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INFLUENCE OF CULTURE CONDITIONS ON GROWTH OF KERATINOPHILIC FUNGAL STRAINS

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Abstract

The aim of this study was to investigate the influence of culture conditions on growth of keratinophilic fungal strain of Chrysosporium sp. in the presence or absence of keratin substrate. The effect of pH, temperature, carbon and nitrogen sources on fungal growth and sporulation was evaluated. The pH values ranged from 4 to 9.5 and the incubation temperature ranged from 20°C to 35°C. Glucose, fructose, maltose, sucrose, starch and cellulose were used as carbon sources. As nitrogen source, yeast extract, ammonium salts, urea and vitamin B12 were tested. All tests were also performed with basal mineral culture medium supplemented with keratin powder from chicken feathers. The feathers were cleaned with ethylic alcohol, washed with distilled water, dried at 60°C and finally grounded several times with a Retsch ball mill until a fine powder was obtained. The influence of the culture conditions on growth was assessed by measuring the diameter of the colonies grown on the solid medium after 5 and 10 days of incubation. The colony sporulation degree was appreciated macroscopically and microscopically. The presence of keratin in the culture media stimulated distinctly the fungal growth as compared to the culture media without keratin. Alkaline pH and temperatures between 27 and 30°C are optimal for its growth. Certain C and N sources can stimulate the fungal growth, but this seems to be influenced by the incubation time.

Key words*: fungal growth, keratin, keratinophilic fungi.*

INTRODUCTION

Biodegradative properties are widespread in the living world, from bacteria (Kumar and Takagi, 1999; Korkmaz et al., 2004; Moniruzzaman et al., 2007), actinomycetes (Laba and Rodziewicz, 2010; Jayalakshni et al., 2011) to fungi (Mushin et al., 1997; Mushin and Aubaid, 2000; Riffel and Brandell, 2006; Singh, 2011). A number of fungi can use the hard biodegradable materials such as keratin as substrate, due to their enzymatic equipment. These fungi are able to use the keratinaceous substrate as unique source of carbon and nitrogen and play an important role on biodegradation of keratin waste in the environment (Kunert, 2000; Moallaei et al., 2006; Sharma et al., 2011). The main types of fungal genera with keratinolytic properties are: *Alternaria*, *Aspergillus* (Kim, 2003; Ali et al., 2011), *Chrysosporium* (Singh, 2002), *Cladosporium*, *Curvularia*, *Fusarium*, *Myrothecium*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, *Sepedonium*, *Stachybotrys*, *Ulocladium*, dermatophyte fungi (Gupta and Ramnani, 2006; Monod, 2008; Saber et al., 2010). Members of the keratinolytic fungi group are found in soil (geophilic) as decomposers of the keratin materials (hair, claws, feathers, horns etc.) (Kanaahi and Ancy, 2012). The sources of keratin are numerous, such as feathers, wool, horns, hair etc. Keratin results in large amounts from the meat industry and accumulates in the environment due to its high stability, becoming a source of contamination and environmental pollution (Balakumar et al., 2013; Sharma and Gupta, 2016; Kumawat et al., 2016). Traditional methods of keratin degradation are expensive, consume large amounts of energy and can destroy some essential amino acids like methionine and lysine. Therefore

biodegradation of keratin waste and conversion of some wastes from food industry into accessible animal feed has a biotechnological importance (Lange et al., 2016).
Environmental factors have a significant role on the growth of keratinophilic fungi and influence the keratinase activity of keratinolytic fungi (Kadhim et al., 2015; Sharma and Sharma, 2009; Sharma et al., 2012).
Therefore, the aim of this study was to investigate the influence of culture conditions on growth of keratinophilic fungi in the presence or absence of keratin substrate.

MATERIALS AND METHODS

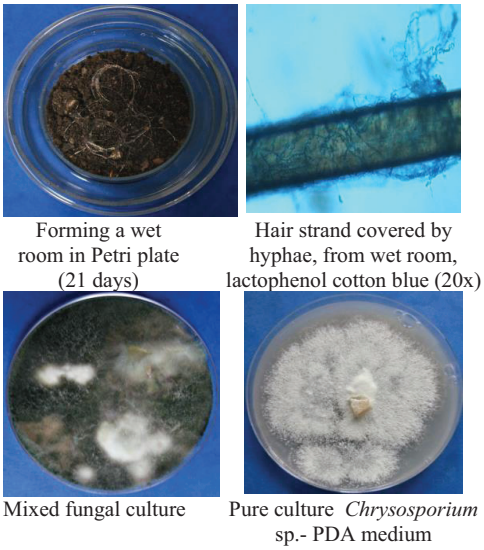
Fungal strains

The tests were carried out with *Chrysosporium* sp. isolated from farm soil (Figure 1) by Vanbreuseghem hair bait technique. The tested strain was grown and maintained on potato dextrose agar (PDA) slants at 4°C.

Soil rich in keratin materials — Collecting soil samples

Figure 1. Soil sampling

Vanbreuseghem hair bait technique. This technique consists in placing collected soil samples in sterile Petri plates. Soil samples rich in keratinic material were collected from several areas in sterile containers using sterile tools and tagged appropriately. The samples were stored at 4°C until their processing in the laboratory. Over the soil samples few sterile hair strands were added. To prevent the soil from drying out, the Petri plate was placed in other Petri plate and about 10 ml of sterile distilled water was added, forming a wet room. The Petri plates were incubated at room temperature for 4 weeks and observed daily until growth of fungal mycelium was observed on the surface of hair strands.
The hair strands covered with fungal mycelium were cultivated on culture media and mixed cultures followed by pure culture were obtained (Figure 2).



Forming a wet room in Petri plate (21 days) — Hair strand covered by hyphae, from wet room, lactophenol cotton blue (20x)

Mixed fungal culture — Pure culture *Chrysosporium* sp.- PDA medium

Figure 2. Steps for fungal strain isolation by Vambreuseghem hair bait method

Keratin substrate: keratin powder
The keratin powder was obtained as follows: chicken feathers were cleaned, sterilized with 3% ethanol, washed and dried at 60°C. They were then cut into small pieces and grounded several times with a Retsch ball mill until a fine powder was obtained.

Conditions of fungal cultivation

A mineral culture medium with a specific formula served as control in all experiments and was used as basal culture medium (B.C.M.) in the assays with different carbon (C) and nitrogen (N) sources. The basal culture medium had the following composition (g/L): 0.1, KH_2PO_4; 0.1, $CaCl_2$; 0.1, $FeSO_4 \cdot 7H_2O$; 0.005, $ZnSO_4 \cdot 7H_20$; 15, agar, pH 7.0, autoclaved at 121°C for 15 min. The effect of pH, temperature, carbon and nitrogen sources on fungal growth and sporulation was evaluated. The basal culture medium was supplement with 1% of each carbon and nitrogen source, respectively (Balakumar et al., 2013). The carbon sources used in the experiment were: glucose, fructose, maltose, sucrose, starch and cellulose.
The nitrogen sources used: ammonium salts (potassium nitrate, potassium sulphate,

potassium phosphate), yeast extract, urea and vitamin B12.

To test the influence of pH on fungal colony development culture media (basal culture medium with or without keratin) with the following pH values were used: pH 4; pH 4.5; pH 5; pH 5.5; pH 6; pH 6.5; pH 7; pH 7.5; pH 8; pH 8.5; pH 9 and pH 9.5.

The incubation temperatures for the plates with the basal culture medium ranged from 20°C to 35°C. Different variants of the culture medium were used, namely: basal culture medium with or without keratin powder, basal culture medium supplemented with C or N source and basal culture medium supplemented with C or N source and keratin powder. The medium was inoculated with 10 µl of fungal suspension. The plates were incubated at 27°C for 10 days.

The influence of the culture conditions on growth was assessed by measuring the diameter of the colonies grown on the solid medium after 5 and 10 days of incubation. The colony sporulation degree was appreciated macroscopically and microscopically. Assays were performed in triplicate, using three-compartment Petri plates, with approximately 5 ml of culture medium in each compartment.

All tests were performed in the ICECHIM laboratories.

RESULTS AND DISCUSSIONS

The isolated fungal strain was identified by macroscopic and microscopic examination as *Chrysosporium* sp. Macroscopically this strain showed a moderate growth, was flat, white to light beige in averse colour, and a powdery surface texture. Microscopically it produced hyaline, smooth, one-celled pyriform to clavate conidia. These features are characteristic for *Chrysosporium* genus.

For *Chrysosporium* sp., carbon sources stimulated the fungal growth to a certain degree compared with the control, when measured after five days.

As can be seen in Figure 3, after 10 days of incubation on solid culture media on Petri plates, a better growth was observed in the presence of different C sources.

However, the presence of keratin powder stimulated the fungal growth (Figure 3 and 4) and sporulation compared to C sources which did not stimulate. The average diameter of the fungal colony was 2.6 cm and 2 cm after 10 days of incubation, in the presence and absence of keratin powder, respectively (Figure 4).

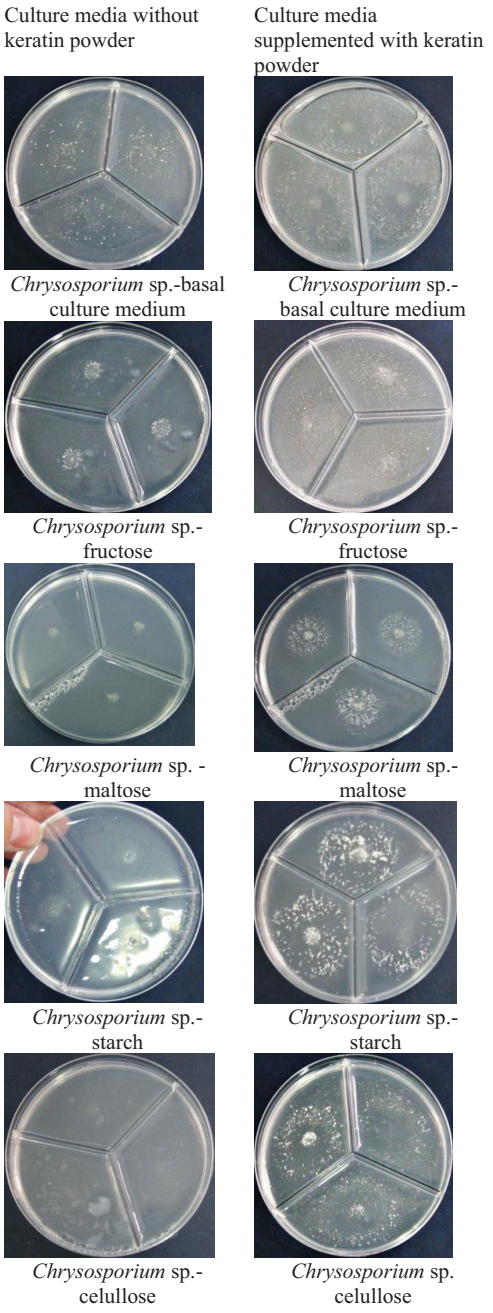


Figure 3. Carbon source (10 days of incubation)

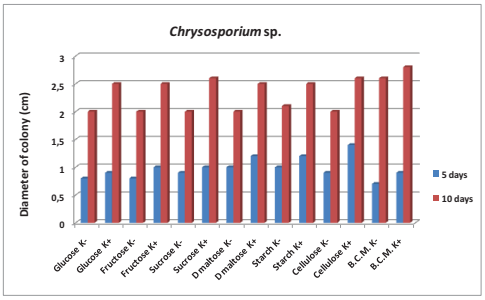


Figure 4. Effect of carbon source on fungal growth (10 days of incubation)

In the experiment with different sources of nitrogen, the addition of urea induced the largest growth after 5 days, while addition of yeast extract resulted in the largest growth after 10 days (Figures 5 and 6).

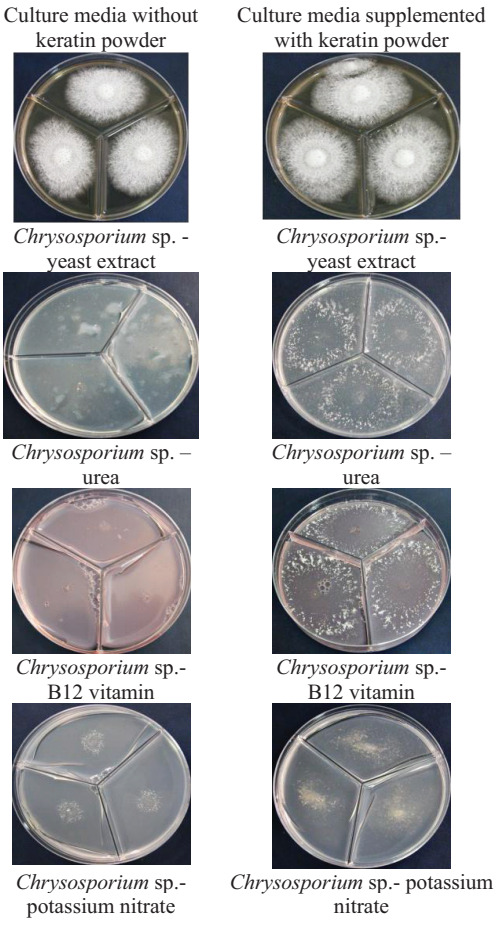


Figure 5. Nitrogen source (10 days of incubation)

Again, the presence of keratin had a positive effect on fungal growth. The average diameter of the fungal colony was 3.5 cm and 3 cm after 10 days of incubation, in the presence and absence of keratin powder, respectively (Figure 6).

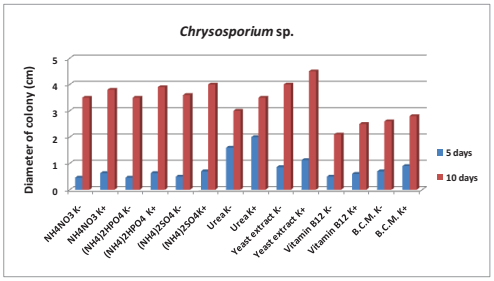
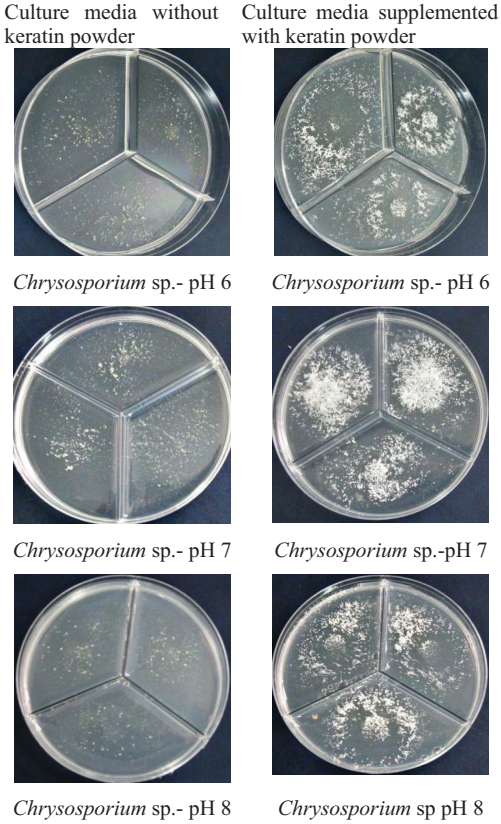
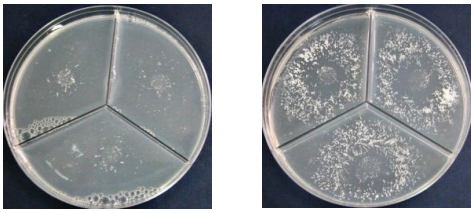


Figure 6. Effect of nitrogen source on fungal growth (10 days of incubation)

Chrysosporium sp. developed better at alkaline pH (Figure 7).





Chrysosporium sp.-pH 9 *Chrysosporium* sp. pH 9

Figure 7. Influence of pH value (10 days of incubation)

The presence of keratin powder in the culture medium positively influenced the growth of *Chrysosporium* sp. strain (Figure 8).

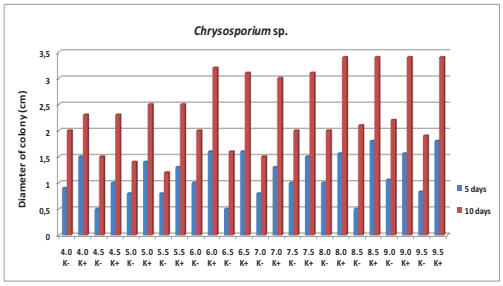
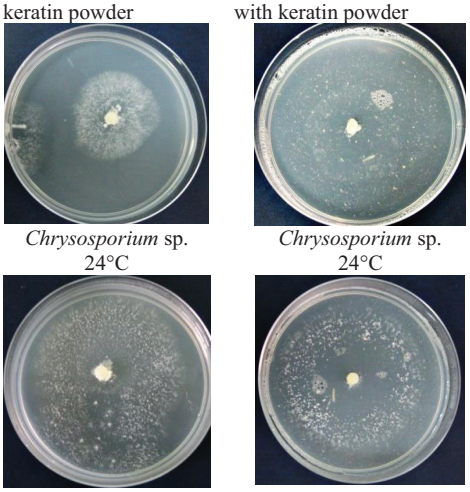


Figure 8. Effect of pH values on fungal growth (10 days of incubation)

Keratin had a positive effect, especially at 27°C-30°C (Figure 9 and 10).

Culture media without keratin powder

Culture media supplemented with keratin powder



Chrysosporium sp. 24°C *Chrysosporium* sp. 24°C

Chrysosporium sp. 30°C *Chrysosporium* sp. 30°C

Figure 9. Influence of incubation temperature value (10 days of incubation)

The optimum growth temperature was in the range 27-30°C (Figure 10). Our results are similar to those reported by other researchers (Sharma et. al, 2016).

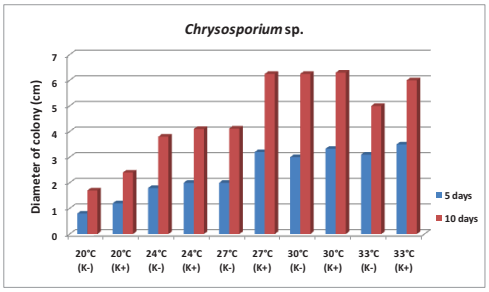


Figure 10. Effect of temperature on fungal growth

CONCLUSIONS

The presence of keratin in the culture media stimulates the growth of *Chrysosporium* sp. as compared to the culture media without keratin, but the degree of stimulation also depends on other factors, such as temperature, pH and the presence or absence of various C and N sources. Alkaline pH and temperatures between 27 and 30°C are optimal for its growth.

Certain C and N sources can stimulate the fungal growth, but this seems to be influenced by the incubation time. More studies are needed to understand this behaviour.

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