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Extracellular production and characterization of red pigment from *Penicillium purpurogenum* BKS9

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Abstract: Fifteen fungal species were isolated from waste soil and used for the production of red pigment. Among the fifteen fungal isolates only *Penicillium sp.* BKS 9 displayed excellent production ability of red pigment in sabouraud's dextrose agar medium as well as broth medium. After 28S rRNA sequencing the fungus *Penicillium* sp. BKS9 was identified as *Penicillium purpurogenum* and submitted to NCBI (Gene Bank) with Gene Bank accession number KT222270. Further, optimization of incubation period and pH was performed and it was concluded that an incubation for 18 days with pH 6.0 at 30°C are the most favorable conditions for biomass (0.877 g/50 ml) and red pigment (0.79 Abs/ml) production. The pigment used for the study was soluble in all the organic solvents taken. The effect of red pigment on germination of fifty numbers of viable and healthy seeds of *Cicer arietinum* was studied and confirmed that the pigment has no significant negative effect on the germination of the seeds.

Keywords: Optimization; Incubation; pigment; solvents

I. Introduction

There is global interest in process development for the extracellular biosynthesis of pigments from microorganism owing to a serious safety issues with many artificial synthetic colorants, which have widely been employed in cosmetic, pharmaceutical manufacturing and foodstuff processes[1]. The natural food colorants are derived either from plant or animal and have numerous disadvantages such as instability against light, low water solubility and are also

non-available throughout the year. In this context the pigments from microbial origin are the excellent alternative. Natural pigments have several activities such as, anticancer activity, stability to light, heat and pH[2]. Furthermore, natural colorants will not only be helpful to the health of humans but it will be a blessing for the preservation and protection of biodiversity from harmful chemicals released into the environment while producing synthetic colorants. These natural colorants are employed for the production of baby foods, breakfast cereals, pastas, sauces, processed cheese, fruit drinks, vitamin-enriched milk products and some energy drinks. Hence, natural colors are environment friendly and can also serve the dual need for visually appealing colors and probiotic health benefits in food products [3.]

Generally, well textured food, prosperous rich in nutrients and flavor cannot be consumed unless it has the right color. The requirement for natural source of such pigments is increasing every day because of the awareness of positive health benefits. As a result, it is essential to explore various microbial sources for the production of food grade colorants and their potentials. Though many natural colors are available, still microbial colorants play a crucial role as food colorant, because of its production and easy down streaming process. Thus, the food processing industry has bound to use microbial technology to produce colors for use in foods.

Microorganisms are well known for the production of a variety of pigments; therefore they are the potent source of food colorants [4, 5]. Although the roles of many secondary metabolites are still unexplored, it is generally believed that pigment likely protect fungi from exposure to environmental stress like UV light [6]. This class of compounds has a high spectrum of their biological activities, such as antibacterial, antifungal, phytotoxic, insecticidal and cytotoxic properties [7]. The synthesis of many secondary metabolites is governed by environmental factors, such as various culture conditions like temperature, pH and incubation period [8] that play a crucial role on growth and production of microbial pigments [9] by microorganisms. Most of the bacteria and fungi are widely employed for their potential as the source of food colorants. Many fungi have been studied to produce non-carotenoid pigments but only a few of those have been explored as possible food colorants [10]. Many species of fungus also have also received special attention owing to their capability of secreting different coloured pigments displaying high chemical stability [11].

Therefore, the aim of this study was to select and identify strains of filamentous fungi as potential pigment producers. An attempt has also been made for optimisation as well as characterisation of the pigment to evaluate its biotechnological potential

II. Experimental Section
II.1 Isolation of fungi

The fungi were isolated from collected soil samples of Gobaraghati, Kalinganagar, Jajpur, Odisha (Site-1) and three different location (Site-2, 3 & 4) of Patia Industrial area, Bhubaneswar, Odisha by serial dilution and pour-plate technique [12]. The mixed fungal cultures were obtained by pour-plating respective diluted soil samples on potato dextrose agar (PDA) and sabouraued's dextrose agar (SDA) plates amended with sefixime (50 g/100ml) under sterile conditions at 30±1°C for 7 days.

II.2 Qualitative screening for pigment production

All the fifteen isolated fungal species were analyzed for their colour producing ability. Sterilized Sabouraud's Dextrose agar media (pH 7.0) supplemented with 50 g/ 100 ml of cefixime (to inhibit bacterial growth) were cooled to 45 °C and aseptically transferred to presterilized Petri plate. After proper mixing and proper solidification, 0.7 cm^2 plugs were point inoculated at the centre of the pour plated plates having media in triplicates. The plates were incubated at 30 ± 1 °C in the incubator for 7 days. Their pattern of growth and pigment-producing ability of fungal colonies was studied in alternative days (after 1, 3, 5, 7 and 9 days). After completion of each incubation, period the colonies were studied for hyphal development and pigment production. The colony morphology and intensity of pigment production were evaluated.

The fungal species which were found to be the producer of red pigment on plates were again screened for their pigment production on broth. Hence, the fungal species were inoculated into Sabouraud's Dextrose broth medium (pH 7.0). 50 ml of fermentation broth was transferred to 150 ml Erlenmeyer flasks (Borosil) and autoclaved as per the standard procedure. The flasks having broth medium were inoculated aseptically with approximately 1 × 10^7 cells/ml from seven days   old cultures. The inoculated flasks were incubated in incubator at static condition under dark condition at 30± 1 °C for 15 days with intermittent observation at different time interval.

II.3 Morphological identification of isolated fungi

All isolated fungi were identified by morphological examinations as per Alexopoulos and Mims [13] and Watanabe [14] based on macro and microscopic characteristics. Identification based on colony morphological study includes form, pattern, quantity of aerial hyphae, diameter, colony colour and texture, margin, elevation, colour of colony reverse, presence of exudates, organs formed and soluble pigment. For micromorphological observations, microscopic mounts were made in lactophenol-cotton blue solution by taking fungi from PDA plates and a drop of alcohol was added to remove air bubbles and excess conidia. Microscopic study was carried out to study the shape and size of head, vesicles, conidiophore and conidia, thickness of stipes and presence of phialides and metulas.

II.4 Molecular identification of the hyper producer fungus

Genomic DNA was isolated from the fungal culture by the method of Moller et al. [15] and confirmed by 1.2 % Agarose gel electrophoresis.The DNA was amplified and sequenced at Xcelris Labs Ltd., Ahmedabad, India. Fragment of 28S rDNA gene was amplified by Eppendorf Thermal Cycler using **DF_S014421_C07_059.ab1** and **DR_S014421_D07_057.ab1** primers. A single discrete PCR amplicon band of 700 bp was observed. The PCR amplicon was purified using QIA quick PCR purification kit (QIAGEN, UK) according to the manufacturer's protocol. Forward and Reverse DNA sequencing reaction of PCR amplicon was carried out with 535F and 541R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). Consensus sequence of **574 bp** of D2 region of LSU gene was

generated from forward and reverse sequence data using aligner software. The D2 region of 28S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. To reveal evolutionary relationships between isolates, DNA sequences were aligned using CLUSTALW (http://www.ebi.ac.uk/clustalw/).The evolutionary history was inferred using the Neighbor-Joining method [16]. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed [17]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [17]. The evolutionary distances were computed using the Kimura 2-parameter method [18] and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of **527** positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [19].

II.5 Working fungus and inoculum build up

This fungus was maintained on PDA/Sabouraud dextrose agar slants at 4°C. Spore suspension (1 ml) having spore concentration of about 1×10^7 cells ml^{-1} from 7 days old culture was used as inoculum in the subsequent experiments [20].

II.6 Quantitative optimization of pigment production in different incubation period

Fifty milliliter of sterilized sabouraud's dextrose broth medium was inoculated aseptically with 1ml of spore suspension from respective seven days old fungal culture as described above to make final spore count of 1×10^7 spores/ml. The inoculated flasks were incubated and studied at different incubation period ranging from 24 h-360 h at 30±1°C and pH 7.0 in static condition. Growth of the fungal strains and pigment production was monitored at 48 h interval up to 360 h. At the interval of 48 h, the fermented broth was filtered through the whatman No-1 filter paper to obtain the dissolved pigment without biomass. The filtrate was centrifuged at 1000rpm for 15 min to obtain cell free extract. Absorbance of the coloured extract or pigment was measured at 500 nm by taking 95% ethanol or distilled water as blank and pigment yield was expressed as Abs/ml [21]. The biomass remained in the filter paper was used for the dry biomass weight analysis.

II.7 Quantitative optimization of pigment production in different pH

For the study, 50ml 0f sabouraud's dextrose broth medium was dispensed into the sterilized flasks (Borosil) of 150 ml capacity and the desired pH values (pH 3.0 to 9.0) of the medium was adjusted using either 1 N HCl and 1 N NaOH and /or then autoclaved at 121 °C for 15 min. After cooling to room temperature, every 50 ml medium was inoculated aseptically with 1ml of spore suspension from respective seven days old fungal culture as described above to make final spore count of 1×10^7 spores/ml. The inoculated flasks were incubated at 30 ±1°C for 15 days in static condition. After 15 days of incubation, the fermented broth was filtered through

the Whatman No-1 filter paper to obtain the pigment without of biomass. The filtrate was centrifuged at 10,000rpm to get the cell free coloured pigment. Absorbance of the coloured extract or pigment was measured at 500 nm by taking 95% ethanol or distilled water as blank and pigment yield was expressed as Abs/ml [21]. The biomass remained in the filter paper was used for the dry biomass weight analysis.

II.8 In vitro pigment extraction and assay

The pigment was extracted from the biomss as per the method of   Velmurugan et al. [22]. In brief 5 g of chilled fresh mycelial mat was taken carefully and washed with sterile distilled water repeatedly changing the water for every wash until the flow of water becomes clear. Ethanol (90%) was added to the test tube at 1:10 ratio (10 ml ethanol per gm of biomass). The mycelia mats immersed in ethanol were heated on a boiling water bath for 30 min to extract the pigments. Thereafter, the mycelia mat was homogenized to make slurry in a clean sterilized mortar and pestle with a pinch of acid washed and oven sterilized sand to remove any pigments that may linger in the biomass/ mycelial mats. The slurry was shaken using an orbital shaker at 200 rpm for 1 h, allowed to stand for 15 min and filtered through Whatman No. 1 filter paper. Absorbance of the coloured extract or pigment obtained was measured at 500 nm by taking 95% ethanol or distilled water as blank and pigment yield was expressed as Abs/ml [21].

II.9 Characterization of chemical parameters of isolated pigment

The chemical parameters on the pigment stability were evaluated. The colour, colour content, water solubility, solubility in organic solvents (acetone, methanol and ethanol), water-acetone (1:1; 1:2; 1:3 and 1:4), water-ethanol (1:1; 1:2; 1:3 and 1:4), water-methanol (1:1; 1:2; 1:3 and 1:4), acetone-methanol (1:1; 1:2; 1:3 and 1:4), methano-ethanol (1:1; 1:2; 1:3 and 1:4), solubility, hue and hygroscopy were studied using the method of Velmurugan et al. [23].

II.10 Effect of pigment on germination of Cicer aeriatinum

Fifty numbers of viable and healthy seeds of *Cicer arietinum* (chick pea) were taken in plastic glass pot and soaked in 100 ml of distilled water up to 24 h. In another set of experiment, 50 numbers of viable and healthy seeds of *C. arietinum* were taken and soaked in 100 ml of distilled water mixed with 1% to 5% (v/v) extracted pigment for a period of 24 h. After 24 h of soaking, the swollen seeds were wrapped with a moist cotton cloth and incubated in the dark for another 24 h at room temperature. After completion of the incubation for germination, the well germinated seeds were counted and the percentage of germination was calculated.

II.11 Statistical analysis
Each set of the experiments were conducted in duplicate and repeated three times. The final result represented is the mean of the actual findings. The ± values in tables and error bars in graphs indicate standard deviation among the replicates.

III. Results
III.1 Isolation of mixed fungal population

Fungi were isolated from soil samples of industrial areas of Jajpur and Bhubaneswar, Odisha and are presented in table-1. Maximum numbers of fungi were found from site-1 (2×10^7 CFU/g) followed by site-4 (2×10^6 CFU/g), site-2.

Table 1 Colony forming units (CFU/g) in polluted soils of few industrial areas of Odisha

Site of collection of soil	Number of soil samples collected	Soil samples having pigment producing fungi	% of samples having pigment producing fungi	Colony forming units in original sample (CFU/g)
Site-1	10	8	80[a]	2×10^7
Site-2	10	7	70[b]	1×10^5
Site-3	10	5	50[c]	2×10^5
Site-4	10	7	70[b]	1×10^6

[a] *Data pooled from a total of 3 separate experiments each comprising of 3 replicates. Means within a column with different superscripts are significantly different at p d≤ 0.05 tested through Duncan's New Multiple Range Test.*

III.2 Pure culture maintenance of fungi

Mixed fungal colonies were aseptically streaked on Saboraud's Dextrose agar (SDA) plates and individual culture of each was maintained. A total 15 fungal colonies were isolated and named as BKS 1 to BKS15.

III.3 Qualitative screening for pigment production

The ability of pigment production by isolated 15 fungal species was investigated on SDA plates. Out of the fifteen fungal isolates, only two isolates, BKS-9 and BKS-12 were found to be positive for red pigment production (Fig 1). However, plate method was not suitable for quantification of red pigment. Therefore, the strains were further screened on SDA broth medium and maximum pigment production was observed by the fungal isolates BKS 9 (Fig 2). Hence the strain BKS9 was selected for further study.
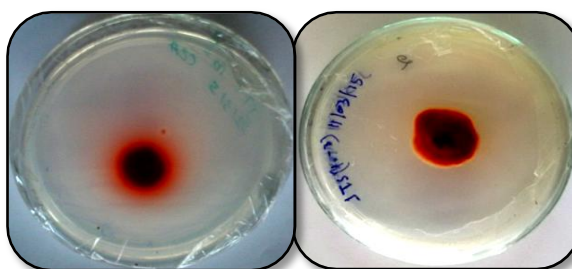

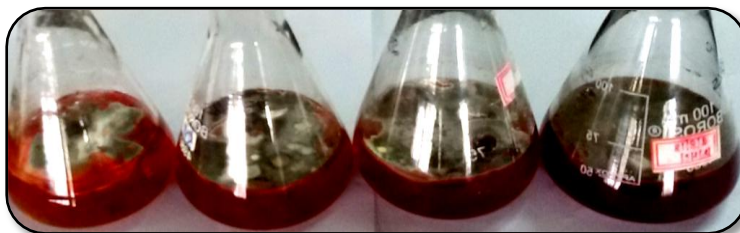*Fig. 1* Red pigment production on SDA plates by fungal isolate BKS9 (left) and BKS 12 (Right)

Fig. 2 Screening of red pigment production on SDA broth medium

III.4 Micro morphological identification of Fungi

Based on microscopic and macro and micro morphological characteristics the fungal strains are tentatively identified belongs to *Alternaria* sp. (BKS1), *Aspergillus* sp. (BKS 2, BKS 3 and BKS 4), *Fusarium* sp. (BKS5), *Mucor* sp. (BKS6 and BKS 7), *Penicillium* sp. (BKS 8, BKS 9, BKS 10, BKS 11, BKS 12 and BKS 13) and *Rhizopus* sp. (BKS 14 and BKS 15).

III.5 Molecular identification of *Penicillium* sp. (BKS9)

Further confirmation of genus of most efficient red pigment producing strain, BKS 9 was done by BLAST analysis data of the 28S rRNA gene sequence which showed similarity with the genus *Penicillium* sp. The sequences were submitted to gene bank. The gene bank accession number of the strain BKS 9 is KT222270. Phylogenetic tree were constructed by comparing nucleotide sequences of 28S rRNA gene of the isolate, BKS 9 with different *Penicillium* sp. We found that the isolate BKS 9 is most closely related to *Penicillium purpurogenum* (Fig. 3)
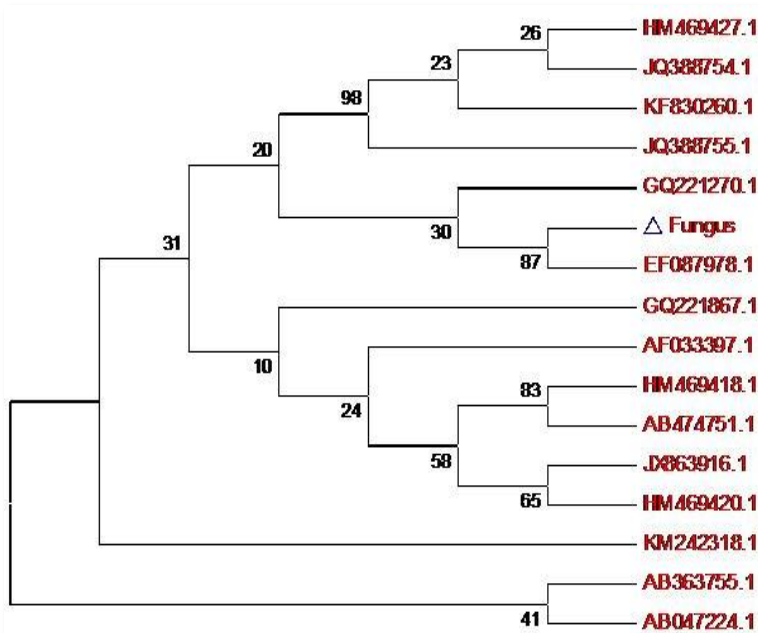

Fig. 3 Phylogenetic Tree of Penicillium purpurogenum (BKS9)

III.6 Quantitative optimization of pigment production

Effect of incubation period on growth and pigment production by *Penicillium purpurogenum* BKS9 was studied. It was observed that the production of biomass was commenced from second day and continued up to fifteenth day of inoculation (0.847 g/50ml) (Fig. 4). The production of biomass was remained almost constant thereafter (Fig. 7). Spectroscopic analysis revealed that red pigment production was recorded from fifth day of incubation (0.176 Abs/ml) with maximum production of pigment (0.761 Abs/ml) at eighteenth day of incubation (Fig.5).
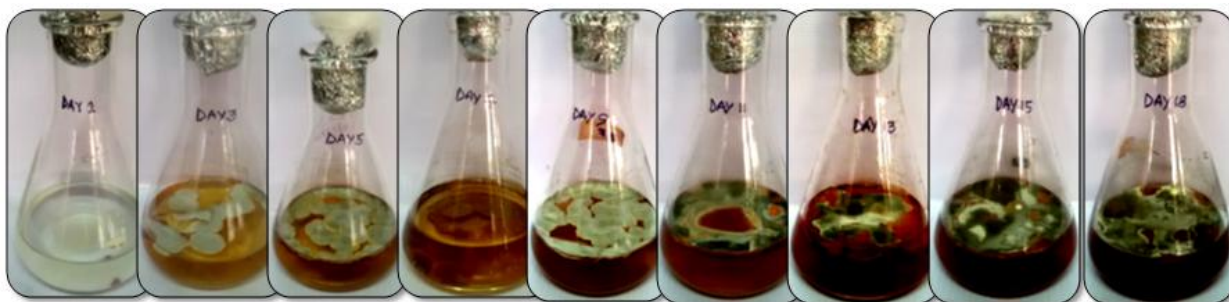

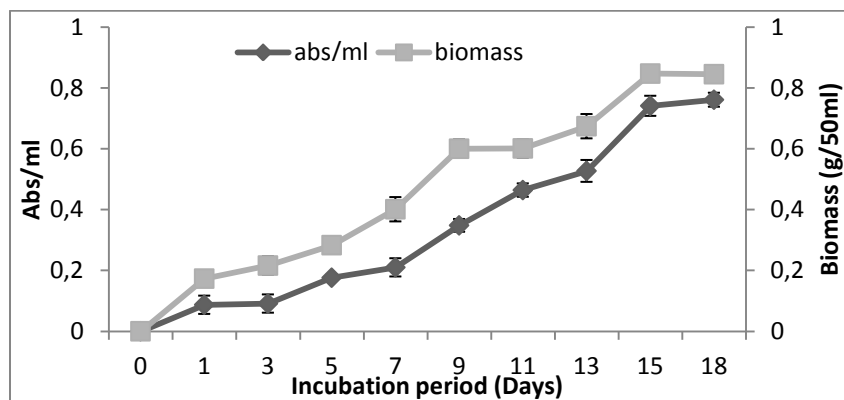*Fig. 4: Biomass and pigment production at varying incubation period by P. purpurogenum BKS9*

Fig. 5: *Biomass and pigment production by P. purpurogenum BKS9 in varying incubation period*

III.7 Effect of pH on pigment production

From the present study it has been confirmed that pH had a greater influence on biomass production as well as on production of pigment. Both biomass and pigment production was very less at initial pH value of 3.0 but gradually increase with increase in pH. Maximum production of red pigment (0.79 Abs/ml) and biomass (0.877 g/50ml) was attained at pH 6.0 and eighteenth day of incubation but decreased thereafter (Fig. 6).
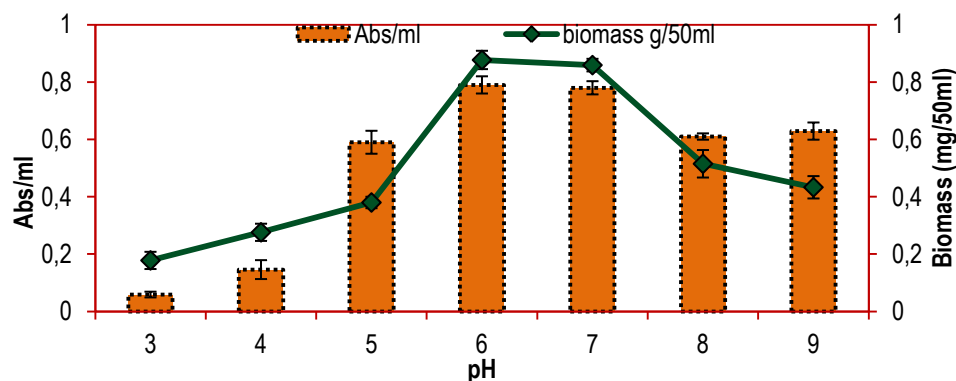
Fig. 6 *Growth and pigment production by P. purpurogenum BKS9 at varying pH*

III.8 *In vitro* pigment assay

In vitro pigment extraction was performed by homogenization of the harvested biomass. *In vitro* pigment assay was performed to estimate the pigment. It was concluded that *P. purpurogenum* BKS9 was able to produce the red pigment from fifth day (0.163 Abs/ml) and this production was continued up to thirteenth day (0.526 Abs/ml) (Table 2).

Table 2 *Invitro pigment produced by P. purpurogenum BKS9 at different incubation period*

Incubation period (days)	Absorbance at 500nm (Abs/ml)
5	0.163±0.09
7	0.420±0.21
9	0.431±0.33
11	0.482±0.27
13	0.526±0.46

N.B. *The submerged fermentation was conducted at pH 6.0 and temperature 30°C.*

III.9 Characterization of extracted red pigment

The red pigment of *Penicillium purpurogenum* BKS9 was subjected for characterization study to evaluate its industrial suitability. Pigment characterization study was performed under various chemical parameters and results are presented in Table 3. The pigment is soluble in all the above solvents showing complete solubility with water and color was red at 500 nm.

Table 3 *Physical properties of the isolated red pigment*

Parameters	Properties
Colour	Red
Colour content	10.22
Water solubility	Yes
Acetone solubility	Yes
Water-acetone (1:1)	Yes
Water-acetone (1:2)	Yes
Water-acetone (1:3)	Yes
Water-acetone (1:4)	Yes
water-methanol (1:1)	Yes

water-methanol (1:2)	Yes
water-methanol (1:3)	Yes
water-methanol (1:4)	Yes
water-ethanol (1:1)	Yes
water-ethanol (1:2)	Yes
water-ethanol (1:3)	Yes
water-ethanol (1:4)	Yes
acetone-methanol (1:1)	Yes
acetone-methanol (1:2)	Yes
acetone-methanol (1:3)	Yes
acetone-methanol (1:4)	Yes
methanol-ethanol (1:1)	Yes
methanol-ethanol (1:1)	Yes
methanol-ethanol (1:1)	Yes
methanol-ethanol (1:1)	Yes
HUE (0.1%, v/v)	Faint reddish
Hygroscopy	Negative

III.10 Effect of isolated pigment on the germination of Cicer aeriatinum

The effect of red pigment on germination of fifty numbers of viable and healthy seeds of *Cicer arietinum* was studied for any inhibitory effect. It was confirmed that the pigment has no significant negative effect on the germination of the seeds. However, the cause of variation in percentage of germination is not confirmed till now and further research is required to disclose the exact mechanism (Table 4; Fig. 7).
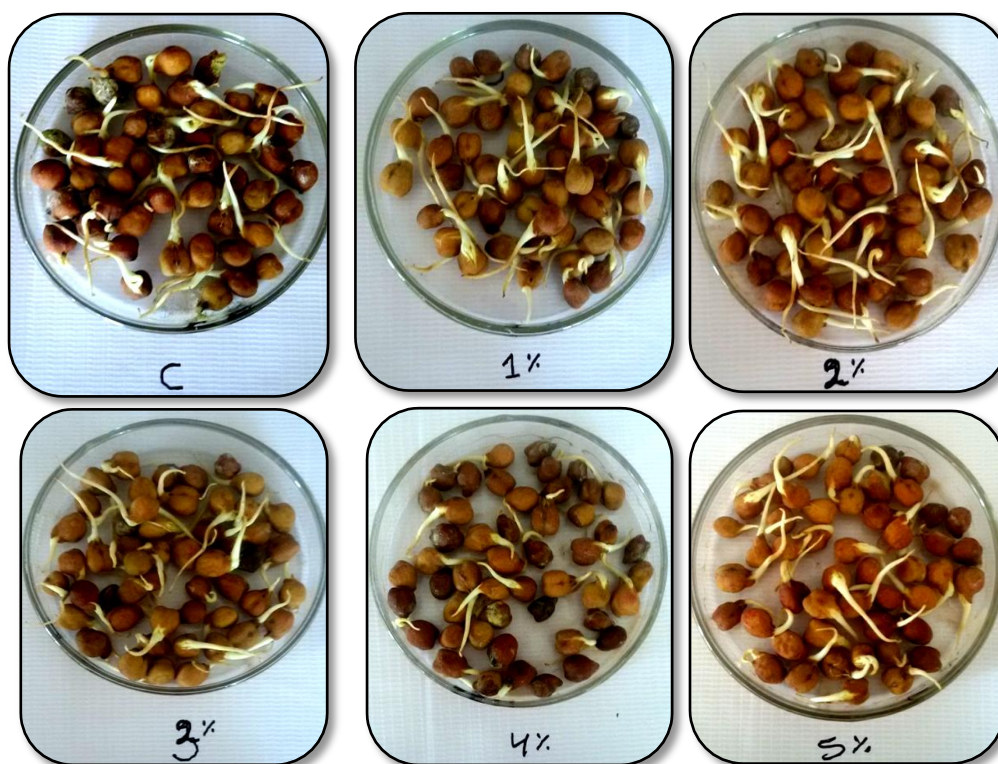

Fig. 13: *Effect of red pigment on the germination pattern of healthy seeds of Cicer arietinum*

Table 4 Germination pattern of healthy seeds of Cicer arietinum

Concentration of pigment (%)	No. of seeds taken	No. of seeds germinated	Percentage of germination
Control (C)	50	46	92
1	50	48	98
2	50	48	98
3	50	48	98
4	50	47	94
5	50	48	98

IV. Discussion

In the present study 15 fungal colonies were isolated from soil samples at different study sites of Jajpur and Bhubaneswar, Odisha, India. All the fungal isolate are identified through micro morphological and physiological basis. Out of the 15 fungal isolates only one isolates found to be produced maximum red pigment, hence selected for further study. The fungal isolate was identified as *Penicillium purpurogenum* BKS9 through 28S rRNA sequencing. Similarly Petit et al. [24] also enumerated and isolated several *Penicillium* species from soil and examined their ability for the secretion of novel secondary metabolites. *Penicillium* species NIOM-02 was also isolated from the sediment and studied for the production of red pigment [25]. Jiang et al. [26] reported *Penicillium* sp. (HKUCC 8070) which was able to produce the water soluble wine red pigment on the reverse of the potato dextrose agar medium. Mapari et al. [27] screened for *Monascus*-like food grade pigments from *Penicillium* species using computer-based- screening method and reported two isolates species of *Penicillium* sp. have the capability to produce *Monascus* pigments.

In this study, pigment production was just started after 5th day of incubation and reached its maximum at 18th days of incubation. Similarly, Mabrouk et al. [28] have reported that the production of the dye was begun just initiated after the seventh day of incubation. A similar finding was also reported by Marova et al. [29] for yeast strains. Maximum beta-carotene production was attained only in the late logarithm and in stationary phase of growth in all the strains. Highest production of red pigment in seventh day of incubation was also reported by Velmurugan et a*l.* [23].

Red pigment production was strongly influence by the action of pH [30]. Color content and intensity of the pigment depends on the initial pH of the fermentation medium [31]. In the present study maximum red pigment production by *Penicillium purpurogenum* BKS9 was observed at pH 6.0. The result obtained is at par the findings of Chen & Johns [32], who reported highest red pigment production by *Monascus purpureus* culture at pH levels 6.0. Maximum red pigment production at lower pH (pH 5.0) was also reported by Mendez et al. [33]. Babitha et al. [34] reported that optimum pigment production was attained with a pH range of 5.5 to 7.5 using *M. purpureus* which is similar with the present findings.

In vitro pigment extraction was performed to study the extracellular pigment production. It was found that the intracellular pigment production is higher than the extracellular pigments. Lin et al. [35] also reported that the pigments were present mainly in the intracellular state in *Monascus* strains. Generally, the intracellular pigments content were higher as compared to extracellular pigment which is at par the present findings.

The chemical parameters such as colour, colour content, water solubility, solubility in organic solvents, hue and hygroscopy on the pigment stability was also evaluated. It was found that the isolated pigment is soluble in most of the solvent systems. Hailei et al. [36] also isolated pigment from co-culture of *Penicillium* sp. HSD07B and *Candida tropicalis* which was soluble with water, ethanol, acetic acid, acetone, glycerol, 1-butanol and methanol, but insoluble in ether, chloroform and non-polar solvents such as petroleum ether and n-hexane. Similar finding was also reported by Velmurugan et al. [23]. Biological activity study was performed using fifty numbers of viable and healthy seeds of *Cicer arietinum* and it was concluded that the pigment has no toxic effect on the germination of the seeds. Both the control and treated seeds showed at par germination pattern.

V. Conclusion

In this work, a novel hyper-producer *Penicillium purpurogenum* BKS9 was isolated that exhibited exuberant production of natural water- soluble red pigment. This isolated fungus can be a potential candidate in food and/or textile industries. Further, the production of red pigment by *P. purpurogenum* BKS9 in economic and synthetic medium will be of great significance in view of its commercial exploitation. Due to its stability in different chemical parameters and non-toxic nature, this novel red pigment may be utilized as a potential food colorant in food industries for the better food quality and positive health effects. But, prior to its industrial exploitation, a detail study of this pigment is required. In addition to this, a complete research is essential for elucidation of expression pattern and regulation of key genes regulating the biosynthesis of such secondary metabolites.

VI. References

[1]   Kim SW, Hwang HJ, Xu CP, Na YS, Song SK, Yun JW., Influence of nutrition conditions on the mycelial growth and exopolysaccharide production in *Paecilomyces sinclairii*, Lett. Appl. Microbiol. 34 (2002) 389-393.
[2]   Joshi VK., Attri D., Bala A., Bhushan S., Microbial Pigments, Ind. J. Biotech. 2 (2003) 362-367.
[3]   Nagpal N., Munjal N., Chatterjee S., Microbial Pigments with Health Benefits - A Mini Review, Trends Biosci. 4: (2011)157-160.
[4]   Aberoumand A., (2011) A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food Industry, World. J. Dairy Food Sci. 6:1(2011)71-78.
[5]   Ahmad WA., Ahmad WYW., Zakaria ZA., Yusof NZ., Application of bacterial pigments as Colorant. Springer Briefs in Molecular Science, (2012) pp 57-74.
[6]   Mendentsev AG., Arinbasarova AY., Akimenko VK., Biosynthesis of naphthoquinone pigment by fungi of the genus *Fusarium*, Appl. Biochem. Microbiol. 41: 5 (2005) 573-577.
[7]   Jittra K., Kulvadee D., Nattawut B., Tanapong B., Pranee R., Samran P., Palangpon K., Chawanee T., Dihydronaphthalenones from endophytic fungus *Fusarium sp.* BCC14842, Tetrahed. 67 (2011) 7540–7547.

[8]   Moita C., Feio SS., Nunes L., Joa M., Curto M., Optimization of physical factors on the production of active metabolites by *Bacillus subtilis* 355 against wood surface contaminant fungi, Int. Biodet. Biodegrad. 55 (2005) 261–269.

[9]   Vahidi H., Kobarfard F., Namjoyan F., Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*, Afr. J. Biotechnol. 3 (2004) 606-609.

[10]   Sameer AM., Anne SM., Ulf T., Colorimetric Characterization for Comparative Analysis of Fungal Pigments and Natural Food Colorants, J. Agric. Food Chem. 54 (2006) 7027-7035.

[11]   Hajjaj H., Klaebe A., Loret MO., Tzedakis T., Goma G., Blanc JP., Production and identification of N-Glucosylrubropuntamine and N-Glucosylmonascorubramine from *Monascus ruber* and occurance of electron donor-acceptor complexes in these red pigments, Appl. Environ. Microbiol. 63 (1997) 2671-2678.

[12]   Sohail M., Naseeb S., Sherwani SK., Khan S., Distribution of hydrolytic enzymes among native fungi: Aspergillus the pre-dominant genus of hydrolase producer., Pak. J. Bot. 41:5 (2009) 2567-2582.

[13]   Alexopoulos C.J., Mims CW., Introductory Mycology, 3rd eds, John Wiley & Sons, (1979) pp. 1-613.

[14]   Watanabe T., Pictorial atlas of soil and seed fungi - morphologies of cultured fungi and key to species. 2nd ed. Boca Ratón: CRC Press; 2002.

[15]   Möller EM., Bahnweg G., San dermann H., Geiger HH., A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucl Acids Res. 22 (1992) 6115 - 6116.

[16]   Saitou N., Nei M.,The Neighbor-joining method: A new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4:4 (1987) 406-425.

[17]   Felsenstein J., Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 39 (1985)783–791.

[18]   Kimura M., A simple method for estimating evolution-ary rates of base substitutions through comparative studies of nucleotide sequences, J. Mol. Evol.16 (1980) 111–120.

[19]   Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.

[20]   Sethi BK., Singh S., Nanda PK., Sahoo SL., Extracellular biosynthesis of amylase by *Aspergillus terreus* NCFT 4269.10 using agro-residues. J. Micrbiol. Biotechnol. Res. 4 (2014) 8-14.

[21]   Said FM., Chisti Y., Brooks J., The effects of forced aeration and initial moisture level on red pigment and biomass production by Monascus ruber in packed bed solid state fermentation, Int. J. Environ. Sci. Develop. 1:1 (2010) 1-4.

[22]   Velmurugan P., Kamala-Kannan S., Balachandar V., Lakshmana- perumalsamy P., Chae JC., Oh BT., Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather, Carbohydr. Polym. 79 (2010) 262–268.

[23]   Velmurugan P., Hur H., Balachandar V., Monascus pigment production by solid-state fermentation with corn cob substrate, J. Biosci. Bioeng. 112 (2011) 590-594.

[24]   Petit P., Lucas EMF., Breu LMA., Flenning LHP., Takahashi JA., Novel antimicrobial secondary metabolites from a *Penicillium* sp. isolated from Brazilian cerrado soil, Elect. J. Biotechnol. 12 (2009) 1–9.

[25]   Dhale MA., Vijay-Raj AS., Pigment and amylase production in *Penicillium* sp NIOM-02 and its radical scavenging activity, Int. J. Food Sci. Technol. 44:12 (2009) 2424-2430.

[26]   Jiang Y., Li HB., Chen F., Hyde K.D., Production potential of water-soluble *Monascus* red pigment by a newly isolated *Penicillium* sp., J. Agric. Technol., 1: 1 (2005)113-126.

[27]   Mapari SAS., Hansen ME., Meyer AS., Thrane U., Computerized screening for novel producers of Monascus-like food pigments in *Penicillium* species. J. Agric. Food. Chem. 56 (2008) 9981-9989.

[28]   Mabrouk AM., El-khrisy EAM., Youssef YA., Asem AM., Production of textile reddish brown dyes by fungi, Mal. J. Microbiol. 7:1 (2011) 33-40.

[29]   Marova I., Carnecka M., Halienova A., Certik M., Dvorakova T., Haronikova A., Use of several waste substrates for carotenoid-rich yeast biomass production, J. Env. Manag. 95 (2011) 338-342.

[30]   Mukherjee G., Singh SK., Purification and characterization of a new red pigment from *Monascus purpureus* in submerged fermentation, Proc. Biochem. 46 (2011)188-192,

[31]   Cho YJ., Park JP., Hwang HJ., Kim SW., Choi JWM., Yun JW., Production of red pigment by submerged culture of *Paecilomyces sinclairii*, Lett. Appl. Microbiol. 35 (2002) 195-202.

[32]   Chen MH., Johns MR., Effect of pH and nitrogen source on pigment production by *Monascus purpureus*, Appl. Microbiol. Biotechnol. 40 (1993)132-138.
[33]   Méndez A., Pérez C., Montañéz JC., Martínez G., Aguilar CN., Red pigment production by *Penicillium purpurogenum* GH2 is influenced by pH and temperature, J. Zhejiang Univ. Sci. B 12:12 (2011) 961-968.
[34]   Babitha S., Soccol CR., Pandey A., Effect of stress on growth, pigment production and morphology of *Monascus* sp in solid cultures, J. Basic Microbiol. 47:2 (2007) 118-26.
[35]   Lin TF., Demain AL., Effect of nutrition of *Monascus* sp. on formation of red pigments, Appl. Microbiol. Biotechnol. 36:1 1991.70-75.
[36]   Hailei W., Zhifang R., Ping L., Yanchang G., Guosheng L., Jianming Y., Improvement of the production of a red pigment in *Penicillium*sp HSD07B synthesized during co-culture with Candida tropicalis, Biores. Technol. 102 (2011) 6082-6087.

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