Correlative characterization of changes in hyphal morphology during xylanase production in submerged culture by Thermomyces lanuginosus SS-8

S Shrivastava, P Shukla, K Mukhopadhyay

Citation

S Shrivastava, P Shukla, K Mukhopadhyay. *Correlative characterization of changes in hyphal morphology during xylanase production in submerged culture by Thermomyces lanuginosus SS-8*. The Internet Journal of Microbiology. 2007 Volume 4 Number 2.

Abstract

Most of the industrial enzymes are produced by filamentous fungi which often display different morphological forms during their growth in different stages of submerged cultivation as well as in solid media. In the present study the characterization in hyphal morphology of indigenous xylanase producing Thermomyces lanuginosus SS-8 was investigated during submerged cultivation along with the change in pigmentation of colony during the growth in solid media. During submerged culture conditions it was noted that different morphological forms in hyphae and conidia in T. lanuginosus SS-8 has an impact on its xylanase producing capability. During the course of growth for about 72 hrs the hyphal characteristics changed in a significant way with a characteristic change in the pigmentation of the colony on solid media. Further it was noted that the overall diameter increase of hyphal elements was three times from 24 hrs to 72 hrs of incubation time. Interestingly there was fourteen times increase in conidial diameter of T. lanuginosus SS-8. The enormous increase in the conidial diameter by a factor of 14 leads to a significant explanation towards the physiological changes during the course of xylanase production by this fungus and it was most striking finding during the present studies. Furthermore we have also noticed that aleuroconidia are smooth walled with multiple layers as the increase in incubation time and later on with the progressive increase in the incubation time the aleuroconidia matures and they form thick outer membrane. Finally after the 72 hrs of incubation time the conidial elements and aleuroconidia seems to be globose in shape and remarkably wrinkled with a diameter range between 10 µm - 11 µm. However after 72 hrs of incubation there was gradual decrease in number of hyphal elements and aleuriospores detaches itself from filaments. These studies supports that these two events viz. characteristic changes in hyphal and conidial elements along with colonial pigmentation and extracellular release of enzyme in one of our indigenous isolate T. lanuginosus SS-8 may be somewhere linked with capability of the fungus producing xylanases.

INTRODUCTION

Xylanases (Endo-1,4-Beta-xylanase or XYN, EC 3.2.1.8) are glycosidase which catalyzes the endohydrolysis of 1,4-β-D-xylosidic linkages in backbone of complex plant polysaccharides xylan. Filamentous fungi produce number industrial enzymes which have wide variety of applications (Kirk et.al 2002; Sharma et.al 2001) and hence studies on filamentous fungi have always been of prime importance. Among microorganisms filamentous microbes are one of the most prolific producers of extra cellular enzymes and they are also widely used for industrial enzyme production. Some other xylanase producing organisms are Humicola griesa, Trichoderma, Penicillium, Humicola insolens, Aspergillus sp., etc (Collins et al, 2005). Hemicellulytic microorganisms play a significant role in nature by recycling hemicelluloses. Thermomyces lanuginosus (formerly known as Humicola lanuginosa) is one such widely distributed filamentous thermophilic fungi. Mycelium is the characteristic growth form of filamentous fungi and it enables them to explore and exploit new environments and substrates. T. lanuginosus SS-8 is a filamentous fungus producing extracellular enzyme xylanases which has varied industrial application.

In the present study different growth forms of T. lanuginosus have been reported. During growth period the hyphal color of T. lanuginosus changes from white to dark brown as it matures.

It is a widely distributed thermophilic fungus commonly isolated from self heating masses of organic debris (Emerson, 1968). It is classified as a Deuteromycetes (imperfect fungus) that is unicellular or septate and reproduces asexually by forming aleurioconidia (Singh et al.,

2003). This organism is non-cellullolytic and it has been reported that it probably grows commensally in composts with cellulolytic fungi by utilizing some of the sugars generated by the enzymes of these fungi and also by using their mycelial breakdown products (Deacon, 1997). T. lanuginosus are hyperproducers of extracellular xylanases. Along with this they also produce other hemicellulases. Apart from xylanase most glycosyl hydrolases are expressed in very low amount. T. lanuginosus also produces range of other secreted degradative enzymes such as alpha-amylase, glucoamylase, pectinase, phytase, protease and lipase (Singh et al., 2003).

Initially the colonies of T. lanuginosus appear white and felt like which turn grey or greenish grey, commencing from the centre of colony. Subsequently the colony turns purplish brown and the agar substratum stains a deep pink or wine colour. Mature colonies appear dull dark brown to black. Fungal hyphae grow by polarized (apical) growth (Cristina, 2005).

T. lanuginosus produces xylanases which are glycosidases which catalyze the endohydrolysis of 1, 4-β-D-xylosidic linkages in xylan; the major structural polysaccharide in plant cell and is the second most abundant polysaccharide in nature. Xylanase has varied industrial applications such as fruit and vegetable processing, brewing, wine production, baking, animal feed, paper and pulp, starch, textiles, bioremediation/bioconversion. Xylanases produced from T. lanuginosus are. thermostable and good activity at varied pH range have made this enzyme applicable in various industries. The present study was conducted to understand the various morphological forms of T. lanuginosus SS-8 isolated from garden soil sample with organic debris and correlative study of change in hyphal morphology to xylanase production.

Despite considerable progress in understanding the different stages in hyphal developments during enzyme production by various morphological forms of filamentous fungi the present hyperxylanolytic isolate T. lanuginosus SS-8 is yet to be explored by various workers. Consequently in the present study we investigated the various morphological forms of T. lanuginosus SS-8 and its correlation with xylanase production.

MATERIALS AND METHODS

MICROORGANISM AND GROWTH CONDITIONS

T. lanuginosus SS-8 was isolated from garden soil samples of Jharkhand (India). From different soil samples thermophilic fungi growing at ~50?C have been isolated and they have been screened for xylanase production. Out of all the strains isolated SS-8 was found to be most potential xylanase producer. A detailed study of this organism is going on in the microbiology laboratory of the department. T. lanuginosus SS-8 was routinely grown in YpSs (Yeast extract starch) medium. Media contains Yeast Extract, Soluble Starch, Dipotassium Hydrogen Phosphate and Magnesium Sulphate. Optimum temperature and pH for growth are 50°C and 6.5 respectively. For xylanase production Thermomyces lanuginosus was grown in production medium (yeast extract:1.5 g/100ml, KH_2PO_4: 0.5g/ml, substrate(Wheat Bran): 1.5 g/100ml; pH:6.5) and activity of the enzyme produced was assayed periodically.

MICROSCOPY & IMAGE ANALYSIS

The microscopy and image analysis was carried out with the help of Leica FW 4000 (Leica Stereozoom at X3.2.)Light microscope at 40X and 100X magnifications. One milliliter samples of T. lanuginosus SS-8 were taken during growth at submerged conditions. Lactophenol cotton blue stain was used to visualize the hyphal changes during the growth by taking the samples from the grown mycelia fragments in solid media which were collected at various incubation times.

ENZYME ACTIVITY ASSAY

Xylanase activity was routinely determined by mixing 100µl of enzyme solution with 500µl of Oat spelt xylan (2%, w:v) in 50mM Citrate buffer, pH 6.5 at 50°C for 10 min. The release of reducing sugar was measured using the dinitrosalicylic reagent method (Miller, 1959). Xylose was used as the standard. Xylanase activity was expressed as µmoles of reducing sugar formed per milliliter of enzyme solution per minute, i.e. as IU ml^{-1} min^{-1} .

RESULTS

The indigenous T. lanuginosus SS8 colony was white in color which with maturity changed to yellowish followed by brown and then blackish brown colony [Fig a]. Light microscopic study of white colony suggested that these were only filamentous. The filaments were of 1µm and the conidia are of about 0.75µm [Fig b]. As the colony matured it turned to yellowish. At this stage young aleurioconidia of about 1µm emerged laterally from the mycelial hyphae and matures to about 2.5µm. [Fig c] and [Fig d]. The hyphal width at this stage is about 2µm. This feature is novel to the

organism as it does not turn greenish or purplish like other reported organisms of same species. On further maturation the colonies turn brown. At this stage the aleurioconidia matures and they are of about 6µm to 9µm in diameter [Fig d], [Fig e] and [Fig f]. The hyphal diameter at this stage is about 2.5µm.

When the colonies completely mature, they appear blackish brown with dark brown globbose aleurioconidia of diameter size 10µm to 11µm. The hyphal diameter at this stage is about 3µm [Fig g]. These aleurioconidia have wrinkled surface. Finally the conidia detaches from hyphae [Fig h] and [Fig i].

The changes in hyphal width with respect to different colony color have been plotted in Graph 1. Changes in aleurioconidia diameter with change in colony color have been plotted in Graph 2. Activity of crude extracellular enzyme collected after 48, 72, 96, 120, 144 and 168 hr incubation was calculated [Table 1] in IUml $^{-1}$ min $^{-1}$ and change in enzyme activity with respect to change in fungal morphology have been plotted in Graph 3.

Figure 1

Figure 1: (a) SS-8 in YpSs Medium. (b) Aleuroconidia (0.75 Âµm Hyphae: 1 Âµm. at 40X (c) Aleuroconidia (1 -2.5 Âµm), hyphae: 2 Âµm. at 40X (d) Hyphae and Aleuroconidia at 100X oil emersion. (e) Hyphae and mature aleuroconidia at 100X, oil emersion, Aleurioconidia: 6-9 Âµm and hyphae: 2.5 Âµm. (f) Mature aleuroconidia on the verge of detaching from hyphae (100X oil immersion). (g) Hyphal elements and aleuroconidia from mature colony at 40X. (h) Aleuroconidia: 11 Âµm; Hyphae 3 Âµm. (i) Mature aleuroconidia detached from hyphae.

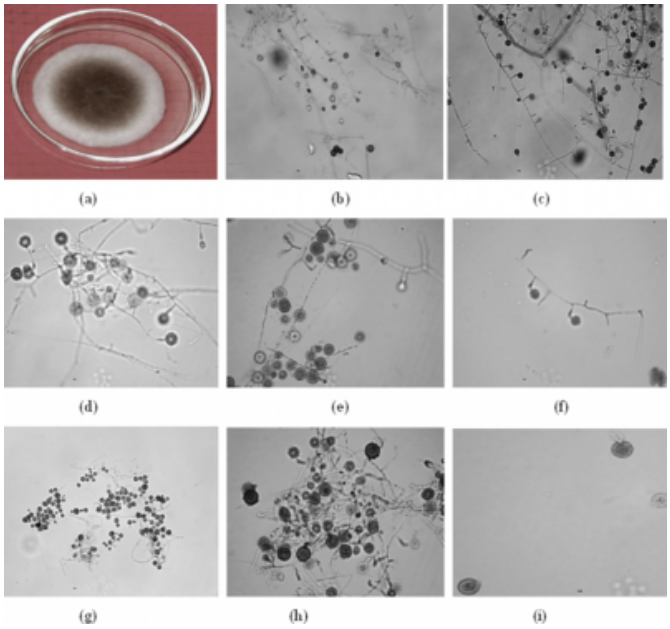


Figure 2

Table 1: Change in colony color, hyphal width, aleurioconidia diameter and enzyme production with respect to incubation time

INCUBATION TIME (Hr)	COLONY COLOUR	HYPHAL WIDTH (µm)	ALEURIOCONIDIA DIAMETER (µm)	ENZYME ACTIVITY OF CRUDE SAMPLE (IU $ml^{-1}min^{-1}$)
24	White	~1	~1	NIL
48	Yellow	~2	~2	0.889
72	Brown	~2.5	~7-8	5.543
96	Brown Black	~3	~11	6.297
120	Brown Black	~3	~11	6.677
144	Brown Black	~3	~11	7.687
168	Brown Black	~3	~11	7.031

Figure 3

Graph 1

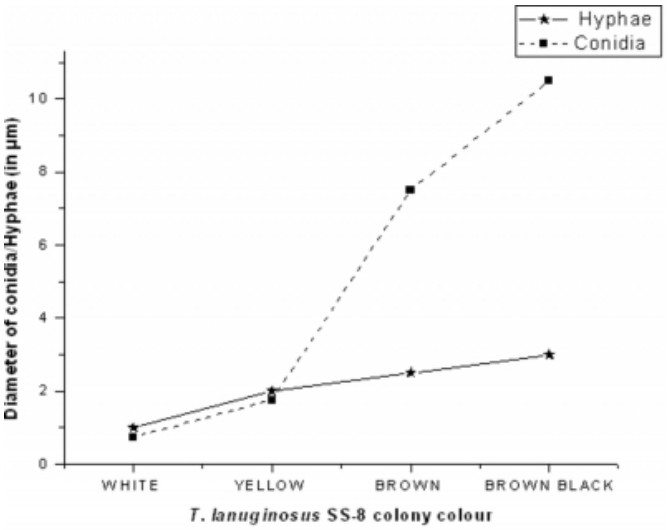


Figure 10: Variation in Colour Appearance of T. lanuginosus SS-8

Figure 4

Graph 2

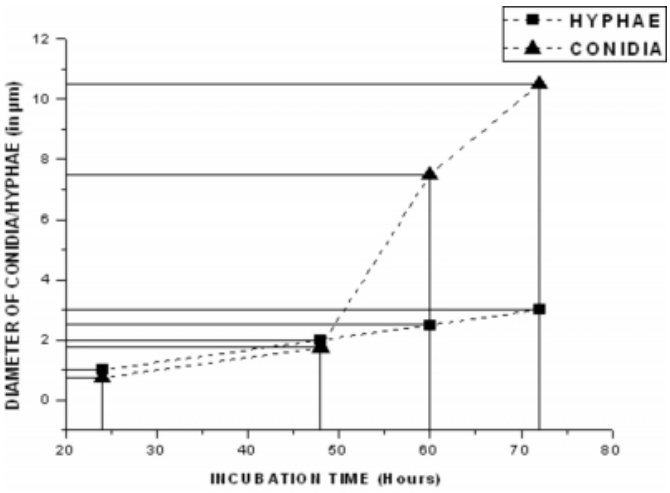
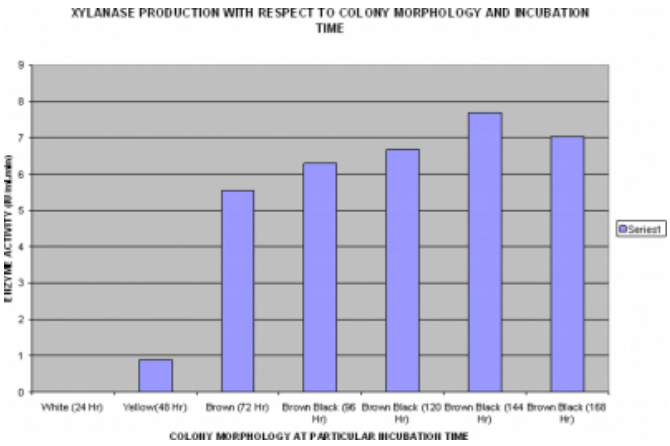


Figure-11: Variation in diameter with respect to Incubation time

Figure 5

Graph 3



DISCUSSION

From the study it was concluded that as fungal cells mature the enzyme production increases. There was no enzyme present in production medium upon 24hr incubation. Thus suggesting that white colony with budding conidia could not produce any enzyme. Subsequent incubation for 48, 72, 96, 120, 144 and 168 hr showed gradual increase in enzyme activity. It was seen that as conidia matures its capacity of enzyme production increases and amount of enzyme produced shows a measurable increase with the change in colony color from yellow to brown.

The increase in hyphal diameter from white to yellowish colony is by a factor of 2. The complete increase in hyphal diameter from white to blackish brown colony is by a factor of 3. The yellow color colony of the organism is unique to this novel form as no earlier literature reports this feature. Previous literatures suggest the purplish or green colony which is absent in this strain.

ACKNOWLEDGEMENT

We gratefully acknowledged the support from Department of Agriculture, Government of Jharkhand & Sub-Distributed Information Center (BTISnet SubDIC) of Department of Biotechnology, Government of India in the form of R & D Grants for our department.

References

r-0. Cristina, G., Reynaga-Pena., Salomon Bartnicki-Garcia. (2005) Cytoplasmic contraction in growing fungal hyphae and their morphogenetic consequences. Arch Microbiol 183; 292-300.
r-1. Collins, T., Gerday, C., Fellar, G. (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiology Reviews 29, 3-23.
r-2. Deacon, J.W. (1997) Environmental conditions for

growth, and tolerance of extremes. In: Modern Mycology, 3 rd Edn., pp. 121-135. Blackwell Science, Oxford.
r-3. Emerson, R. (1968) Thermophiles. In: The Fungi – An advanced Treaties (Answorth, G.C. and Sussman, A.S., Eds), pp. 105-128. Academic press, London.
r-4. Kirk, O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13: 345-351.
r-5. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31, 426–428.
r-6. Sharma R, Chisti Y, Bannerjee UC (2001) Production, purification, characterization and applications of lipases. Biotechnol Adv 19:627-662.
r-7. Singh, S., Mandala, A.M., Prior, B.A. (2003) Thermomyces lanuginosus: properties of strains and their hemicellulases. FEMS Microbiology Reviews 27, 3-16.

Author Information

Smriti Shrivastava, M.Sc.
Department of Biotechnology, Birla Institute of Technology, (Deemed University)

Pratyoosh Shukla, Ph.D.
Department of Biotechnology, Birla Institute of Technology, (Deemed University)

Kunal Mukhopadhyay, Ph.D.
Department of Biotechnology, Birla Institute of Technology, (Deemed University)