Research Article

Influence of Liquid Culture Media, Temperature and Hydrogen Ion Concentration on the Growth of Mycelium and Sporulation of *Arthroderma multifidum*

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Accepted on: 26-09-2016; **Finalized on:** 30-11-2016.

ABSTRACT

The influence of the liquid culture media (synthetic and natural), pH and temperature on the growth of mycelium and sporulation of a keratinophilic fungus *Arthroderma multifidum* (KU560574) isolated from soils of poultry farmhouse of Rajasthan, India was examined. Six liquid culture media, pH (4.00-10.00) and temperature (5°C-55°C) were tested to measure the mycelial growth and sporulation. In the present research, sabouraud's dextrose broth was the most suitable medium for mycelial growth (1.229±0.12 gm) of *A. multifidum* due to its good and balanced nutrient content. Maximum sporulation was observed in malt extract broth medium. The results of the experimentations showed that the mycelium growth of *A. multifidum* was maximum at pH 8.00 (1.629±0.04 gm) followed by pH 7.0 (1.320±0.10 gm). Temperature also played the significant role in mycelial growth and sporulation. The highest mycelial growth, as well as sporulation, was observed at 25°C (1.672±0.10 gm). The *A. multifidum* fungus tolerates high temperatures 35°C (1.131±0.12 gm). The results suggest that *A. multifidum* naturally selected for the semiarid situation, where it could serve as an important role in the natural degradation of keratinous wastes. In the present study, an attempt was made to study some factors which influence the growth and sporulation of *A. multifidum*. This would help us to know the immense role of environmental factors for the growth of *A. multifidum* and degradation of keratinous wastes produced annually from poultry processing plants and slaughterhouses.

Keywords: *Arthroderma multifidum,* Mycelial Growth, Sporulation, Degradation, Keratinous wastes

INTRODUCTION

The keratinous wastes are generated from the poultry processing plants, leather industries, and slaughter houses in huge amounts[1]. The accumulation of keratinous wastes in the environment can result in the pollution and environmental contamination[2]. Keratinous substrates, *i.e.* chicken feathers are the source of nutrients for plant life and animals. Various species of keratinophilic fungi have a very important role in the degradation of keratinous wastes and subsequent production of useful products like animal feeds and fertilizers[3].The growth of keratinophilic fungi in different *in-vitro* environmental conditions could reveal some physiological characteristics. *Arthroderma multifidum* belongs to the family *Arthroderma taceae* and issoil saprotrophs as well as an effective keratin degrader. This species was distributed worldwide and isolated from keratinous waste contaminated soils, rabbit burrows soils and from domestic fowl (*Gallus gallus domesticus*)[4-6].

After insects, fungi are the next biggest group of microorganisms[7].Fungi being saprophytic grow up in different habitats in the environment and require numerous specific component and source of nutrition for growth and reproduction[8, 9]. Environmental factor stake part in the growth and sporulation of keratinophilic fungi[10].Fungi are susceptible to nutritional and physiological factors[11] and slight variations in these factors may stimulated if ferences in their morphological characters[12,13].The nutritional necessities for the growth of fungi are generally not complex[14], but various fungal species require different chemical, physical and nutritional conditions[15]. Spore analysis is the main character in fungal classification, even though various isolates are not capable to sporulate on common artificial media[16].Fungal spores are usually mass-produced in huge liquid culture fermentation[17]. Several studies and researches have been performed to evaluate the influence of different culture media components along with important physiological parameters that direct to maximum growth and sporulation[18, 19].

Temperature is an abiotic environmental factor, which influences the germination of fungi[20,21].It is the well-known reality for all the fungus that, there is lowest, optimal and highest temperature for growth and sporulation[12].Generally,several fungi grow at the temperature ranging from 15°C to 35°C; some of the fungi require high temperature for growth[22].Hydrogen ion concentration (pH) of the culture media significantly influence the growth of fungi either directly by its action on the cell exterior or indirectly by its effect on the availability of nutrients[23,24].Studies on hydrogen ion concentration suggest that fungi grow up at pH neutral tothe weak acidic environment[25], with the greatest production of dehydrated mycelial weight. Optimum pH 5.0-8.0 is suitable for conidial production and sporulation in liquid media[19, 26].

Studies on the nutritional requirement and environmental factors on *Arthroderma multifidum* are limited as well as very little information is available on the fungus as a keratin. With these perspectives, the present study was undertaken to observe the influence of liquid cultures, pH and temperature of *A. multifidum* onmycelial growth and sporulation. Thus, this paper reports new elementsabout the production of this fungal species in different liquidculture as well as in various environmental factors.

MATERIALS AND METHODS

Fungal culture

The fungal culture of *Arthroderma multifidum* (KU560574) was isolated from poultry farm soil in Jaipur district of Rajasthan, India. The strain was maintained on Sabouraud's dextrose agar (SDA).

Influence of various culture media

Mycelium growth and sporulation of *A. multifidum* was studied on six culture media, *i.e.*Sabouraud's dextrose broth (SDB), Richard's synthetic broth (RSB), Czapek dox broth (CDB), Mannitol salt broth (MSB), Yeast extract broth (YEB), Malt extract broth (MEB). The 100ml of culture media were prepared in 250 ml flasks and final pH was adjusted to 6.5. Flasks were later inoculated aseptically with a 10 mm actively grown culture disc of the *A. multifidum* cultured on Sabouraud's dextrose agar plates. The flasks were incubated at 28±2°C in the incubator. The mycelium dry weight and sporulation were recorded after 14 days. The experiment was carried out in triplicates.

Influence of different temperatures

The Sabouraud's dextrose broth (SDB) medium was used for the study of the influence of different temperatures. The 100 ml of culture media were prepared in 250 ml conical flasks. 10 mm discs of mycelia culture of *A. multifidum* cultured on Sabouraud's dextrose agar(SDA) plates was inoculated in the experimental flasks and incubated at different temperatures (5°C to 55°C at intervals of 10°C) in the incubator.Each treatment was replicated thrice. Mycelial dry weight and sporulation was recorded after 14 days.

Influence of various pH

For the influence of various pH the Sabouraud's dextrose broth (SDB) medium was used. The 100 ml of culture media were prepared in 250 ml conical flasks and was adjusted to different pH, ranging from 4.0 to 10.0, by adding 1N HCl or 1N NaOH. 10 mm discs of mycelia culture of *A. multifidum* cultured on Sabouraud's dextrose agar (SDA) plates was inoculated in the experimental flasks and incubated at 28±2°C in the incubator. For each pH condition, the treatment was replicated thrice. Mycelial dry weight and sporulation was recorded after 14 days.

Assessment of dry weight of the fungus/biomass production and sporulation

For assessing the growth of the fungus, mycelial mat was collected after the incubation days by filtering them through preweighed Whatman no. 1 filter paper individually. It was dry inside an incubator at a temperature of 50±2°C until a constant weight was obtained.

The actual weight of dry fungal mycelium was then calculated using the formula[27]:

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)

The degree of sporulation of fungi was determined using standard methods as recommended by Wilson and Knight, 1952[28] and Tuite, 1969[29].

pH variation over time

The pH of the culture filtrate was taken after 14 days using a digital pH meter (Model 181, Electronics India). A standard curve was prepared for the pH of culture filtrate over different media and temperature.

RESULTS

Effect of different liquid culture media, temperature and pH on *Arthroderma multifidum* growth was analyzed by dry mycelium weight and sporulation. In the present research, the rate of growth of *A. multifidum* has been compared in various liquid culture media types *viz.* SDB, RSB, CDB, MSB, YEB and MEB. The results showed that SDB (1.229±0.12) is the most suitable liquid culture media followed by MEB (0.976±0.08) for the growth of *A. multifidum.* Limited mycelial growth was observed in MSB, and moderate mycelial growth was observed in CDB, RSB and YEB (Fig.1). Media significantly affected mycelial growth and sporulation of *A. multifidum.*Highest sporulation was observed in MEB followed by SDB (Table 1). It was also observed that the initial pH (6.5) of the culture filtrate was drifted towards the neutrality or alkaline range after 14 days of incubation. pH of culture filtrate of MEB medium reached at 7.44 wherwas SDB medium was 7.36 (Fig.2). Fungi differ in their metabolic activity and rate of growth; the pH changes brought about in the culture medium also differ.

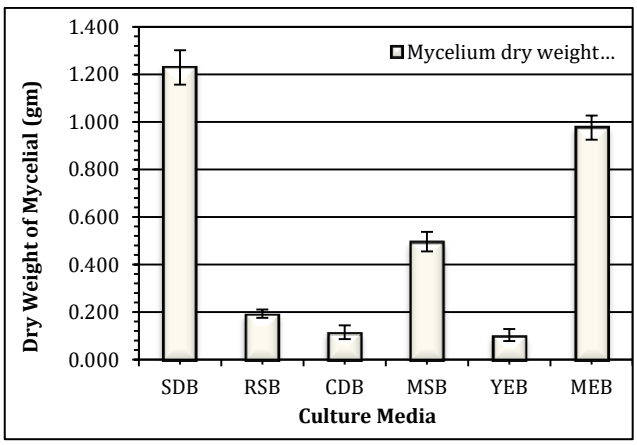

Figure 1: Influence of different liquid culture media on the biomass production of *Arthroderma multifidum*

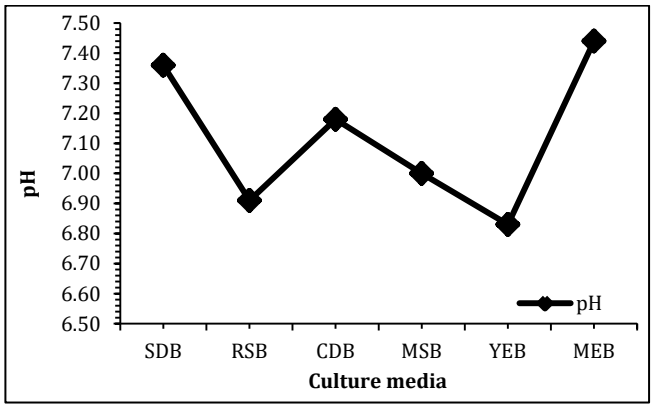

Figure 2: Graph depicting pH of culture filtrate of various media after 14 days

In case of the effect of temperature, the optimal temperature for mycelial growth and sporulation was 25°C (1.672±0.10). At 5 and 55°C poor mycelial growth occurred after fourteen days with no spore production. Mycelial growth was reduced when temperature was less than 25°C or higher than 35°C (Table 1; Fig.3). The initial pH was floated towards the neutrality or alkaline range after incubation (Fig.4).

Table 1: Sporulation of *A. multifidum*on liquid culture media and temperature

Culture media	Sporulation	Temperature	Sporulation
SDB	+++	5 °C	+
RSB	+	15 °C	++
CDB	++	25 °C	++++
MSB	+++	35 °C	+++
YEB	++	45 °C	+
MEB	++++	55 °C	+

Note: Sporulation grades: + = Poor growth and sporulation, ++ = fair growth and sporulation, +++ = Good growth and Sporulation, ++++ = Excellent growth and Sporulation.

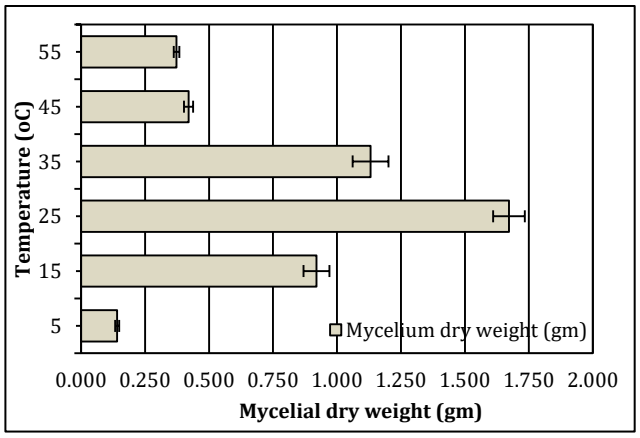

Figure 3: Influence of temperature on mycelial growth (gm) of *A. multifidum* after 14 days

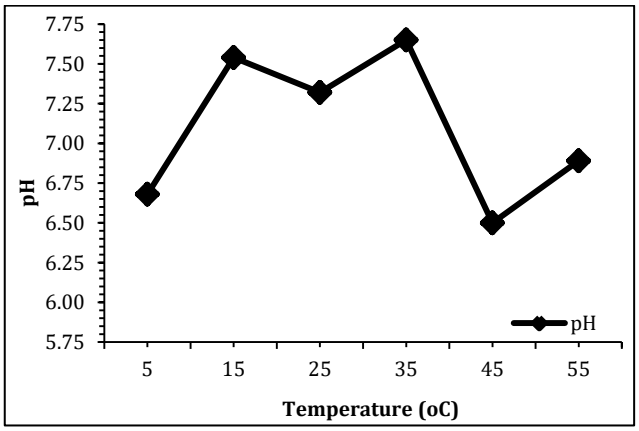

Figure 4: Graph depicting pH of culture filtrate on temperature after 14 days

In case of influence of Hydrogen ion concentration, neutral to weak base environment was suitable for mycelial growth with optimum pH 7-8. pH 6-7 was favorable for sporulation. It was also noticed that when the initial pH was low, it drifted towards the neutrality or alkaline range, whereas in highly alkaline media this was reverse (Table 2; Fig.5).

Table 2: Influence of variouspH on mycelial growth and sporulation of *Arthroderma multifidum* SDB medium after 14 days of incubation

pH	Mycelium dry weight (gm)	Sporulation	pH of the filtrate
4.0	0.621±0.06	+	5.56
5.0	0.935±0.07	+	5.89
6.0	1.107±0.16	++++	6.80
7.0	1.320±0.10	++++	7.39
8.0	1.629±0.04	++	7.56
9.0	1.257±0.06	++	7.01
10.0	0.869±0.12	+	7.94

Note: Mean±SD (n=3); Sporulation grades: + = Poor growth and sporulation, ++ = fair growth and sporulation, +++ = Good growth and Sporulation, ++++ = Excellent growth and Sporulation

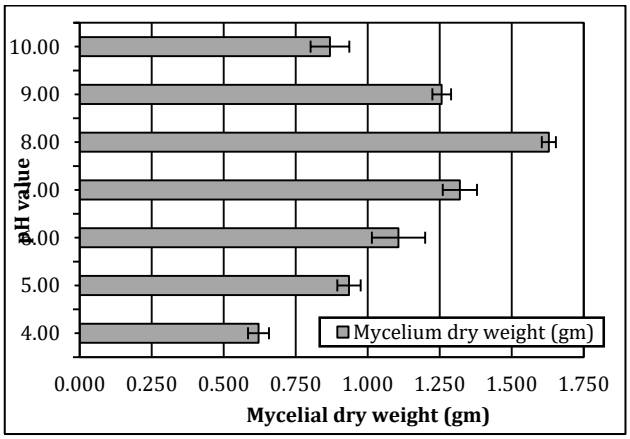

Figure 5: Influence of various pH on mycelial growth (gm) of *A. multifidum*

DISCUSSION

The media components are significant criteria for fungal culture and study, along with important physiological parameters that lead the maximum sporulation[18]. Every living being require food for growth and fungi are not an exception to it. Fungi secure food and energy from the inhibitory substrate uponwhich they live in the nature[30].

In the present investigation, different types of culture media affected the mycelial growth rate and conidial production in *Arthroderm multifidum*. The results showed that SDB is the most suitable liquid culture media followed by MEB. Furthermore, in this study, when the fungus was cultured in RSB, CDB and YEB there was limited mycelial growth and conidial production after 14 days of incubation at 28±2°C (Figure: 1). Gupta[31] studied the growth of keratinophilic fungi on five different media. Out of all media, SDA, found the best for all *Chrysosporium carmichaelii, Chrysosporium georgii, Chrysosporium indicum, Chrysosporium keratinophilum, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium queenslandicum* and *Chrysosporium tropicum* followed by PDA. Ingle[32]reported that SDB yielded second highest fungal biomass (0.609 g) followed by BCY broth (0.590 g) of *Nomuraea rileyi.* Zhao[26] reported that media significantly affected mycelial growth and conidial production of *Diplocarp onmali*. Generally, CSB, PCDB and PCSB were more favorable than other media for mycelial growth, and PCDB, PCSB, PCB and CDB were favorable for conidial production. Ara[33] reported that Richard's medium was the most suitable with respect to growth and sporulation of *Fusarium semitectum* and *Alternaria alternata*. pH 6.0 was the best with respect to the weight of dry mycelia, whereas 30°C temperature was the most suitable for the same. Kadhim[34] reported the effect of culture media (SDA, PDA, CMA, and YEA) in growth rate of eight isolates of *Trichophyton rubrum* during different periods of incubation and 30°C. The optimal growth for all tested isolates (No. 1-8) were (4, 4.5, 4.3, 4.7, 9, 4.3, 7.3 and 6.5 cm), respectively, on SDA rather than other used media and after 7 days of incubation. Mishra and Khan[9] reported that the best growth of *Trichoderma viride* was observed on Sabouraud Malt Yeast extract Agar (SYMA) medium with colony diameter of 2 cm after 5 days of incubation. Wiriya[35] reported that *Termitomyces sp.*CMUTM002 showed the largest colony diameter (46.0±1.00 mm) and biomass yield (60.66±6.93 mg/plate) on malt extract agar.

In temperature case in our study, the optimum temperature for *A. multifidum* was 25°C. Their sporulation was best at 25-35°C. The growth of the fungus was increased with rise of temperature up to 25°C but above 25° it decreased (Figure: 3).Similarly Verma[12]working on *Alternaria tennis*, *Alternaria solani* and *Colletotrichum gloeosporioides* reported that the growth of the fungi was highest at 25°C. Simpfendorfer[23] studied the effects of temperature and pH on the growth and sporangial production of the four known races of *Phytophthora clandestina* Taylor, Pascoe & Greenhalgh. The highest mycelial growth occurred at 25°C. Yang and Liau[36]reported the effects of environmental conditions on the mycelial growth of *Ganoderma lucidum* in shake flask cultures and observed the optimal temperature 30-35 °C. The maximum mycelial concentration reached to 350 mg/100 ml. Miao[37] reported that the marine-derived fungus *Arthrinium* c.f. *saccharicola* isolated from seawater in a mangrove habitat grew faster at 30°C, at pH 6.5 and in freshwater medium. According to Saha[19]*Lasiodiplodiatheobromae* (Pat.) Griffon and Maubl was capable of growing at temperatures that range between 8°-36°C. Best growth was recorded at 28°C. Sharma and Sharma[10] reported the mycelial growth and sporulation onkeratinophilic fungi *i.e. Chrysosporium tropicum* and *Trichophyton mentagrophytes* isolated from public parks soil. These fungi showed their maximum growth at 28-30°C temperature and best sporulation at 25°C-35°C.Onilude[17] reported the maximum growth and sporulation of *Trichoderma viride* between 30 and 37°C were good for mycelium growth while temperatures between 30 to 45°C were good for sporulation.Sharma[16] reported that *Trichophyton mentagrophytes* showed maximum growth at 30°C but the excellent sporulation was observed at 30°C-35°C temperature. *Trichophyton rubrum* showed maximum growth and sporulation at 20°C to 35°C.Singh and Chauhan[38] studied the effect of temperature on the growth of *Aspergillus flavus* and *Penicillium chrysogenum in vitro.* The most suitable temperature for the growth of *A. flavus* and *P. chrysogenum* was observed on 25°C (0.228±.030) and 30°C (0.393±.015) respectively. Kaur and Aggarwal[39] studied on *Alternaria macrospora* and observed that *m*aximum dry weight of *A. macrospora* was recovered at temperature 25°C after five days. At this temperature highest biomass was shown by *A. macrospora* MKP3 (1.25gm) followed by *A. macrospora* MKP4 (0.52 gm), *A. macrospora* MKP1 (0.43) and *A. macrospora* MKP2 (0.25).

In case of effect of Hydrogen ion concentration, mycelial growth was highest at pH 8.00 and pH 6.00-7.00 was most favorable for sporulation. It was evident from the present results that the *A. multifidum* changed the pH of the medium by the end of the incubation days. Saha[19] reported that the optimum pH for growth of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl was at the range of pH 5.5-6.5and the results indicated that slightly acidic pH to neutral pH was optimum for the growth of the organism. Singh and Chauhan[38] studied the effect of pH on the growth of *Aspergillus flavus* and *Penicillium chrysogenum in vitro.* Maximum growth of *A. flavus* was observed on pH 6 (0.506±.030) and whereas pH 7 (0.356±.013) was most suitable for the growth of *P. chrysogenum*. Abubakar[24] reported the highest dried mycelial weight (355.67mg) was at pH 4.0 followed by 353.3mg at pH 7.0 of *Aspergillus parasiticus*. The lowest mycelia dry weight (302.73mg) was obtained at pH 10.0.

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. Tyagi and Paudel[40] observed that pH level 6.0 is the optimum pH for the growth as well as sporulation of the *Fusarium oxysporum.* Singh[41] reported that maximum number of *Trichoderma* Species showed high biomass production at pH 6.5 followed by 7.5 and 5.5 and minimum at pH 4.0 and 4.5. Sharma[22]reported thatpH 7.0 was most suitable for *Trichophyton rubrum* in terms of dry weight of mycelium (0.823gm) as well as in colony diameter (3.2cm).

CONCLUSION

In summary, in this study, we identified media and environmental conditions suitable for rapid growth and sporulation of the fungus *Arthroderma multifidum*. The information generated will facilitate mycological research on the fungus. The highest mycelium growth and sporulation on different liquid culture media, temperature and pH helps to maximize the degradation of keratinous wastes.

Acknowledgements: We thank to Director, School of Sciences for encouragement and Head, Department of Biotechnology, JECRC University, Jaipur and Department of Medical Microbiology, PGIMER, Chandigarh (India) for providing the laboratory facilities.

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Source of Support: Nil, **Conflict of Interest:** None.