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Dimorphism in *Benjaminiella poitrasii*: Involvement of Intracellular Endochitinase and *N*-Acetylglucosaminidase Activities in the Yeast–Mycelium Transition

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ABSTRACT. The chitinase and *N*-acetylglucosaminidase activities in cell-wall-bound and free fractions in the dimorphic fungus *Benjaminiella poitrasii* were studied as a function of morphological (unicellular yeast–mycelium) transition. The specific activities of chitinases of cell-wall-free, particularly in the membrane fraction, were significantly different in the yeast and mycelial forms. During the yeast–mycelium transition, the *N*-acetylglucosaminidase activity isolated in a membrane preparation increased steadily. The activity of the yeast cells (0.83 ± 0.17 nkat/mg protein) increased 17-fold to 14.2 ± 1.7 nkat/mg protein in 1-d-old mycelial cells. The endochitinase activity increased 12-fold between 6 and 12 h and thereafter practically remained unchanged up to 24 h. A reverse trend in the chitinolytic activities was observed during the mycelium–yeast transition. Isoelectrofocussing (pH range 3.5–10) of mixed membrane fraction free of particulate fraction of parent and morphological (Y-5, yeast-form) mutant cells separated endochitinase and *N*-acetylglucosaminidase activity into two pH ranges, *viz.* 4.3–5.7 and 6.1–7.7, respectively. The predominant *N*-acetylglucosaminidase activity observed at pH 6.9 and 7.1 for the parent strain membrane fraction was undetected in the mutant preparation. The results suggested that the membrane-bound (either tightly or loosely) chitinolytic enzymes, particularly, *N*-acetylglucosaminidase, significantly contributed to the morphological changes in *B. poitrasii*.

Chitin, a 1,4-β-D-*N*-acetylglucosamine-linked polymer, is the main structural component of most fungal cell walls (*e.g.*, Kopeček and Raclavský 1999; Žnidaršič *et al.* 1999). The chitinase complex comprising endochitinase (EC 3.2.1.14) and *N*-acetylglucosaminidase (EC 3.2.1.52) activities has been implicated in several aspects of the life cycle of fungi, such as hyphal tip growth, spore germination, cell separation and autolysis (Kuranda and Robbins 1991; Rast *et al.* 1991; Sahai and Manocha 1993; Yanai *et al.* 1992; Lima *et al.* 1999). These enzymes are either extracellular or are present intracellularly as cell-wall-bound, microsomal or cytosolic (Adams *et al.* 1993; Balasubramanian and Manocha 1992; Binks *et al.* 1990; Humphreys and Gooday 1984*a*; Kuranda and Robbins 1991). As a function of age, Pedraza-Reyes and Lopez-Romero (1989) reported two distinct cytosolic chitinases in extracts of mycelial cells of *Mucor rouxii*. Later they detected nine chitinases in germinating cells (Pedraza-Reyes and Lopez-Romero 1991). However, the functional implications of the chitinase multiplicity is still to be understood completely. According to Humphreys and Gooday (1984*a*), membrane-bound chitinases in *Mucor mucedo* contributed significantly in the hyphal growth. Similarly, in a number of other taxonomically different filamentous and dimorphic fungi, membrane-bound chitinolytic enzymes were reported (Adams *et al.* 1993; Balasubramanian and Manocha 1992; Binks *et al.* 1990; Jackson *et al.* 1996).

Our earlier studies on the cell wall chemistry of the zygomycetous, dimorphic fungus *Benjaminiella poitrasii*, revealed that the mycelial cell walls contain three times more chitin than the yeast cell walls (Khale and Deshpande 1992). The present communication deals with the localization of endochitinase and *N*-acetylglucosaminidase activities in the mycelial and yeast-form cells and their possible involvement in the yeast–mycelium transition in *B. poitrasii*.

MATERIALS AND METHODS

Chemicals. The substrates for chitinase estimation, *viz.* ethylene glycol chitin (EGC), 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide (pNP-GlcNAc), 4-methyl-umbelliferyl-*N*-acetyl-β-D-glucos-

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aminide (4-MU-GlcNAc), 4-MU-*N'*,*N''*,*N'''*-triacetyl-chitotrioside (4-MU-(GlcNAc)$_3$) and D-glucono-1,5-lactone were purchased from *Sigma Chemicals* (St. Louis, MO, USA). Pharmalytes (pH 3–10) were obtained from *Pharmacia Chemicals* (Uppsala, Sweden). Yeast extract and peptone were obtained from *Difco Industries* (Detroit, MI, USA). All other chemicals used were of analytical grade.

Organism and growth conditions. Glucose concentration in the growth medium has an effect on the dimorphic behavior of *B. poitrasii* (Khale *et al.* 1990). Therefore, yeast extract–peptone (YP: yeast extract 0.3 %, Bactopeptone 0.5 %) media containing different concentrations of glucose used in the present studies are designated as YPG (% glucose). Stock cultures of *B. poitrasii* were maintained by subculturing weekly on slants of YPG (1 % glucose) containing 2 % agar. The stable yeast-form mutant (Y-5) was isolated after mutagenesis (using nitrosoguanidine) of spores of the parent strain (Khale *et al.* 1990). The reversion studies were also carried out as described earlier (Khale *et al.* 1990). The Y-5 mutant was maintained as parent strain on YPG slants (Khale and Deshpande 1992; Khale *et al.* 1990). The spores (5000/μL) were inoculated in YP and YPG (YP containing 0.5 % glucose) media under shaking conditions (3 Hz) at 28 °C for 1 d to obtain mycelial and yeast-form cells. The slow-growing Y-5 mutant was grown in YPG (0.5 % glucose) for 2 d at 28 °C.

Yeast–mycelium transition. The transition was studied in 200 mL YPG (0.1 % glucose) medium by inoculating yeast cells (50/nL) and incubating at 28 °C, under shaking conditions (3 Hz) for 1 d. Morphology and counting of the cells on a hemocytometer were carried out as described earlier (Khale *et al.* 1992). Single or budding cells were counted as one yeast morphological unit and cells with one or more germ tubes were counted as one hyphal morphological unit.

Mycelium–yeast transition. The transition was studied in YPG (0.5 % glucose) medium inoculated with mycelium-form cells (50/nL), and incubated with shaking at 28 °C for 30 h. The morphological differentiation was closely monitored throughout the shift. The mycelial strands were counted once, for each hemocytometer square in which they appeared. During transition to the yeast-form cells, the number of mycelial cells remaining was expressed as a percentage of the total cell count (Khale *et al.* 1992).

Isolation of different cell fractions for localization studies. The mycelial and yeast-form cells grown for 1 d in respective media were separated by centrifugation (2 000 ***g***, 5 min) and the supernatant was used to estimate extracellular chitinolytic enzyme activities. All further operations were carried out at 4 °C, unless otherwise stated. The cells (2.5 g, wet mass) were homogenized in 10 mL 50 mmol/L sodium acetate buffer at pH 5.0, using a cell homogenizer (Braun) with glass beads (Khale *et al.* 1992). The homogenate was centrifuged (2 000 ***g***, 5 min) to remove the cell walls. The cell-wall pellet was suspended in 5 mL of the same buffer and used for the estimation of enzyme activities. The cell-wall-free homogenate was centrifuged at 100 000 ***g*** for 40 min to separate the mixed membrane fraction and cytosol. The membrane pellet was resuspended in 50 mmol/L sodium acetate buffer at pH 5.0 and homogeneous suspension was used for enzyme assays unless otherwise mentioned (Deshpande *et al.* 1997). However, for solubilization, the mixed membrane fraction was resuspended in 1 % digitonin in the same buffer, incubated at 0 °C for 1½ h and centrifuged (160 000 ***g***, 1 h) and the supernatant was used for the estimation of enzyme activities.

Chitinase and N-acetylglucosaminidase assays. Acid swollen-chitin (ASC) for total chitinase activity was prepared using phosphoric acid (Vyas and Deshpande 1989). The 0.3 mL of 1 % (*W*/*V*) ASC or EGC (for endochitinase) was incubated with 0.2 mL of enzyme preparation at 37 °C for 1 h and the hexosamine liberated was estimated according to Reissing *et al.* (1955). One unit of specific enzyme activity is 1 nkat/mg (1 nmol *N*-acetylglucosamine per mg protein per s).

The endochitinase and *N*-acetylglucosaminidase assays were performed using fluorogenic glycosides, 4-MU-(GlcNAc)$_{1 \text{ and } 3}$ prepared in 50 % (*V*/*V*) ethanol. The activities measured on 4-MU-(GlcNAc)$_3$ and on 4-MU(GlcNAc) were designated as endochitinase and *N*-acetylglucosaminidase, respectively (Jackson *et al.* 1996). Twenty μL of 700 μmol/L substrate was incubated with 50 μL of suspended mixed membrane fraction (MMF) in 130 μL of 50 mmol/L sodium acetate buffer at pH 5.0 or 5.5 for mycelium or yeast cell preparations, respectively, at 37 °C for ½ h. The reaction was then stopped by adding 2.3 mL 0.2 mol/L Na_2CO_3. The fluorescence was measured in a Perkin-Elmer fluorescence spectrophotometer, using excitation at 360 nm and emission at 445 nm. One unit of specific enzyme activity is 1 nat/mL (1 nmol of 4-methylumbelliferone per mg protein per min).

N-Acetylglucosaminidase activity was also estimated by incubating 0.3 mL of 4 % (*W/V*) pNP-(GlcNAc) with 0.2 mL of MMF at 37 °C for 1 h. The reaction was terminated by adding 2 mL of 0.2 mol/L Na_2CO_3. The 4-nitrophenol liberated was measured spectrophotometrically at 405 nm. One unit of specific enzyme activity was defined as 1 nmol of 4-nitrophenol per mg protein per min.

In situ chitinase activity in the whole cell was estimated according to O'Donnell (1991). The reaction mixture contained 5 mg (wet mass) of whole cells suspended in 300 µL 50 mmol/L sodium acetate buffer at pH 5.0 and 20 µL of 700 µmol/L fluorogenic substrate. After incubation at 37 °C for ½ h the reaction was terminated by the addition of 2.2 mL 0.2 mol/L Na_2CO_3. After centrifugation (2000 ***g***, 10 min), the supernatant was collected and the fluorescence was measured (*see above*). One unit of enzyme activity was defined as 1 nmol of 4-methylumbelliferone per mg wet mass of whole cells per min.

Isoelectrofocussing. The membrane pellet was suspended in 50 mmol/L sodium acetate buffer at pH 5.0 and the fraction of MMF free of particulate matter was used for isoelectrofocussing which was carried out in a mini-scale IEF unit using Pharmalytes in the pH range of 3–10 for rapid (8–10 h) separation as described by Sathivel *et al.* (1995). The fractions were collected and the pH and activity on 4-MU-(GlcNAc) and 4-MU-$(GlcNAc)_3$ were estimated.

Protein was estimated according to Lowry method using bovine serum albumin as standard.

All the experiments were carried out three times in triplicate unless otherwise mentioned.

RESULTS

Localization of chitinolytic activities

To find out the biochemical correlation between morphology and chitinolytic enzyme activity, different fractions, *viz.* extracellular, cell-wall-bound, mixed membrane-fraction and cytosol, were isolated and used for estimation of enzyme activity (*see above*). The extracellular endochitinase and *N*-acetylglucosaminidase activity was not detected in the respective supernatants. The chitinolytic activity was found to be present in all three fractions, *viz.* cell wall, mixed membrane and cytosol (Table I). The MMF was the fraction separated from the cytosol and resuspended in the buffer and used as homogeneous suspension for activity determination. The *N*-acetylglucosaminidase activity in cell wall, MMF and cytosol were 42, 37 and 21 % for mycelial cells and 46, 31 and 23 % for yeast cells, respectively. The distribution of endochitinase activities of mycelial cells was 53, 24 and 23 % in cell wall, mixed membrane and cytosolic fraction, respectively, while for yeast-form cells it was 39–41 and 20 %, respectively. For the Y-5 mutant, however, the distribution of chitinolytic enzyme activities was different from the parent strain. The relatively higher *N*-acetylglucosaminidase and endochitinase activity (54 and 62 %, respectively) was observed in the MMF as compared to the parent strain. The cell wall fraction contained 28–30 % both activities. As these activities were found to be distributed in all three fractions, to understand their relationship with the morphological outcome, the differences in the specific activity of both cell forms were studied. *N*-Acetylglucosaminidase activities in the MMF of mycelial-form cells were 18-fold higher than the yeast-form cells while differences for activities in the other two fractions were 2–5-fold. Similarly, endochitinase activity ranges over 3-fold differences in the MMF of yeast and mycelial cells. Surprisingly, the mutant showed lower *N*-acetylglucosaminidase activity as compared to the parent yeast while the endochitinase was 2.5 times higher and comparable to the mycelial-form cells. In view of the significant differences in the membrane-bound activities of the two forms, further experiments were carried out using MMF.

After digitonin solubilization, the total *N*-acetylglucosaminidase activity of membrane fraction increased by 12 % (1.22 ± 0.25 nkat/mg protein) and 30 % (16.3 ± 3.33 nkat/mg protein) in the case of yeast and mycelial cells, respectively, as compared to their respective controls (Table I). However, the endochitinase activity was found to be decreased by 45 % (1.23 ± 0.17 nkat/mg protein) for the yeast fraction and 65 % (4.72 ± 0.83 nkat/mg protein) for the mycelium fraction. For the Y-5 mutant, digitonin treatment affected chitinolytic activities marginally (a 2 % increase in *N*-acetylglucosaminidase activity and a 9 % decrease in the endochitinase activity as compared with the control). The treatment with Triton X-100 (1 %) also revealed similar changes in activity (*data not shown*).

Table I. Localization and specific activity of different fractions of chitinolytic enzymes in *B. poitrasii*[a]

	Total activity, nkat[b]			Specific activity, nkat/mg[c]		
	mycelium	yeast	Y-5 mutant	mycelium	yeast	Y-5 mutant
			N-Acetylglucosaminidase			
Cell wall fraction	158 ± 23.8 (42)	43.6 ± 11.9 (46)	7.4 ± 1.21 (29)	12.2 ± 1.83	4.35 ± 1.18	0.52 ± 0.08
MMF	141 ± 20.8 (37)	28.9 ± 10.4 (31)	14.0 ± 3.02 (54)	12.7 ± 2.42	1.08 ± 0.20	0.63 ± 0.13
Digitonin-solubilized MMF	–	–	–	16.3 ± 3.33	1.22 ± 0.25	0.65 ± 0.18
Cytosol	79.4 ± 22.3 (21)	21.3 ± 2.21 (23)	4.42 ± 1.14 (17)	7.12 ± 2.00	0.80 ± 0.083	0.20 ± 0.050
			Endochitinase			
Cell wall fraction	180 ± 25.9 (53)	69.0 ± 25.1 (39)	74.2 ± 10.8 (30)	13.9 ± 2.00	6.88 ± 2.50	5.18 ± 0.75
MMF	80.9 ± 9.2 (24)	73.2 ± 15.5 (41)	156 ± 22.4 (62)	7.27 ± 0.83	2.75 ± 0.58	7.05 ± 1.02
Digitonin-solubilized MMF	–	–	–	4.72 ± 0.83	1.23 ± 0.17	6.42 ± 0.87
Cytosol	77.1 ± 9.6 (23)	34.7 ± 4.4 (20)	20.3 ± 2.9 (8.2)	6.92 ± 0.87	1.30 ± 0.17	0.92 ± 0.13

[a]The percentage activities are given in *parentheses*. Mycelium and yeast cells were grown in YP and YPG (0.5 % glucose), respectively for 1 d at 28 °C. The Y-5 mutant was grown in YPG (0.5 % glucose) for 2 d at 28 °C.
[b]nmol of 4-methylumbelliferone per s of 1 g wet mass of cells; means ± SD of 2 sets of duplicate experiments.
[c]Means ± SD of 3 sets of duplicate experiments.

Chitinolytic activities of suspended membrane fractions of mycelium and yeast on various substrates were also measured. The total chitinase activity measured on ASC (10.9 ± 0.67 and 1.00 ± 0.67 nkat/mg protein), EGC (9.08 ± 0.33 and 0.78 ± 0.17, and pNP-(GlcNAc) (82.0 ± 1.7 and 14.0 ± 0.67) were higher (6–10 times) in the mycelium membrane preparation than the yeast one.

The optimum temperature for the activities on fluorogenic substrates was 37 °C for all the 3 MMF. However, pH optima for endochitinase and *N*-acetylglucosaminidase activity of mycelium and yeast-form preparations (both parent and mutant) were 5.0 and 5.5, respectively.

Yeast–mycelium transition. During the yeast–mycelium transition, the *N*-acetylglucosaminidase activity of the membrane preparation increased steadily throughout the transition (Fig. 1). The activity (0.83 ± 0.17 nkat/mg protein) in the yeast cells was increased in 1-d-old mycelial cells 17-fold (14.2 ± 1.70). The activity at 50 % germ-tube formation (12 h) was 6 times higher (1.7 ± 0.83) than at time zero. The endochitinase activity did not show any increase in the first 6 h but increased steeply (12-fold) between 6 h (0.83 ± 0.17) and 12 h (9.83 ± 0.83) and thereafter practically remained unchanged up to 1 d.

Mycelium–yeast transition. A reverse trend in the specific activity of chitinolytic enzymes of membrane fractions was observed during mycelium–yeast transition. As compared with the yeast–mycelium transition, this process was slow. For a complete mycelium–yeast transition, the time period required was 1¼ d.

Isoelectrofocussing. The separation of chitinolytic enzyme activities of the soluble portion of the membrane fractions was carried out by isoelectrofocussing. Endochitinases and *N*-acetylglucosaminidases were resolved into two pH ranges, *viz.* 4.3–5.7 and 6.1–7.7 (6.1–6.5 and 6.7–7.7), respectively (Fig. 2). The endochitinases of mycelial and yeast-like cells (both parent and Y-5) showed

a similar pattern of resolution. In contrast, for the *N*-acetylglucosaminidase activity a distinct yeast-form specific peak was noted at pH 6.3–6.4 in the parent, as well as in the mutant. Another peak was observed between 7.3 and 7.4 for the yeast-form cells. While the mycelial fraction appeared at pH 7.6. It was interesting to note that the predominant *N*-acetylglucosaminidase activity observed at pH 6.9 for the yeast and 7.1 for the mycelium fraction of the parent strain could not be detected in the MMF of the Y-5 mutant.

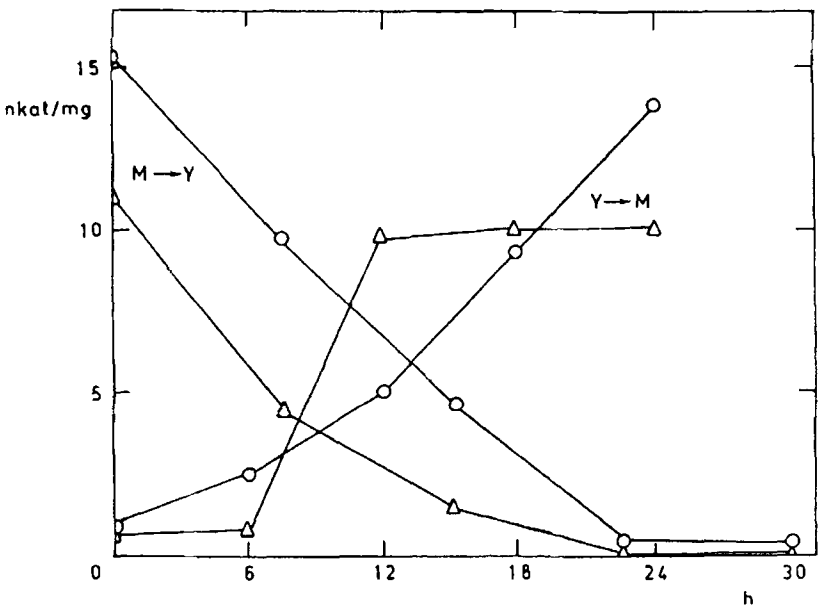

Fig. 1. Changes in the chitinolytic activities (nkat/mg) of soluble mixed membrane fraction during yeast–mycelium (Y→M) and mycelium–yeast (M→Y) transition in *B. poitrasii*; *circles* — *N*-acetylglucosaminidase, *triangles* — endochitinase.

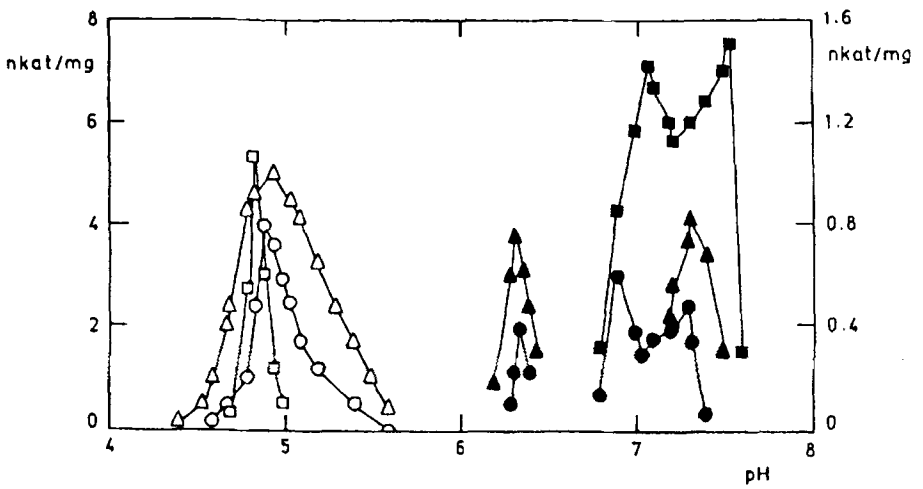

Fig. 2. Isoelectrofocussing of particulate-fraction-free mixed membrane fractions of *B. poitrasii* mycelium (*squares*), yeast (*circles*) and Y-5 mutant (*triangles*) cell (nkat/mg total membrane fraction protein); *open symbols* — endochitinase (*left axis*), solid symbols — *N*-acetylglucosaminidase (*right axis*).

Effect of β-glycosidase inhibitor on whole-cell chitinolytic activities and yeast–mycelium transition

Glucono-1,5-lactone (a known inhibitor of β-glycosidases) was used to study its effect on the intracellular endochitinase and *N*-acetylglucosaminidase activity and subsequently on the yeast–mycelium transition. The enzyme activities were estimated by permeabilizing whole cells with 50 % (*V*/*V*) (O'Donnell 1991). The presence of increasing concentrations of gluconolactone (2.5–10 mg/mL) during the yeast–mycelium transition increasingly inhibited enzyme activities and retarded germ-tube formation. At 2.5 mg/mL, the *N*-acetylglucosaminidase activity measured with 4-MU-(GlcNAc) was inhibited by 65 % (*i.e.*, 1.47 ± 0.17 and 4.35 ± 0.83 nmol/mg wet mass in presence and absence of gluconolactone, respectively). The endochitinase activity assayed with 4-MU-$(GlcNAc)_3$ was inhibited by 45 % as compared with the control (3.52 ± 0.17 nkat/mg wet mass and 6.48 ± 1.33 nkat/mg wet mass, respectively). In the presence of 2.5 mg gluconolactone per mL a 60 % inhibition in germ-tube formation was observed relative to the control (22 ± 4 % in the presence of gluconolactone, as compared with the absence of the inhibitor, *i.e.* 55 ± 8 % germ-tube formation) during the initial ½ d of transition. When the concentration of gluconolactone was further increased to 10 mg/mL, the *N*-acetylglucosaminidase activity was found to be inhibited by more than 80 % (0.73 ± 0.067 nkat/mg wet mass) while endochitinase was inhibited by 65 % (2.18 ± 0.20 nkat/mg wet mass) and the yeast–mycelium transition was not detected. There was no effect of gluconolactone on the growth of yeast by budding.

DISCUSSION

The chitinolytic enzymes present as cell-wall-bound, microsomal or cytosolic significantly influence the morphogenetic event in fungi (Adams *et al.* 1993). In *Saccharomyces cerevisiae* it has been observed that most of the chitinase was secreted into the growth medium. However, in *B. poitrasii*, detectable levels of extracellular chitinolytic activity was not observed. The intracellular distribution of these activities was found to be significantly different. The chitinolytic activity bound (either tightly or loosely) to the membranes isolated following identical protocols indeed exhibited a morphology-related difference in the levels (Table I). In addition, reports available in the literature suggested that membrane-bound chitinases significantly contributed in the morphogenetic event in fungi (Adams *et al.* 1993; Humphreys and Gooday 1984*a*,*b*); therefore, membrane-bound chitinase levels were measured during transition in *B. poitrasii*.

The microsomal chitinases of *M. mucedo* were reported to be solubilized most effectively by Triton X-100 (Humphreys and Gooday 1984*b*). In *B. poitrasii*, the treatment of the MMF with Triton X-100 also increased the *N*-acetylglucosaminidase activity. Furthermore, after digitonin solubilization, the total *N*-acetylglucosaminidase activities of the membrane fraction increased by 12 and 30 % in the case of yeast and mycelial cells (Table I). However, the endochitinase activity was found to be decreased by 45–65 % for yeast-like and mycelial fractions. This can be attributed to the possible requirement of lipophilic environment for the endochitinases in *B. poitrasii* as observed in *M. mucedo* by Humphreys and Gooday (1984*b*).

During the early stages of yeast–mycelium transition in *B. poitrasii*, a sudden increase in the endochitinase activity was observed (Fig. 1). This initial increase can be correlated with its hydrolytic role in weakening cell walls for germ-tube formation. In *C. albicans* germ-tube formation was reported to be accompanied by an increase in *N*-acetylglucosaminidase activity (Sullivan *et al.* 1984). Rast *et al.* (1991) suggested that *N*-acetylglucosaminidase activity may participate in chitin synthesis by supplying *N*-acetylglucosamine for wall synthesis. The increase in the *N*-acetylglucosaminidase activity at a later stage may, therefore, be important in mycelial proliferation of *B. poitrasii*. During mycelium–yeast transition (Fig. 1) the trend of the specific activities was reverse to the trend observed for yeast–mycelium transition. The high quantities of chitinolytic enzymes of mycelial cells appeared to be decreased. There was no detectable extracellular secretion of chitinolytic enzymes during transition. The decrease in the specific activities could be attributed to either proteolysis of the enzymes that were not required at the given point of time or the inhibition by specific inhibitors, if any (Deshpande 1992).

Sullivan *et al.* (1984) reported that in *C. albicans*, *N*-acetylglucosamine and some of its related derivatives favored germ-tube formation and induced *N*-acetylglucosaminidase activity. The enzyme activity *in situ* is in a privileged state, protected especially from inhibitors and pH changes (Gooday *et al.* 1992). Therefore, the effect of chitinase inducer like GlcNAc and the inhibitor, gluconolactone, was studied in whole cells. The *in situ* measurements of enzyme activities showed that in *B. poitrasii* GlcNAc enhanced the germ-tube formation and increased *N*-acetylglucosaminidase activity (*data not presented*). On the other hand, glucono-1,5-lactone, a powerful competitive inhibitor of glycosidases (Reese and Maguire 1971), drastically reduced germ-tube formation. This can be correlated with the inhibition of *N*-acetylglucosaminidase activity.

Isoelectrofocussing of the soluble portion of membrane fractions of mycelium, yeast and of mutant cells repeatedly exhibited distinct profiles for endochitinases in the pH range of 4.3–5.7 and for *N*-acetylglucosaminidases in the pH ranges 6.1–6.5 and 6.7–7.7. The endochitinases of mycelium- and yeast-like cells (both parent and Y-5) showed a similar pattern of resolution. In contrast, a distinct yeast-form-specific peak for the *N*-acetylglucosaminidase activity was noted at pH 6.3–6.4 in the parent as well as in the mutant (Fig. 2). The predominant *N*-acetylglucosaminidase activity observed at pH 6.9 for the yeast and 7.1 for the mycelium fraction of parent strain could not be detected in the mixed membrane preparation of Y-5 mutant. Further purification and characterization of *N*-acetylglucosaminidases of the parent-strain membrane fraction may be useful to understand their definite role in the morphological transition in *B. poitrasii*.

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