**	**22.83±0.76**	**20.16±0.29**
23	Isolate 23	39.50 ± 1.00	30.17 ± 0.29	20.66±0.58	20.16±0.29
24	ZPTO	43.00±1.00	43.66±0.57	49.00±1.00	50.00±1.00
25	Placebo shampoo	0	0	0	0

ZPTO has strong antifungal activity. Zone of inhibition is >40 mm in shampoo formulation. Whereas, zone of inhibition was found to be >20 mm in case of pigments in shampoo formulation.

STUDY OF MINIMUM INHIBITORY CONCENTRATION:

MIC study was performed for the DMSO suspended pigment using tube method various concentration were prepared and inoculated with the test organism. ZPTO was used as standard antidandruff agent. MIC value isolate was higher i.e. 750 µg/ml in case of Isolate I against *Malassezia furfur* and C*andida albicans* and Isolate 21 against *Malassezia furfur* compared to other Isolates. ZPTO showed 100 µg/ml MIC value against *Malassezia furfur* and *Candida albicans*. Details are captured in table II.

Table no II : Study of the Minimum Inhibitory Concentration of the pigments against *Malassezia furfur* MTCC 1374 & *Candida albicans* ATCC 10231

Sr. No.	Isolate ID	Minimum Inhibitory Concentration (µg/ml)	
		Malassezia furfur MTCC 1374	*Candida albicans ATCC 10231*
1	Isolate 1	750	750
2	Isolate 10	500	500
3	Isolate 22	500	500
4	Isolate 21	750	500
5	Isolate 23	500	500
6	Isolate 20	500	500
7	Isolate 19	500	500
8	Isolate 16	500	500
9	ZPTO	100	100

TIME KILL METHOD

The reduction in culture count in presence of the DMSO suspended pigment was evaluated using time kill study. The Isolate 16 showed 100 % reduction at 5 min for *Malassezia furfur MTCC 1374*. Isolate 20 and 22 showed 99.99 % (4 log) and 99.999% (5 log) reduction respectively. ZPTO showed 100 % reduction in 30 sec for *Malassezia furfur MTCC 1374*. (Table III)

For *Candida albicans ATCC 10231* isolate 22 and isolate 20 showed 99.98 % reduction whereas isolate 16 showed 99.88 % reduction. Isolate 20, isolate 22 and isolate 16 are showing antidandruff activity (Table III) ZPTO showed 100 % reduction in 30 sec for *Candida albicans.* The results are comparable with ZPTO.

Table III: Percent reduction in count after 5 min of contact time

Microbial Strain	***Malassezia furfur MTCC 1374***				***Candida albicans ATCC 10231***			
Isolated Pigment	Isolate 22	Isolate 20	Isolate 16	ZPTO	Isolate 22	Isolate 20	Isolate 16	ZPTO
Percent inhibition (%)	**99.999**	**99.992**	**100**	**100**	**99.986**	**99.982**	**99.883**	**100**

CONCLUSION:

The samples collected from different ecological niche of Maharashtra were screened for the pigment producing bacteria. Total 33 isolates were obtained from 13 different samples. The colony characteristics were studied and absorption maxima was determined, it was found that extracted pigments were giving absorption maxima in the range of 490-515 nm wavelength. Prodigiosin pigment also gives the absorption maxima in at 490 nm. Thus the extracted pigments resemble the Prodigiosin like structure.

The antidandruff activity was also studied against *Malassezia furfur* MTCC 1374 and *Candida albicans* ATCC 10231 and it is found that the 4 isolates produce pigment having antidandruff activity. Time kill study was also performed to evaluate the time required for reduction in count. The results indicate that the isolate 20, isolate 22 and isolate 16 are potent strains with respect to pigment production for antidandruff application. Isolate 16 was isolated from water system, isolate 20 was isolated from effluent of the industrial vessel and isolate 22 isolated from the settling vessel tank. These strains are producing pigment having antidandruff activity can be used for the antidandruff shampoo with natural antimicrobial agent. These pigments are natural compounds therefore don't have any adverse effect on the health. This is considered as new opportunity in development of antidandruff shampoo.

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