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Diversity of Keratin Degrading and Keratinolytic Potential of *Fusarium redolens* and *Penicillium chrysogenum*

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POOJA MISHRA, NEELAM TRIPATHI, DEEPAK MITHAL, SUNIL K SHAH*

Department of Microbiology, University Teaching Department, Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India.

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ABSTRACT

Keratinophilic fungi are small but well-defined and important group of fungi that live in soil. Keratinophilic fungi play an important ecological role in decomposing keratins. They produce the keratinase enzyme, which is consisted of disulphide and hydrogen bonds. The soil keratinophilic fungi are responsible for the breakdown of any keratin containing wastes such as hair, nail, fur and feather. The keratinophilic fungi are biggest group of organisms that can utilize keratin as the sole source of carbon and nitrogen. *Fusarium spp.*, was the most frequently isolated keratinophilic fungus present in all the soil samples examined followed by *Penicillium chrysogenum*, natural, synthetic and semi synthetic media were will be thoroughly screened for selection of suitable media for quantitative production of enzyme, most of the fungi were found to exhibit maximum mycelial growth and excellent sporulation in Natural Medium -Potato dextrose agar (PDA), which contains 1.0% nitrogen and 4.0% dextrose as carbon source. Potato dextrose media (PDA) remained good for the dry mycelia weight and Czapek's agar and Neurospora culture agar media is remained second but maximum sporulation was observed in the Czapek's agar media than the potato dextrose media, where the minimum mycelia dry weight and sporulation was with and Neurospora culture agar media, among the different liquid media, tested for the growth and sporulation activity of the pathogenic fungus. In case of the effect of temperature, the optimal temperature for mycelial growth was 25°C (1.15±0.005mg). Maximum sporulation was recorded at 25°C. In case of influence of Hydrogen ion concentration, pH 6-8 was suitable for the mycelium growth. Maximum sporulation was recorded at neutral pH. It was also noticed that in high alkaline media the initial pH drifted towards neutrality.

INTRODUCTION

Keratinophilic fungi are the important group of fungi and useful for natural degradation of keratin substrates. Ecological factors play the significant role in growth and sporulation of Keratinophilic fungi[1]. Environmental conditions i.e. culture media, temperature and pH affecting the sexual reproduction in Keratinophilic fungi. Fungi are susceptible to nutritional and physiological factors, and little variation in these factors may induce differences in their morphological characters, growth, and sporulation [2].

The requirement of nutrition for fungal germination are not complex[3], but some fungal species require different chemical, physical and nutritional conditions. Morphology of spore is the most important part in fungal taxonomy, although many isolates are not able to sporulates on common artificial media[4]. Spores of fungi are normally mass-produced in large liquid culture fermentation. Several researches has been performed on effects of various media along with important physiological parameters that lead to maximum sporulation[5,6].

Temperature is an abiotic parameter which determines the germination and growth potential of the microorganism. This is a very important environmental factor, which influences the growth and sporulation of fungi[7]. It is an established fact that for each fungus there is a minimum, optimum and maximum temperature for growth and sporulation. The growth of fungi on temperature ranging from 15°C to 35°C and some require a range of high temperature for optimum growth[8]. Hydrogen ion concentration (pH) of the culture media considerably influences the growth of fungi by its action on the cell surfaces. Fungi grow at pH neutral to the weak acidic environment, with the highest production mycelial. Optimum pH 5.0-8.0 is suitable for conidial production and sporulation in liquid media.

The Physico-chemical properties of keratinase were analysed, the optimum pH and stability of the purified keratinase was determined. Various investigations in our study showed that keratinases are valuable enzymes to degrade the recalcitrant protein keratin. The knowledge on keratinolytic microorganisms and the biochemical properties of their keratinases are robustly increased[9], since keratin degradation is facilitated at high temperatures and hydrogen ion concentration. The thermostable hydrolases are employed in various industrial processes which are of great interest nowadays. Therefore, the utilization of keratinolytic enzyme might contribute to the production of high quality leather, also resulting in the improvement of wastewater quality and reduced pollution [10].

MATERIALS AND METHODS

The Sehore district was divided into 20 zones and each zone five location were selected and each location soil samples were collected randomly from different sites viz, gardens, schools, poultry farms, rivers, hospitals and garbage dumping sites roots where most of the microbial activity is concentrated. Soil samples (approximately 5g) were collected with clean dry and sterile polythene bags along with sterile spatula[11]. The collected samples brought to the laboratory and preserved for further studies. The soil samples were collected from the India during month of August 2016 to February 2017 in Sehore District (extends between the parallels of Latitude 22'31 to 23'40 North and between the meridians of Longitude 76'22 and 78'08 East) at various locations. The soil samples collected from twenty different zones of Sehore district mentioned in **Table 1.**

Procedure for sample collection

Before collection of soil samples, superficial debris and other vegetative materials were removed from the soil surface. All the soil samples were collected from the superficial layer (depth not exceeding 3-5 cm) with the help of sterilized spoon in pre-sterilized polythene bags (10 x 20 cm). Each polythene bag was labeled indicating the date and site of collection[8]. These samples were then tightly closed to maintain the original moisture and were brought to the laboratory and stored at 4°C till further processing.

Collection and preparation of baits

Keratin rich baits were collected from different places; the baits included human hair, animal hair, human nails and chicken feathers. Defatting of the baits was done by soaking all the baits for 24 hrs in either diethyl ether or in a chloroform/methanol (1:1) mixture and later rinsed 4-5 times with distilled water and air dried[12]. After defatting, baits were sterilised by the process of tyndallisation which involve heating the baits at 80°C for 1 hour followed by incubation for 24 hours at 28°C. The process was repeated thrice for three consecutive days[13].

Baiting of soil samples

Hair baiting technique was used for isolating the Keratinophilic fungi from the collected soil samples[14]. The samples were processed rapidly after collection[15]. Each of the collected soil samples as homogenized thoroughly and a required amount of soil was taken in sterilized Petri dishes under sterilized conditions and moistened with water. The plates were baited by

keeping sterile human hair, animal's hair, human nails and chicken feathers on the soil. These Petri dishes were incubated 28°C ± 2°C and examined daily from the fifth day for fungal growth over a period of 4 weeks. Sterilized water was added to each plate to keep the soil moist. The plates were monitored regularly and sterilized distilled water was added as and when required to maintain the moisture level[16].

Screening the incubated plates for the presence of fungal hyphae

The incubated plates were monitored regularly to observe any fungal growth on them. Once the fungal growth was observed on any plate, a small portion of the fungal mycelia was picked up for fungal examination with the help of a sterilized needle, mounted on a slide under glass coverslip containing a drop of sterilized distilled water or lactophenol cotton blue, and The purity of the isolated fungus was confirmed by examined under a microscope of the culture at 400X magnification using light microscope for the identification of the fungi[17]. The fungal mycelium was also inoculated on appropriate media for growth of the culture. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc. The characteristics were compared with the standard descriptions.

Isolation of the pure fungal isolates from the samples

Sabouraud's Dextrose Agar (SDA) media supplemented with chloramphenicol (0.05mg/ml) and cycloheximide (0.5mg/ml) was used for culturing. Media was prepared and autoclaved at 121°C for 15 min and after a ~~Sehore district~~ preliminary examination of fungal growth on baits, the fungal mycelium was subsequently transferred to the petri plates of Sabouraud's dextrose agar (SDA). The petri dishes were incubated at 28±2°C for two weeks and routinely checked for fungal growth. The samples were subcultured to obtain pure cultures[18]. Pure cultures thus obtained were maintained on SDA slants at 4°C.

Standardization of Physiological Condition for Keratinase Enzyme Production by Selected Potential Strain[20-23]

Selection Suitable Basal Medium

Natural, synthetic and semi synthetic media will be thoroughly screened for selection of suitable media for quantitative production of enzyme according. [19] Composition of different media used for selection of the basal medium will be based on standard microbiological literature and also will be modified if required.

Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37˚C. There are three general types of fungal culture media: natural semi-synthetic and synthetic media[20].

Natural media are composed of natural substrates, such as herbaceous or woody stems, seeds, leaves, cornmeal, wheat germ, and oatmeal etc. Natural media are usually easy to prepare but they have the disadvantage of their unknown composition. Some examples include corn meal agar, potato dextrose agar, V-8 juice agar, and dung agar[21].

Table No. 1: Composition of Potato Dextrose Agar (PDA)

Sr. No.	Ingredients	Gm/Ltr.
1	Potato infusion	200 gm
2	Dextrose	20 gm
3	Agar	20 gm
4	Distilled water	1 liter

Prepare potato infusion, boil 200 g sliced, unpeeled potatoes in 1 liter distilled water for 30 min. Filter through cheesecloth, saving effluent, which is potato infusion (or use commercial dehydrated form). Mix with Dextrose, Agar and Water and boil to dissolve. Autoclave 15 min at 121°C. Dispense 20-25 ml portions into sterile 15 × 100 mm petri dishes and Final pH, 5.6 ± 0.2.

Semi-synthetic medium CZAPEK DOX AGAR is used as semisynthetic medium for general cultivation of fungi.

Table No. 2: Composition of CZAPEK DOX AGAR is used

Sr. No.	Ingredients	Gm/Ltr.
1	Sucrose	30.00
2	Agar	15.00
3	Sodium nitrate	2.00
4	Dipotassium phosphate	1.00
5	Magnesium sulphate	0.50
6	Potassium chloride	0.50
7	Ferrous sulphate	0.01

Dehydrated powder, store in a dry place, in tightly-sealed containers at 24°C and protect from direct Sunlight.

Preparation of Semi-synthetic medium CZAPEK DOX AGAR

Dissolve 49.00 gm in 1000 ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121ºC) for 15 minutes. Cool to 45 - 50°C, mix well and dispense as desired.

Synthetic Media, on the other hand, contain ingredients of known composition. These types of media can be duplicated with precision each time they are made and contain defined amounts of carbohydrates, nitrogen, and vitamin sources. Czapek-Dox medium, glucose-asparagine and Neurospora crassa minimal medium fall in this category. Two synthetic media are now in general use as standards for culturing and crossing Neurospora Medium N (Vogel 1956, 1964) for growth, and Synthetic Cross Medium (SC) (Westergaard and Mitchell 1947) for crosses and mating type tests. Variations of these basic media have been derived to meet special needs."Neurospora culture agar" (Difco) Dissolve in 1 liter water:

Table No. 3: Composition of "Neurospora culture agar" (Difco) is used

Sr. No.	Ingredients	Gm/Ltr.
1	Yeast extract	5 g
2	Proteose-Peptone No. 3 (Difco)	5 g
3	Maltose	40 g
4	Agar	15 g

This medium supports growth of a wide range of auxotrophs (tyrosine excepted). Conidiation is generally poor.

Influence of different physiological factors effect for standardizing enzyme production

Effect of pH and temperature on enzyme activity and stability

In the present investigation, the pH effect on the mycelial growth of five fungi was evaluated. For influence of various pH, the Potato Dextrose Agar (PDA) medium was used. The 100 ml of culture media was prepared in different pH, ranging from 4.0 to 10.0, by adding 1N HCl or 1N NaOH. 10 mm discs of mycelia culture of selected keratinophilic fungal strain were inoculated in the experimental flasks and incubated at 28±2.0°C in the incubator. For each pH condition, the treatment was replicated thrice. Mycelial dry weight and sporulation was recorded after 14 days[24].

Effect of temperature on keratinase

To study the effect of different temperature on the mycelial growth and sporulation, the test organisms were grown in the Potato Dextrose Agar (PDA) medium. The 100 ml of culture media was prepared in 250 ml conical flask. 10 mm discs of mycelia culture of selected keratinophilic fungal strains cultured on Potato Dextrose Agar (PDA) plates were inoculated in the experimental flask and incubated at different temperatures (5°C to 55°C at intervals of 10°C). Mycelial dry weight and sporulation was recorded after 14 days. Each treatment was replicated thrice[25,26].

RESULTS AND DISCUSSION

The soil samples were collected from districts Sehore of Madhya Pradesh, India during August 2016 to February 2017. The soil samples were collected from roadside, public parks, poultry farm, slaughterhouses and barbershop dump area. The soil samples were processed in the Microbiology Research Laboratory, University, (India). The soil samples were shade dried and sieved for pH analysis[16]. The pH range of collected soil samples was founded in minimum 6.4 and maximum 10.0 pH.

Isolation of Keratinophilic Microflora

During the course of the present study, the isolation of keratinophilic fungi was completed by the well-known Hair-Baiting Technique of Vanbreuseghem. All the soil samples were processed for the isolation of keratinophilic fungi. Apart from hair, animal hair, human nail clipping and Chicken feathers used as bait for the isolation of keratinophilic fungi. Among the baits used for the isolation of keratinophilic fungi, feather bait was highly utilized by the fungus followed by nails, and hair. Feather as bait was found to be most suitable for the growth of keratinophilic fungi as compared to other baits[28]. This differential degradation of the keratin substrates can be attributed to the hardness of the keratin source. The keratin present in feather is comparatively less hard and can be easily broken down by the extracellular enzymes of the fungi. The isolated fungal colonies were cultured and purified on SDA media supplemented with chloramphenicol. All the soil samples collected from different sites of Sehore district of Madhya Pradesh were found to be positive for the growth of keratinophilic fungi.

Isolated fungi were identified on the basis of the monographs of Sigler and Carmichael studying macro and micromorphological characters of these cultures[29]. *Fusarium spp.*, was

the most frequently isolated keratinophilic fungus present in all the soil samples examined followed by Penicillium spp. Many investigations have been carried out in recent years on the distribution and occurrence of keratinophilic fungi in soil in many parts of the world[30]. Reports on the presence of these fungi in different soil habitats from different countries, Egypt, Australia, Palestine, Spain, India, Kuwait, Ukraine and Malaysia, have indicated that they are distributed worldwide. Various workers have reported these fungi from Indian soils.

Identification of Keratinophilic Fungi

From soil sample of keratinophilic fungi were isolated. The fungal isolates *Trichophyton* (10.89%) genera were the most predominant in this study. *Penicillium* (10.2%), *Fusarium spp.* (4.29%).

Selection of Suitable Culture Medium

For energy sources fungi relay upon their environment. Carbon, nitrogen, sulphur, phosphorus, sodium, potassium, calcium, magnesium, iron, manganese, zinc, copper, cobalt, molybdenum and vitamins are some of the important components for the growth of an organism.

Knowledge of the nutrition of the fungi is necessary for culturing them in laboratory or in industry. Thousands of years back man started cultivating fungi. In the beginning, it was unintentional, 1later on developed into an art in connection with the preparation of foodstuffs and beverages. Natural materials were used in the early periods for culturing fungi in laboratories. In the last century, many workers have tried to develop semisynthetic as well as synthetic media to cultivate fungi in laboratories. "There is no universal natural substrate or artificial medium upon which all fungi will grow. Even closely related forms may differ considerably in their nutritional requirements qualitatively as well as quantitatively [31].

In the present investigation, experiments were conducted to evaluate the nutritional media, temperature and the best pH for the optimum mycelial growth and sporulation in selected Keratinophilic fungi *Fusarium redolens, Penicillium spp.* were studied, so that, a suitable basal medium could be selected.

Effect of Culture Broth on Mycelial Growth and Sporulation of Keratinophilic fungi

The nature of a particular medium has great role to play in the growth and sporulation of fungi. The suitability of culture medium depends upon the nature of the organism. Culture

media significantly affected the growth, sporulation and conidial discharge of Keratinophilic fungi. The nutritional requirement of the test fungi varies from species to species. The data (Table 4) showed that most of the fungi prefer natural media for their growth and sporulation. The data showed that some fungi also behaved in a more or less similar manner on the semi-synthetic and synthetic media as well as natural media.

In the present study, most of the fungi were found to exhibit maximum mycelial growth and excellent sporulation in Natural Medium -Potato dextrose agar (PDA), which contains 1.0% nitrogen and 4.0% dextrose as carbon source. The Semi-Synthetic Medium (Czapek's agar) was found as the mycelial growth stimulator but not as Natural Medium for some fungi because of the presence of lower concentration of carbon and nitrogen sources. It contains 30% Sucrose extract, 2% Agar as carbon and 0.1% peptone as a nitrogen source and Synthetics Medium (Neurospora culture agar) was also found as the mycelial supported moderate stimulator growth but not as Natural Medium or semi-synthetic medium for some fungi because of the presence of lower concentration of carbon and nitrogen sources. The sporulation in all the test fungi was found very well in Natural Medium (Potato dextrose agar) among the different media of solid type. However, poor growth was accomplished in Synthetics Medium (Neurospora culture agar) medium.

Table No. 4: Growth and sporulation of different Fungi/ Species on different Natural, Semi-synthetic and synthetic solid media

Fungi/ Species	Culture Medium								
	Natural Medium (Potato dextrose agar)			Semi-Synthetic Medium (Czapek's agar)			Synthetics Medium (Neurospora culture agar)		
	Colony Diameter (mm)	Growth rate (mm/day)	Sporulation (1000/ml)	Colony Diameter (mm)	Growth rate (mm/day)	Sporulation (1000/ml)	Colony Diameter (mm)	Growth rate (mm/day)	Sporulation (1000/ml)
Fusarium redolens	72.7	8.5	40	45.5	2.8	35	28.2	4.5	32
Penicillium chrysogenum	73.5	6.5	25	26	4.2	25	28.4	3.5	18

The results obtained for growth and sporulation in different liquid media was (Table 5) Potato dextrose media (PDA) remained good for the dry mycelia weight and Czapek's agar and Neurospora culture agar media is remained second but maximum sporulation was observed in the Czapek's agar media than the potato dextrose media, where the minimum mycelia dry weight and sporulation was with and Neurospora culture agar media, among the different liquid media, tested for the growth and sporulation activity of the pathogenic fungus.

Table No. 5: Growth and sporulation of different Fungi/ Species on different Natural, Semi-synthetic and Synthetic liquid media

Fungi/ Species	Culture Medium					
	Natural Medium (Potato dextrose agar)		Semi-Synthetic Medium		Synthetics Medium (Neurospora culture agar)	
	Mycellia Dry weight (mg)	Sporulation (1000/ml)	Mycellia Dry weight (mg)	Sporulation (1000/ml)	Mycellia Dry weight (mg)	Sporulation (1000/ml)
Fusarium redolens	375	35	280	30	245	25
Penicillium chrysogenum	280	42	285	35	180	28

Effect of pH on growth and sporulation of different Keratinophilic fungi

Effect of different, pH and liquid culture media on of different Keratinophilic fungi growth was analyzed by dry mycelium weight and sporulation. In case of the effect of temperature, the optimal temperature for mycelial growth was 25°C (1.15±0.005mg). Maximum sporulation was recorded at 25°C. In case of influence of hydrogen ion concentration, pH 6-8 was suitable for the mycelium growth. Maximum sporulation was recorded at neutral pH. It was also noticed that in high alkaline media the initial pH drifted towards the neutrality.

In case of effect of Hydrogen ion concentration, mycelial growth and sporulation was highest at pH 6-7. It was evident from the present results that the different Keratinophilic fungi changed the pH of the medium by the end of the incubation days. Reetha 22 studied that the maximum growth of Trichoderma harzianum was observed at pH 7-7.5 and the minimum growth was observed at pH 5. Kotwal 37 recorded that, Metarhizium anisopliae was found best and maximum mycelial growth with abundant sporulation at pH 5.5. Saha 24 reported that the optimum pH for growth of Lasiodiplodia theobromae (Pat.) Griffon and Maubl was at the range of pH 5.5-6.5. Abubakar 23 reported the highest mean of dried mycelial weight (355.67mg) was in pH 4.0 broth medium followed by 353.3mg in pH 7.0 of Aspergillus parasiticus. The lowest mycelia dry weight (302.73mg) was obtained in pH 10.0 broth medium. pH 5.0 produced the highest spores per ml (8.33 × 107), followed by pH 7.0 (7.67×107). The lowest spore formation of 2.83×107 was recorded at pH 10.0.

In the present study, the most favorable pH for the growth of the Keratinophilic fungi was found to be pH 6.0 and 7.0. The range of the pH in which these fungi grew quite well was recorded between pH 6.0 to 8.0. Another important point to be noted is that even in the

presence of a suitable culture medium, the variation in the pH may influence the growth of the Keratinophilic fungi. pH is the most important environmental factors that governing the sporulation of the fungi. A little variation in this factor may induce marked differences in their morphological characters and sporulation. It is an established fact that for each fungus there is a minimum, optimum and maximum pH for sporulation. In this study, the maximum sporulation was recorded at pH 6.0 and 7.0 whereas pH 2.0 showed poor sporulation. It is evident from the present result that all the tested Keratinophilic fungi changed the pH of the medium at the end of the incubation period. It was noticed that the initial pH of the culture media was floated towards the neutrality or an alkaline range after incubation. Since fungi differ in their metabolic activity and rate of growth, the pH changes brought about in the culture media also differ.

Fusarium redolens showed maximum growth at pH 7.0 (0.82±0.05 gm). Mycelial growth of this fungus was reduced when pH was less than 5.0 or higher than 9.0. There was poor growth at pH 2.0 and pH 10.0 i.e., 00.20±0.05 mg and 0.22±0.05 mg respectively, but inoculum started rooting in the liquid media. Excellent sporulation was recorded at pH 7.0. pH 4.0 and pH 9.0 showed the poor sporulation.

The optimal pH for mycelial growth of *Penicillium chrysogenum* was pH 6.0 (1.16±0.02 gm). At lower and higher pH, the mycelial growth was very poor. At 7.0, the mycelial growth was (0.98±0.04 gm). The best sporulation was found at pH 6.0. Other pH 5.0 and 9.0 showed the poor sporulation in this respect.

Effect of Temperature on Average dry weight of mycelium growth and Sporulation of different Keratinophilic fungi

In the present study, the most favorable temperature for the growth of the Keratinophilic fungi was found to be 25°C and 35°C. The range of the temperature in which these fungi grew quite well was recorded between 25°C and 35°C. Another important point to be noted is that even in the presence of a suitable culture media, the fluctuation in the temperature may influence the growth of the Keratinophilic fungi.

Fusarium redolens grew at a temperature of 15-35°C. However, the growth of the fungus was very poor at 5°C and 55°C, because of these temperatures did not favor for growth. The growth of *Fusarium redolens* was obtained maximum at 25°C (1.32±0.08 gm) after 14 days of incubation. The sporulation was of course found to be best at 25°C, next to which were 35°C, 15°C and 45°C.

Penicillium chrysogenum showed maximum growth at 35°C (1.19±0.210 gm). Mycelial growth of this fungus was reduced when temperature was less than 25°C or higher than 35°C. There was no growth at 5ºC and 55°C *i.e.*, 0 gm respectively, but inoculum started rooting in the liquid media. Excellent sporulation was recorded at 35°C and good sporulation was recorded at 25°C; rest of the temperatures *i.e.*, 15°C and 45°C favored the fair sporulation. 5°C and 55°C showed negative sporulation.

Table No. 6: Effect of pH on growth and sporulation of different Keratinophilic fungi

Fungi/ Species	**Effect of pH on growth and sporulation of different Keratinophilic fungi**									
Fusarium redolens	Initial pH	2	3	4	5	6	7	8	9	10
	Final pH	3.2	5.4	6.2	6.2	7.1	7.0	7.5	7.8	7.6 S
	Mycellial Dry weight (gm)	0.20± 0.05	0.32± 0.04	0.52± 0.04	0.51± 0.05	0.72± 0.05	0.82±0 .05	0.71±0. 05	0.32± 0.05	0.22± 0.05
	Sporulatio n (x1000/ml	+++	+	++	++	+	+++	++	+	+
Penicillium chrysogenu m	Initial pH	2	3	4	5	6	7	8	9	10
	Final pH	2.8	3.0	5.1	6.1	6.3	7.0	7.2	7.4	
	Mycellial Dry weight (gm)	0.04± 0.17	1.16± 0.08	1.10± 0.03	1.14± 0.02	1.16± 0.02	0.98±0 .04	0.90±1. 02	0.93± 0.12	0.03± 0.10
	Sporulatio n (x1000/ml	+	++	+++	++	+++	++	+	+	+

Table No. 7: Average dry weight of mycelium and sporulation of different Kerationophilic fungi at different temperature regimes (Initial pH 6.5)

Fungi/ Species	**Average dry weight of mycelium and sporulation of different Keratinophilic fungi at different temperature regimes**						
Fusarium redolens	Temperature	5ºC	15ºC	25ºC	35ºC	45ºC	55ºC
	Mycelium dry weight (gm)	0.12±0 .02	0.76±0.09	1.32±0.0 8	1.10±0.1 0	0.71±0.0 5	0.44±0.0 3
	pH of the filtrate	6.50	6.12	6.36	6.29	6.32	6.25
	Sporulation	+	++	++++	++++	++	+
Penicilliu m chrysoge num	Temperature	5ºC	15ºC	25ºC	35ºC	45ºC	55ºC
	Mycelium dry weight (gm)	0	0.22±0.10	0.75±0.0 2	1.19±0.2 1	1.04±0.0 3	0
	pH of the filtrate	6.50	6.10	7.06	6.50	6.68	7.52
	Sporulation	+	++	++++	+++	+	+

CONCLUSION

It is clear from present investigation that soils of Sehore are ideal environment for the Keratinophilic fungi. This could be attributed to the high organic debris and keratinous substrates present in these soils. However, the isolation of fungi was not uniform as it depends on organic matter. Organic matter content of soils is one of the major factors affecting the presence of Keratinophilic fungi in soils. Thus these fungi which are human/ animal pathogens could be considered as bio-indicators of environmental pollution with keratinous substrate and can pose risk of human and animal mycoses. Keratinophilic fungi mostly preferred slightly acidic to alkaline soil (27). Soil is rich in keratinolytic fungi capable of degrading waste poultry feathers; this capability makes the fungi interesting in terms of their potential for bioconverting residues in food with high protein for animal feed and also for bio remediating environments contaminated with keratin residues. Preservation in mineral oil is sufficient to maintain the viability and taxonomic characteristics of cultures for long periods of time, as shown in our study; in addition, it could maintain the metabolic characteristics of these fungi, all of which retained keratinase activity. *Fusarium redolens* and *Penicillium chrysogenum* showed greater keratinolytic activity, indicating their suitability for keratinase production. The keratinase from *Fusarium redolens* showed greater activity at pH 6.36 and 25ºC. The ability of *Penicillium chrysogenum* to be stable over wide pH and temperature ranges suggests the feasibility of using this strain in commercial biotechnological processes.

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