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SHORT COMMUNICATION

Extracellular chitinase production by some members of the saprophytic Entomophthorales group

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Abstract Thirteen strains were isolated from different habitats, belonging to two genera, namely *Conidiobolus* and *Basidiobolus*, related to saprophytic Entomophthorales. Chitin flake colonization and agar-well diffusion tests were used to screen potential extracellular chitinase-producing strains in plate assays. Preliminary screening resulted in five chitinase producers that were further studied quantitatively. Results indicated that studied isolates of this group produced chitinase at different levels in chitin-containing as well as non-chitin-containing medium. *Conidiobolus coronatus* was found to be the most significant chitinase producer, giving 0.261 U/ml using colloidal chitin as a carbon source, among the isolates under study. This communication also reports the chitinolytic activity of *Basidiobolus haptoporus*, the effect of environmental and nutritional parameters on chitinase production, and utilization of fungal biomass as a carbon source, which hitherto had not been elaborated from this genus.

Keywords *Basidiobolus haptosporus* · Colloidal chitin · *Conidiobolus coronatus* · Native chitin

All the strains have been deposited and accessioned in National Fungal Culture Collection of India (NFCCI) (WDCM-932) At MACS's Agharkar Research Institute, Pune, India.

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Introduction

Chitin, a tough and pliable β (1–4) homopolymer of *N*-acetyl-D-glucosamine (GlcNAc), is the most abundant polysaccharide, after cellulose, existing in nature. It constitutes a major structural component of many biological systems, particularly those of mollusks, insects, crustaceans, fungi, algae, and marine invertebrates (Shaikh and Deshpande 1993). More than 10^{11} tonnes of chitinous waste is obtained per year from the aquatic biosphere alone (Tsujibo et al. 1998). Chitin and its derivatives are of commercial and biotechnological interest because of their wider range of biological activities (Bhushan and Hoondal 1998; Gohel et al. 2006). The enzymatic hydrolysis of chitin to free *N*-acetylglucosamine units is performed by a chitinolytic system, which is found in a variety of organisms such as actinomycetes, bacteria, fungi, yeasts, plants, insects, and also in human beings (Bhattacharya et al. 2007; Mellor et al. 1994; Royer et al. 2002; Tjoelker et al. 2000). In recent years, chitinases (EC 3.2.1.14) have received greater attention for reasons of their wider range of biotechnological applications, especially in the biocontrol of fungal phytopathogens (Mathivanan et al. 1998) and harmful insect pests (Mendonsa et al. 1996; Pinto et al. 1997). Chitinases have also been used in the preparation of sphaeroplasts and protoplasts from yeast and fungal species (Peberdy 1985; Mizuno et al. 1997). Some other significant applications of chitinases include bioconversions of chitin waste to single-cell protein and ethanol (Vyas and Deshpande 1991) and fertilizers (Sakai et al. 1986).

The saprophytic Entomophthorales group has been studied by different workers for its extracellular enzyme profile. However, meager data are available about the extracellular secretion of chitinase from the fungal members of this group. Keeping this in mind, we studied some

members of this group from soil and plant litter, including two strains of *Basidiobolus haptosporus* from intestinal contents of frog, and screened them for extracellular chitinase production.

Leaf detritus collected from different locations was screened for saprophytic Entomophthorales by canopying moistened litter on MGYP (malt extract-glucose-yeast extract-peptone agar) plates (Drechsler 1952; Srinivasan and Thirumalachar 1968). Isolates found in this study were obtained after 7 days, developing from forcibly discharged conidia in isolation plates. Excreta was collected from two frogs captured from the institute's garden and used for the isolation of fungi by the aforementioned technique.

The morphological details of isolates were studied and measurements of different fungal structures were undertaken following the procedure described earlier to confirm the identity of the fungal isolates of saprophytic Entomophthorales (Waingankar et al. 2008). All the fungal strains have been deposited and accessioned in the National Fungal Culture Collection of India (WDCM-932) at MACS Agharkar Research Institute, Pune, India.

Fungal cultures were inoculated onto 0.2% yeast extract agar, and sterile chitin flakes were placed near the fungal inoculum aseptically and incubated at 28° ± 2°C. Flakes were microscopically examined for the presence of fungal mycelia after 24 and 48 h of incubation.

One percent (1%) colloidal chitin medium with 0.2% yeast extract powder was prepared for agar plate assays. An agar punch from the 48-h-old culture growing on MGYP plates was placed in the center of the plate. After incubation at 28° ± 2°C for 3–4 days, plates were observed for the zones of clearance. To enhance the visibility and clarity of zones of clearance, the dye Remazol Brilliant Blue (0.85%) was also added into the medium. For the agar-well diffusion test, crude enzyme was obtained by cultivating fungal isolates on MGYP broth containing 1% colloidal chitin at 28° ± 2°C at 200 rev/min for 48 h. The cell-free supernatant (crude enzyme) was used on 1% colloidal chitin agar.

The production medium for the chitinase contained (in g/l) polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; glucose, 2.5; chitin (native/colloidal), 10.0, with pH 6.5–6.8. Flasks were inoculated with spore suspension containing 1×10^6 cfu and incubated at 28° ± 2°C at 200 rev/min. Acid-swollen chitin, containing about 7 mg chitin/ml, was prepared according to Hackman (1962) and used for the quantitative estimations. For the chitinase assay, crude enzyme was assayed using the colorimetric method with slight modifications (Elson and Morgan 1933). The reaction mixture contained 1 ml each acid-swollen chitin, 0.05 M acetate buffer (pH 5.0), and crude enzyme preparation. *N*-Acetyl-D-glucosamine (NAG) units released were measured at A_{585}. A calibration curve was obtained by using the range of 10–100 µg/ml of standard NAG. A unit of chitinase activity was defined as the amount of enzyme that produced 1 µmole of NAG equivalent per hour under the assay conditions.

Basidiobolus was grown on MGYP agar plates. After sporulation, it was transferred onto MGYP broth containing 1% NH_4Cl for production of darmform cells, up to 48 h, which were used as inoculum (Ingale et al. 2002). Production medium contained MGYP with 1% colloidal chitin or 1% native chitin. Flasks were inoculated with broth containing darmform cells 10% (v/v) and incubated at 28° ± 2°C at 200 rev/min. The effect of different environmental and nutritional parameters such as inoculum size (5–25%), pH (4.0, 6.5, 8.0, 10.0, and 12.0), temperatures (20°, 25°, 30°, and 35°C), sugars (glucose, sucrose, maltose, lactose, and galactose), and types of chitin (colloidal chitin and flakes) on chitinase production by *Basidiobolus haptosporus* was studied using a one factor at a time approach.

Dried fungal mycelia as an alternate carbon source were used for the chitinase production. Different fungi, viz. *Aspergillus niger* (NFCCI 663), *Aspergillus flavus* (NFCCI 664), *Penicillium* sp. (NFCCI 672), *Paecilomyces variotii* (NFCCI 671), and *Acremonium* sp. (NFCCI 662), were grown in potato dextrose broth for 1 week. Mycelium was dried and powdered in a blender. From production medium, chitin was replaced by dried and powdered fungal mycelia. The comparative chitinase production was calculated against chitin-containing medium.

The morphotaxonomic identification of recovered fungal isolates resulted in a total 13 isolates contributing nine *Conidiobolus* and four *Basidiobolus* species (eight *Conidiobolus* and one *Basidiobolus* from plant litter, one isolate each from soil, and two *Basidiobolus* isolates from frog excreta).

During preliminary screening all the isolates showed colonization on chitin flakes when inoculated on agar containing 0.2% yeast extract with sterile chitin flakes. In microscopic observations, the presence of fungal mycelia inside the chitin flakes confirmed the chitinolytic activity (Fig. 1a, b). In plates containing nutrient media with colloidal chitin, each isolate showed a zone of clearance (Fig. 1c, d). Similar results were observed in the presence of the dye Remazol Brilliant Blue (Fig. 1e, f). Confirmation of chitinolytic activity of fungal isolates was done by agar-well diffusion test using crude enzyme on 1% colloidal chitin agar (Fig. 1g, h). The zone of clearance having the largest diameter was observed with *Conidiobolus coronatus* NFCCI 1235 (8 mm), followed by *C. couchii* NFCCI 719 (4.5 mm), *C. coronatus* NFCCI 718 (4 mm), and *Basidiobolus haptosporus* NFCCI 1922 and *B. haptosporus* NFCCI 1923 (4 mm each)

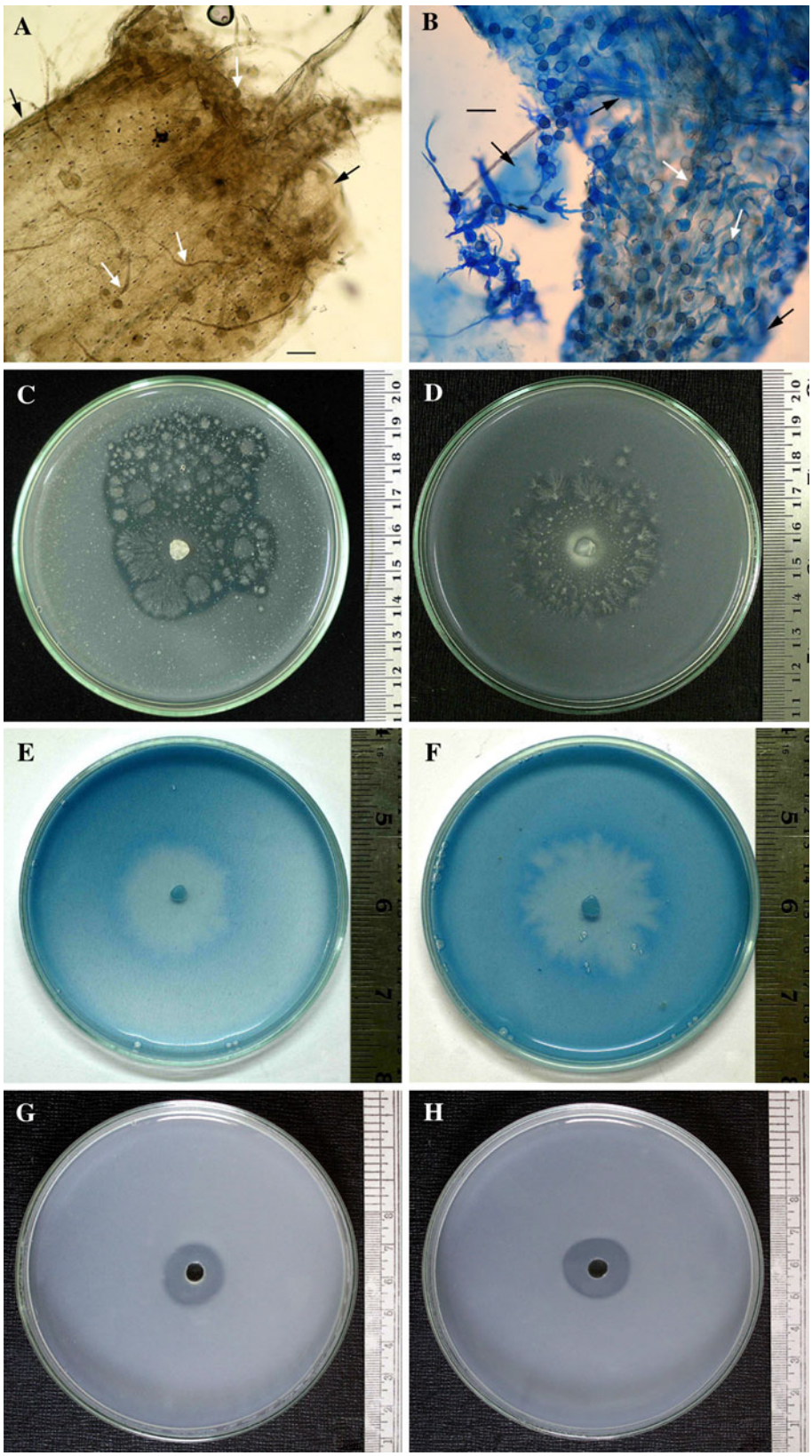

Fig. 1 **a**, **b** Chitin flake colonization by *Basidiobolus haptosporus* NFCCI 1922 and NFCCI 1923 (**b** stained with cotton blue). *White arrows*, chitin flake; *black arrows*, presence of fungal structures. *Bars* **a**, **b** 40 µm; ×40. **c**, **d** Zone of clearance on colloidal chitin by *Conidiobolus coronatus* NFCCI 1235 and *C. couchii* NFCCI 719. **e**, **f** Zone of clearance on colloidal chitin supplemented with dye formed by *B. haptosporus* NFCCI 1922 and *C. coronatus* NFCCI 1235. **g**, **h** Agar-well diffusion test of crude chitinase by *C. coronatus* NFCCI 1235 and *C. couchii* NFCCI 719

Isolates that produced zones of clearance of more than 4 mm were considered significant producers of extracellular chitinase and were selected for flask level studies. During preliminary quantitative estimations, maximum chitinase production was 0.261 U/ml in crude cell-free supernatant of *C. coronatus* NFCCI 1235, followed by

Table 1 Chitinase production by fungal isolates using native and colloidal chitin as a carbon source

Strain no.	NFCCI accession no.	Name of the isolate	Activity with 1% colloidal chitin (48 h) (U ml^{-1})	Activity with 1% native chitin (72 h) (U ml^{-1})
1	NFCCI 1235	*Conidiobolus coronatus*	0.261	0.092
2	NFCCI 719	*Conidiobolus couchii*	0.241	0.077
3	NFCCI 718	*Conidiobolus coronatus*	0.230	0.060
4	NFCCI 1922	*Basidiobolus haptosporus*	0.205	0.067
5	NFCCI 1923	*Basidiobolus haptosporus*	0.189	0.071

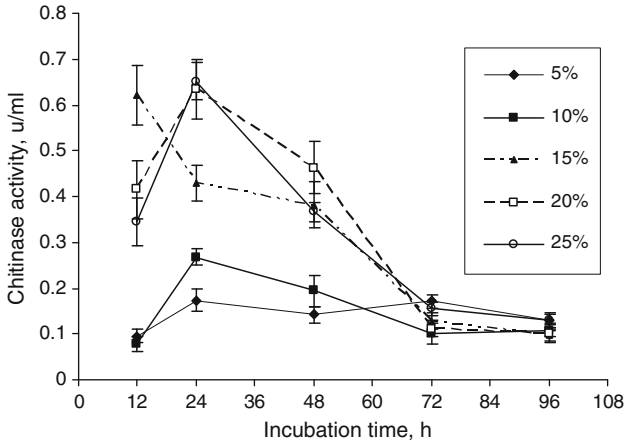

Fig. 2 Effect of inoculum density on chitinase production by *Basidiobolus haptosporus* NFCCI 1922: production medium consisted (in g/l) of polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; glucose, 2.5; colloidal chitin, 10.0 (g/l); pH 6.5, 50 ml production medium per 250-ml Erlenmeyer flask, with different percent inoculum of 1×10^6 cfu, incubated for 96 h at 200 rpm and 28° ± 2°C. *Error bar* represents the mean of triplicate analysis ± SD

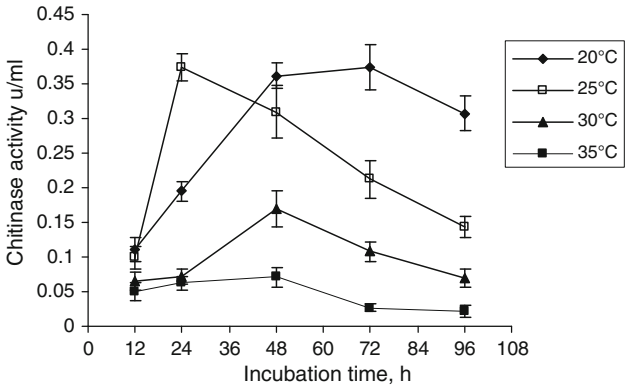

Fig. 3 Effect of temperature on chitinase production by *Basidiobolus haptosporus* NFCCI 1922 on production medium consisting (in g/l) of polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; glucose, 2.5; colloidal chitin, 10.0 (g/l); pH 6.5, 50 ml production medium per 250-ml Erlenmeyer flask, inoculum 1×10^6 cfu, incubated at different temperatures for 96 h at 200 rpm. *Error bar* represents the mean of triplicate analysis ± SD

C. couchii NFCCI 719 (0.241 U/ml) after 48 h incubation in a medium containing 1% colloidal chitin and 0.25% glucose. The two *Basidiobolus haptosporus* isolates, NFCCI 1922 and NFCCI 1923, produced 0.205 and 0.189 U/ml of enzyme, respectively, after 48 h incubation (Table 1). Colloidal chitin was found to be a preferable carbon source over the native chitin by all the isolates in terms of enzyme production.

It was observed during the screening experiments that *B. haptosporus* secreted small amounts of extracellular chitinase even in the absence of chitin in the medium. The inclusion of chitin in the medium increased chitinase production significantly.

An inoculum density of 3×10^6 cfu/flask (15% v/v) was found to be optimal for chitinase production after 12 h incubation (Fig. 2), although maximum chitinase production was observed with 25% (v/v) inoculum after 24 h incubation giving 0.651 U/ml. Chitinase production at 25°C was found to be maximal (0.374 U/ml) after 24 h incubation (Fig. 3). At pH 8.0, chitinase production was the highest, giving 0.409 U/ml after 24 h incubation, although much less difference in chitinase production was observed between pH 4 and 10 (Fig. 4). Based upon these observations, it can be said that the culture under study produced

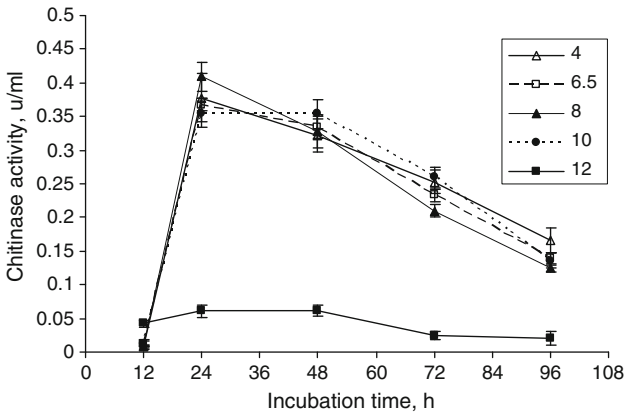

Fig. 4 Effect of pH on chitinase production by *Basidiobolus haptosporus* NFCCI 1922: production medium consisted (in g/l) of polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; glucose, 2.5; colloidal chitin, 10.0 (g/l); 50 ml production medium per 250-ml Erlenmeyer flask, with different pH, inoculum 1×10^6 cfu, incubated for 96 h at 200 rpm and 28° ± 2°C. *Error bar* represents the mean of triplicate analysis ± SD

chitinase that is active in a broad range of pH. It was observed that chitinase activity declined after a particular time period, which may occur because of autolysis of the enzyme or unavailability of the substrate in the production medium. Of the different sugars used in the study, glucose was found to be the most supportive for chitinase production, giving 0.651 U/ml after 24 h incubation, closely followed by lactose (0.64 U/ml) and maltose (0.63 U/ml) (Fig. 5), although maximum activity was observed with galactose (0.778 U/ml) after 72 h incubation.

The organism under study utilized mycelia of all the fungal isolates as a major carbon source for the production of chitinase. Significant hydrolysis of fungal mycelia was observed with mycelium of *Aspergillus niger* (0.572 U/ml) after 72 h incubation. Chitinase produced by utilization of mycelia of *A. niger* was comparable with chitinase production using native chitin (0.623 U/ml) (Fig. 6).

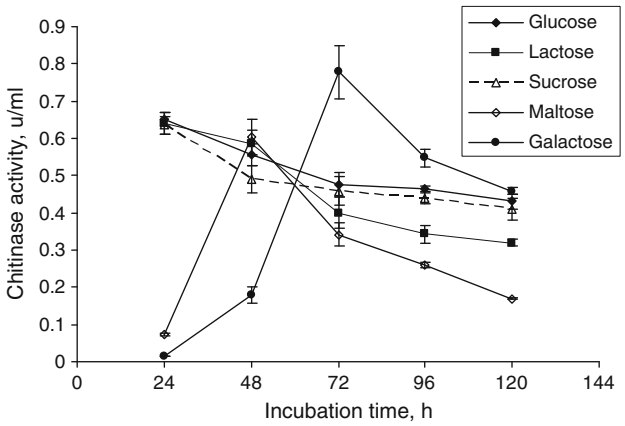

Fig. 5 Effect of different sugars on chitinase production by *Basidiobolus haptosporus* NFCCI 1922: production medium consisted (in g/l) of polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; sugars, 2.5; colloidal chitin, 10.0 (g/l); pH 6.5, 50 ml production medium per 250-ml Erlenmeyer flask, inoculum 1×10^6 cfu, incubated for 96 h at 200 rpm and 28° ± 2°C. *Error bar* represents the mean of triplicate analysis ± SD

The extracellular chitinase system generally involves a complex of hydrolytic enzymes such as *N*-acetylglucosaminidase, *β*-1,3-glucanase, and *β*-glucosidase. This extracellular enzyme system helps the fungi with its sustainability in nature. There are only a couple of reports available on chitinase production from the saprophytic Entomophthorales group of fungi. Ishikawa et al. (1981) reported the activity of ammonium sulfate precipitate of chitinase from different isolates of *Conidiobolus* ranges from 6.33 to 90.6 $\times$ 10^3 U/ml. Feio et al. (1999) reported urease, *N*-acetyl-*β* glucosaminidase, trypsin, lipase, lecithinase, and gelatinase, among others, from some isolates of genus *Basidiobolus*, but chitinase activity remained inconclusive. There are reports of chitinase from other microbial sources, such as different species of *Trichoderma*, which produce chitinases in the range of 0.07–41.44 U/ml (Bruce et al. 1995; El-Katatny et al. 2000), and from *Streptomyces*, 0.0176–0.0833 mU/ml (Taechowisan et al. 2003). This study reveals the chitinolytic potential of *Basidiobolus haptosporus* from frog excrement, which has been elaborated for the first time. The study also provides presumptive information about less explored fungal isolates, namely *Basidiobolus* and *Conidiobolus*, which may have possible applications in the biocontrol of insect pests and phytopathogenic fungi and in the bioconversion of chitin to free *N*-acetyl glucosamine units. Utilization of fungal mycelia for the chitinase production from fungi, actinomycetes, and bacteria has been reported by different workers (Anitha and Rebeeth 2009, 2010; El-Katatny et al. 2000). Our results suggest that this

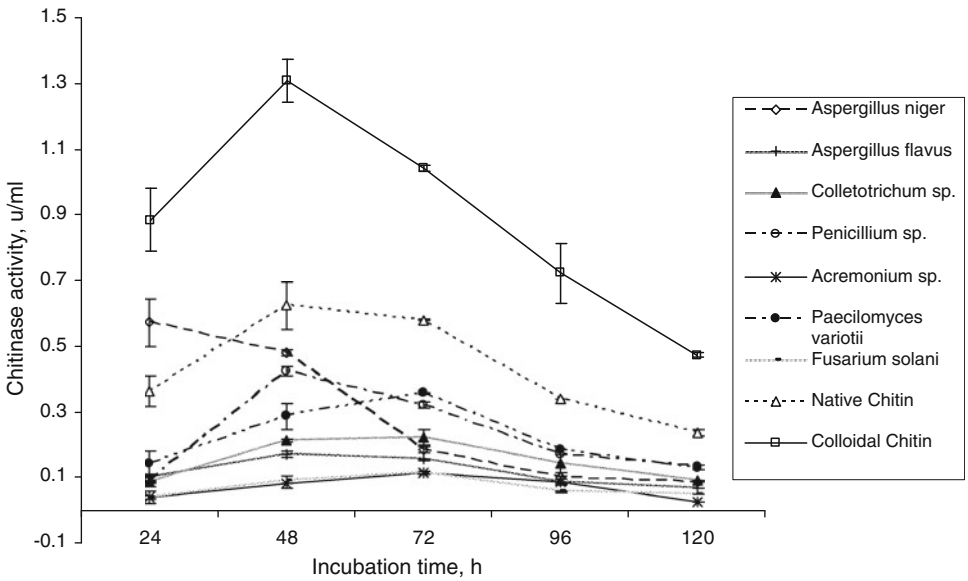

Fig. 6 Utilization of dried fungal mycelia by *Basidiobolus haptosporus* NFCCI 1922 for chitinase production: production medium consisted (in g/l) of polypeptone, 1.25; yeast extract, 1.25; malt extract, 0.75; glucose, 2.5; dried fungal mycelia, 10.0 (g/l), pH 6.5, 50 ml production medium per 250-ml Erlenmeyer flask, inoculum 1×10^6 cfu, incubated for 96 h at 200 rpm and 28°± 2°C. *Error bar* represents the mean of triplicate analysis ± SD

enzyme can be used for utilization of fungal biomass produced during different fermentation processes and thus *N*-acetyl glucosamine units can be produced for various biomedical applications such as in the treatment of osteoarthritis and autoimmune encephalomyelitis (Felson and McAlindon 2000; Reginster et al. 2001; Zhang et al. 2005).

Perusal of the literature indicates that interest in the biotechnological potential of *Conidiobolus* has centered on the production of high-activity alkaline protease (Phadtare et al. 1993; Srinivasan 2007) and the production of polyunsaturated fatty acids (Idemitsu-Petrochem 1990). This is the first systematic study of the chitinase profile of saprophytic Entomophthorales, in particular from *Basidiobolus haptosprous*. As such, the isolation of different isolates in the present study will serve as a valuable gene pool. Further, it attests to the fact that there is an urgent need for sustained exploration of a wide variety of substrates for selective isolation of fungi of one of the most neglected group of saprophytic Entomophthorales and their screening for extracellular chitinase and other biotechnological potential.

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References

Anitha A, Rebeeth M (2009) In vitro antifungal activity of *Streptomyces griseusa* against phytopathogenic fungi of tomato field. Acad J Plant Sci 2(2):119–123

Anitha A, Rebeeth M (2010) Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus*. Afr J Plant Sci 4(3):61–66

Bhattacharya D, Nagpure A, Gupta RK (2007) Bacterial chitinases: properties and potential. Crit Rev Biotechnol 27(1):21–28

Bhushan B, Hoondal GS (1998) Isolation, purification and properties of a thermostable chitinase from an alkalophilic *Bacillus* sp. BG-11. Biotechnol Lett 20(2):157–159

Bruce A, Srinivasan U, Staines HJ, Highley TL (1995) Chitinase and laminarinase production in liquid culture by *Trichoderma* spp. and their role in biocontrol of wood decay fungi. Int Biodeterior Biodegrad 35:337–353

Drechsler C (1952) Widespread distribution of *Delacroixia coronata* and other saprophytic Entomophthoraceae in plant detritus. Science 115:575–576

El-Katatny MH, Somitsch W, Robra KH, El-Katatny MS, Gübitz GM (2000) Production of chitinase and β-1,3-glucanase by *Trichoderma harzianum* for control of the phytopathogenic fungus *Sclerotium rolfsii*. Food Technol Biotechnol 38(3):173–180

Elson LA, Morgan WTJ (1933) A colorimetric method for the determination of glucosamine and chondrosamine. Biochem J 27:114–118

Feio CL, Bauwens L, Swinne D, De Meurichy W (1999) Isolation of *Basidiobolus ranarum* from ectotherms in Antwerp zoo with special reference to characterization of the isolated strains. Mycoses 42:291–296

Felson DT, McAlindon TE (2000) Glucosamine and chondroitin for osteoarthritis: to recommend or not to recommend? Arthritis Care Res 13:179–182

Gohel V, Singh A, Maisuria V, Phadnis A, Chhatpar HS (2006) Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr J Biotechnol 5(2):54–72

Hackman RH (1962) Studies on chitin. V. The effect of mineral acids on chitin. Aust J Biol Sci 15:526–537

Idemitsu-Petrochem (1990) Preparation of lipid containing bishomogammalinolenic acid by culturing *Conidiobolus* sp. in sesame oil culture medium JP-089982. Biotechnol Abstr 91:02817

Ingale SS, Rele MV, Srinivasan MC (2002) Alkaline protease production by *Basidiobolus* (N.C.L. 97.1.1): effect of 'darm-form' morphogenesis and cultural conditions on enzyme production and preliminary enzyme characterization. World J Microbiol Biotechnol 18(5):403–408

Ishikawa F, Oishi K, Ko A (1981) Chitinase production by *Conidiobolus lamprauges* and other *Conidiobolus* species. Agric Biol Chem 45(10):2361–2362

Mathivanan N, Kabilan V, Murugesan K (1998) Purification, characterization and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis*. Can J Microbiol 44:646–651

Mellor KJ, Nicholasb RO, Adams DJ (1994) Purification and characterization of chitinase from *Candida albicans*. FEMS Microbiol Lett 119(1-2):111–117

Mendonsa ES, Vartak PH, Rao JU, Deshpande MV (1996) An enzyme from *Myrothecium verrucaria* that degrades insect cuticles for biocontrol of *Aedes aegypti* mosquito. Biotechnol Lett 18:373–376

Mizuno K, Kimura O, Tachiki T (1997) Protoplast formation from *Schizophyllum commune* by a culture filtrate of *Bacillus circulans* KA-304 grown on a cell-wall preparation of *S. commune* as a carbon source. Biosci Biotechnol Biochem 61:852–857

Peberdy JF (1985) Mycolytic enzymes. In: Peberdy JF, Ferenczy L (eds) Fungal protoplast applications in biochemistry and genetics. Dekker, New York, pp 31–44

Phadtare SU, Deshpande VV, Srinivasan MC (1993) High activity alkaline protease from *Conidiobolus coronatus* (NCL 86-8-20): enzyme production and compatibility with commercial detergents. Enzyme Microb Technol 5:72–76

Pinto AS, Barreto CC, Schrank A, Ulhao CJ, Vainstein MH (1997) Purification and characterization of an extracellular chitinase from the entomopathogen *Metarhizium anisopliae*. Can J Microbiol 43:322–327

Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, Giacovelli G, Henrotin Y, Dacre JE, Gossett C (2001) Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomized, placebo-controlled clinical trial. Lancet 357:251–256

Royer V, Fraichard S, Bouhin H (2002) A novel putative insect chitinase with multiple catalytic domains: hormonal regulation during metamorphosis. Biochem J 366:921–928

Sakai T, Koo K, Saitoh K (1986) Use of the protoplast fusion for the development of rapid starch-fermenting strains of *Saccharomyces diastaticus*. Agric Biol Chem 50(2):297–306

Shaikh SA, Deshpande MV (1993) Chitinolytic enzymes: their contribution to basic and applied research. World J Microbiol Biotechnol 9(4):468–475

Srinivasan MC (2007) Biology and biotechnology potential of saprophytic Entomophthorales. In: Ganguli BN, Deshmukh SK

(eds) Fungi: multifaceted microbes. Anamaya, New Delhi, pp 16–27

Srinivasan MC, Thirumalachar MJ (1968) Two new species of *Conidiobolus* from India. J Elisha Mitchell Sci Soc 84:211–212

Taechowisan T, Peberdy JF, Lumyong S (2003) Chitinase production by endophytic *Streptomyces aureofaciens* CMUAc130 and its antagonism against phytopathogenic fungi. Ann Microbiol 53(4):447–461

Tjoelker LW, Gosting L, Frey S, Hunter CL, Trong HL, Steine B, Brammer H, Gray PW (2000) Structural and functional definition of the human chitinase chitin-binding domain. J Biol Chem 275(1):514–520

Tsujibo H, Orikoshi H, Shiotani K (1998) Characterization of chitinase C from marine bacterium *Alteromonas* sp. strain 0–7, and its corresponding gene and domain structure. Appl Environ Microbiol 64:472–478

Vyas P, Deshpande MV (1991) Enzymatic hydrolysis of chitin by *Myrothecium verrucaria* chitinase complex and its utilization to produce SCP. J Gen Appl Microbiol 37:267–275

Waingankar VM, Singh SK, Srinivasan MC (2008) A new thermophilic species of *Conidiobolus* from India. Mycologia 165:173–177

Zhang GX, Yu S, Gran B, Rostami A (2005) Glucosamine abrogates the acute phase of experimental autoimmune encephalomyelitis by induction of Th2 response. J Immunol 175:7202–7208