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Effect of phosphate deficiency on growth and protein profile in three strains of *Pholiota nameko*

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Changes in mycelial dry weight and soluble protein amounts and acid phosphatase activities on a mycelial dry weight basis in the mycelia and culture supernatants during the Pi-supplied (P^+) and Pi-depleted (P^-) cultures of three strains of *Pholiota nameko* were examined. Mycelial dry weights of the three strains were lower in the P^- culture than in the P^+ culture. However, soluble protein amounts in the culture supernatants and acid phosphatase activities in the mycelia and culture supernatants of the three strains were higher on a mycelial dry weight basis in the P^- culture than in the P^+ culture. Total proteins of strains N2 and N4 were analyzed by two-dimensional-PAGE. Comparison of electrophoretograms of the P^+ and P^- cultures showed that many polypeptides in the two strains were induced and secreted by Pi deficiency, but more than half of them were specific to each strain. Activity staining of acid phosphatase also revealed that two isozymes with the same molecular weights in the three strains were induced and secreted by Pi deficiency. Adaptive mechanisms for Pi deficiency in the three strains were discussed.

Key Words——acid phosphatase; *Pholiota nameko*; phosphate deficiency; protein synthesis; two-dimensional-PAGE.

Phosphorus (P) is an essential nutrient for all organisms. However, plants and microorganisms can directly absorb only inorganic phosphate (Pi). Therefore, Pi is often a limiting element for their growth. To determine the effects of Pi deficiency on organisms, quantitative changes of various cellular constituents have been examined, and the amounts of lipids (Fife et al., 1990), organic acids (Li et al., 1997), nucleotides (Johnson et al., 1996; Ueki and Sato, 1971) and proteins (Bostian et al., 1983; Fife et al., 1990; Goldstein et al., 1989; Nagano and Ashihara, 1993; Theodorou et al., 1991) have been found to be affected. These changes are considered to be metabolic changes that allow the organisms to adapt to Pi deficiency. They have been called the pho regulon in bacterium (Torriani and Ludtke, 1985) and yeast (Yoshida et al., 1987) and the psi rescue system in plant (Goldstein et al., 1988).

The best-known enzyme involved in such metabolic changes is a phosphatase, which plays important roles in the recycling of P in the cell and the acquisition of P from the external environment, and the intracellular and extracellular activities are increased by Pi deficiency in many plants (Goldstein et al., 1988; Lefebvre et al., 1990; Tadano and Sakai, 1991) and microorganisms (Bostian et al., 1983; Elliot et al., 1986; Phongdara et al., 1998). A ribonuclease has been also reported to be induced by Pi deficiency and directly involves in phosphate metabolism (Loffler et al., 1992). In addition, a phosphoenolpyruvate carboxylase (Duff et al., 1989; Johnson et al., 1996), a glucose dehydrogenase (Gyaneshwar et al., 1999) and an ADP-glucose pyrophosphorylase (Ball et al., 1990) are known to be induced by Pi deficiency. However, little is known about the functions of other proteins induced by Pi deficiency.

We previously reported that under Pi deficiency, mycelium of strain N114 of *Pholiota nameko* (T. Ito) S. Ito et Imai in Imai grow well, the intracellular and extracellular activities of acid phosphatase greatly increase, and a number of soluble proteins are specifically synthesized *de novo* in the mycelium (Joh et al., 1996a, 1996b, 1998). The results suggest that the mycelium of *P. nameko* strain N114 has an excellent adaptive mechanism for Pi deficiency and changes its metabolism dramatically when Pi is deficient. However, it is unclear whether other strains of *P. nameko* also have the same adaptive mechanism.

In the present study, we examined the effect of Pi deficiency on growth, acid phosphatase activity and protein profile in three strains of *P. nameko* and revealed that there are differences in their abilities to adapt to Pi deficiency.

Materials and Methods

Strains and culture conditions Three strains, N2, N4 and N301, of *P. nameko* (Onuki Kinjin, Utsunomiya, Japan) were used in the study. The mycelia were subcultured for 2 wk in casamino acids-glucose medium, the Pi-supplied (P^+) medium. The composition of the medium was: glucose, 20.0 g; vitamin assay casamino acids (Difco Laboratories, Detroit, MI, USA), 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; KCl, 0.27 g; $CaCl_2$, 0.1 g;

$FeSO_4 \cdot 7H_2O$, 10 mg; thiamine HCl, 10 mg; $ZnSO_4 \cdot 7H_2O$, 3.0 mg; $MnSO_4 \cdot 5H_2O$, 3.0 mg; $CuSO_4 \cdot 5H_2O$, 1.0 mg; and $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$, 1.0 mg; in 1 liter of distilled water. The Pi-depleted (P^-) medium was the same as the P^+ medium except for the omission of KH_2PO_4. The pH of both media was adjusted to 6.5 with HCl. The P^+ and P^- media contained phosphate at the concentrations of 550 and 50 mg/L as KH_2PO_4, respectively. The mycelia were inoculated into 30 ml of the P^+ or P^- medium in a 200-ml Erlenmeyer flask and grown at 25°C in darkness.

Measurement of dry weight of mycelia The mycelia were collected by centrifugation at 8,000×*g* for 30 min at 4°C and washed three times with about 30 ml of distilled water, and after drying for 12 h at 90°C, the weight was measured.

Preparation of soluble protein The mycelia were collected by centrifugation at 8,000×*g* for 30 min at 4°C and washed three times with 10 mM acetate buffer (pH 5.5). The four supernatants were combined and used as culture soluble protein. After washing, the mycelia were homogenized in the same buffer with a polytron homogenizer (Model K; Kinematica, Luzern, Switzerland) at about 13,000 rpm for 2 min at 4°C. The homogenate was centrifuged at 10,000×*g* for 30 min at 4°C, and the supernatant was used as mycelial soluble protein.

Protein and enzyme assays Protein concentration was determined by the method of Bradford (1976) using Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as standard. Acid phosphatase activity was assayed in 1.5 ml of a reaction mixture containing 0.1 M sodium acetate buffer (pH 5.5), 15 mM *p*-nitrophenylphosphate (Nacalai Tesque, Kyoto, Japan) and the enzyme at 37°C for 10 min as described previously (Yazaki et al., 1998). The absorbance at 400 nm was measured to determine the amount of librated *p*-nitrophenol. One unit of the enzyme activity was defined as the amount of enzyme releasing 1 μmole of *p*-nitrophenol per min under the assay conditions.

Preparation of protein for electrophoresis Soluble proteins from the mycelia and the culture supernatants at 20 d of cultivation were prepared as mentioned above. The soluble protein solution was mixed with an equal volume of 0.125 M 2-amino-2-hydroxymethyl-1,3-propanediol (Tris)-HCl (pH 6.8), 5% (w/v) sodium dodecyl sulphate (SDS), 2% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, heated at 100°C for 3 min, and then analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Total protein was prepared from the mycelia and from the culture supernatants at 20 d of cultivation according to the phenol extraction method (Hurkman and Tanaka, 1986). After washing three times with 10 mM acetate buffer (pH 5.5), the mycelium was homogenized in extraction buffer containing 0.7 M sucrose, 0.5 M Tris, 30 mM HCl, 0.1 M KCl and 2% (v/v) β-mercaptoethanol. The homogenate was incubated for 10 min at 4°C and centrifuged at 10,000×*g* for 30 min at 4°C, and the supernatant was mixed with an equal volume of water-saturated phenol. After shaking for 10 min at room temperature, the mixture was centrifuged at 10,000×*g* for 10 min at 4°C. The phenol phase was taken and 5 volumes of 0.1 M ammonium acetate in methanol was added. Protein was allowed to precipitate at −20°C overnight. After centrifugation for 30 min at 10,000×*g*, the precipitate was washed three times with 0.1 M ammonium acetate in methanol and once with acetone. The pellet was dried under a vacuum for about 15 min and resuspended in an adequate volume of lysis buffer containing 8 M urea, 1% (v/v) Nonidet P-40, 3% (v/v) β-mercaptoethanol and 0.5% (v/v) Ampholine pH 3.5–10 (Amersham Pharmacia Biotech, Buckinghamshire, England). The suspension was centrifuged at 15,000×*g* for 3 min, and the supernatant was used for two-dimensional (2D)-PAGE. The protein from the culture supernatant for 2D-PAGE was prepared by the same method as the mycelial total protein, after mixing the culture supernatant with an equal volume of water-saturated phenol and shaking for 10 min at room temperature.

Gel electrophoresis SDS-PAGE was carried out according to Laemmli's method (1970) in a 12% (w/v) polyacrylamide gel with 0.1% (w/v) SDS. Gel was run at a constant current of 30 mA for 3 h at 4°C.

2D-PAGE was carried out according to the manufacturer's protocol for the IPGphor Isoelectric Focusing System (Amersham Pharmacia Biotech). First-dimension isoelectric focusing (IEF) was performed by use of IPGphor and Immobline DryStrip IPG gel (Amersham Pharmacia Biotech). After the IPG strip had been rehydrated with the lysis buffer containing protein for 15 h at 20°C, IEF was conducted first at 500 V for 1 h, then at 1,000 V for 1 h and finally at 8,000 V for 8 h at 20°C. Subsequently, the IPG strip was equilibrated successively with equilibration buffer A containing 50 mM Tris-HCl (pH 6.8), 6 M urea, 30% (v/v) glycerol, 1% (w/v) SDS and 0.5% (v/v) β-mercaptoethanol and equilibration buffer B containing 50 mM Tris-HCl (pH 6.8), 6 M urea, 30% (v/v) glycerol, 1% (w/v) SDS and 4.5% (w/v) iodoacetamide for 10 min each. The equilibrated IPG strip was transferred onto second-dimension SDS-polyacrylamide gel and fixed with agar. The gel was run at a constant current of 30 mA for 3 h at 4°C.

Gel staining Proteins were visualized by silver staining as described by Oakley et al. (1980). Acid phosphatase activity was detected by the phosphatase activity staining method of Lacks and Springhorn (1980).

Computer analysis of 2D gels Silver-stained 2D electrophoresis gels were scanned with a Color Image Scanner (EPSON GT-7600U, Nagano, Japan). Spot detection, matching, editing and quantification were performed with the 2-D software (pdi, New York, USA) according to the 2-D User's Guide.

Results

Effect of Pi deficiency on growth of the three strains Changes in mycelial dry weights of strains N2, N4 and N301 during the P^+ and P^- cultures were examined. In the P^+ cultures, the increase rate of mycelial dry weight in strain N301 was lower than those in strains N2 and N4

throughout the cultivation (Fig. 1). After 25 d, the dry weight of strain N2 decreased, while those of strains N4 and N301 continued to increase. The rate of increase of mycelial dry weight of strain N4 in the P^- culture was higher than those of strains N2 and N301, and the dry weight at 30 d was 1.4 and 1.5 times those of strains N2 and N301, respectively. In the P^- cultures, the rates of increase of mycelial dry weights of the three strains were lower than those in the P^+ cultures. However, there were differences in the extent of growth depression by Pi deficiency among three strains, and the difference in dry weight between the P^+ and P^- cultures at 25 d of cultivation was largest in strain N2 and smallest in strain N4.

Effect of Pi deficiency on synthesis and secretion of protein of the three strains Changes in soluble protein amounts on a mycelial dry weight basis in the mycelia and the culture supernatant during the P^+ and P^- cultures were examined. The mycelial soluble protein content of strain N2 in the P^+ culture remained almost constant throughout the cultivation (Fig. 2A). That of strain N4 was the highest at 7 d and decreased thereafter (Fig. 2B). That of strain N301 peaked at 10 d, decreased at 15 d, then slowly increased again (Fig. 2C). The mycelial soluble protein content of strain N2 in the P^- culture remarkably increased at 10 d, decreased up to 20 d, and remained constant thereafter (Fig. 2A). Those of strains N4 and N301 in the P^- culture were similar to those in the P^+ culture: the former decreased gradually throughout the cultivation and the latter was high temporarily at 10 d (Fig. 2B, C). Compared with the P^+ culture, the mycelial soluble protein content in the P^- culture was much higher throughout the cultivation only in strain N2; no pronounced difference was found in strains N4 and N301.

The soluble protein amount on a mycelial dry weight basis in the P^+ culture supernatants of strains N2 and N301 increased up to 10 d and then decreased gradually, while that of strain N4 was the highest at 7 d and thereafter decreased remarkably (Fig. 2D–F). The soluble protein amount of strains N2 and N301 in the P^- culture was maximal at 20 and 10 d, respectively, and then decreased (Fig. 2D, F). However, that of strain N4 decreased constantly from 7 d (Fig. 2E). Compared with the P^+ culture, the soluble protein amount in the P^- culture supernatant was higher in all strains after 7 d. However, the difference in the soluble protein amount between the P^+ and P^- cultures varied among the three strains, being largest in strain N2 and smallest in strain N4.

2D-PAGE analysis of mycelial proteins specifically synthesized under Pi deficiency The soluble proteins from the mycelia and the culture supernatants of the P^+ and P^- cultures at 20 d of cultivation were separated by SDS-PAGE and visualized by silver staining. Differences in band patterns between the P^+ and P^- cultures were observed in all the strains, and many polypeptides were changed quantitatively by Pi deficiency. However, the resolution of quantitative changes of many polypeptides by SDS-PAGE is limited. To obtain higher resolution, total mycelial protein at 20 d of cultivation was separated by 2D-PAGE. The proteins from two strains, N2 and N4, cultured in P^+ and P^- media for 20 d were first separated in the pI range of 3 to 10. Total mycelial protein was resolved into more than 600 spots (Fig. 3A–D), many of which clustered in the pI range of 5 to 8. Since this electrophoretogram alone was insufficient to analyze all spots, the proteins were further separated in the pI range of 4 to 7 (Fig. 3E–H). Mycelia of strains N2 and N4 cultured in the P^+ medium contained many common polypeptides (Fig. 3A, C, E, G), some of which differed greatly in relative abundance between the two strains. Specific polypeptides were also found in each strain. Comparison of the electrophoretograms of the P^+ and P^- cultures revealed that Pi deficiency changed the relative intensity of many spots. Because the aim of this study was to find specific proteins involved in the adaptive mechanism for Pi deficiency, we considered only polypeptides that were reproducible and relatively abundant in the P^- culture. In the mycelium of strain N2, 31 spots appeared only in the P^- culture, and 72 spots ap-

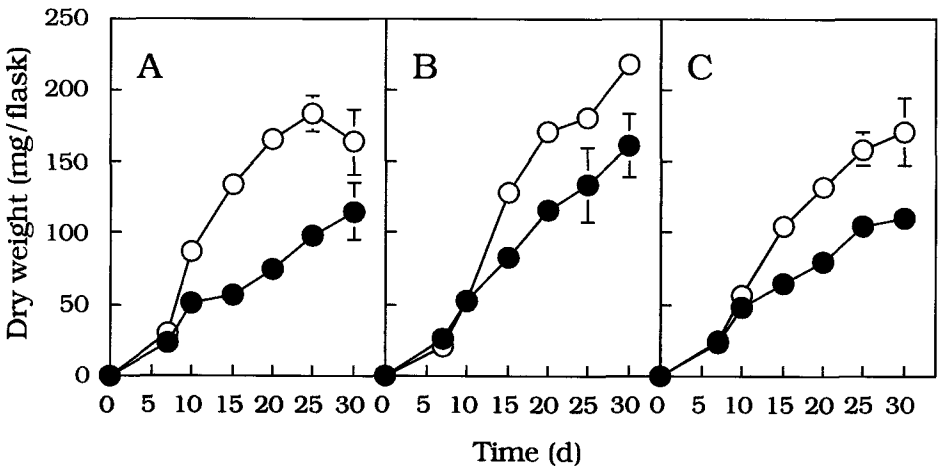

Fig. 1. Changes in mycelial dry weights of strains N2 (A), N4 (B) and N301 (C) during P^+ (○) and P^- (●) cultures. Data are means and SD of three replicates. SD bars are not shown when they are smaller than the symbols.

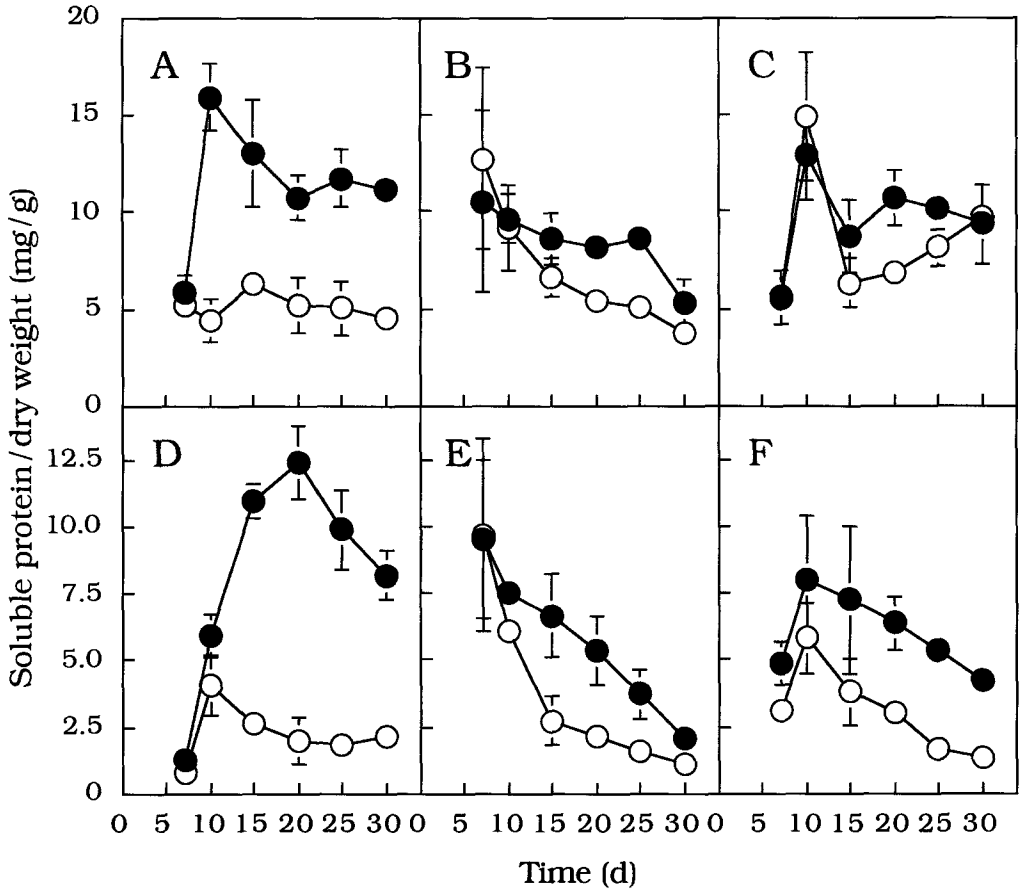

Fig. 2. Changes in amounts of soluble protein on a mycelial dry weight basis in mycelia (A–C) and culture supernatants (D–F) of strains N2 (A, D), N4 (B, E) and N301 (C, F) during P^+ (○) and P^- (●) cultures. Data are means and SD of three replicates. SD bars are not shown when they are smaller than the symbols.

pearing in both cultures had higher relative intensity in the P^- culture. In strain N4, the corresponding figures were 28 spots and 37 spots. Twenty spots were found that were common to both strains.

2D-PAGE analysis of proteins specifically secreted by Pi deficiency Total protein in the culture supernatant at 20 d of cultivation was also analyzed by 2D-PAGE. When the protein was separated in the pI range of 3 to 10, most of spots were detected between pH 4 and 7. Consequently, only the electrophoretograms analyzed in the pI range of 4 to 7 are shown in Fig. 4. Total proteins in the culture supernatants of the P^+ and P^- cultures were resolved into about 100 and 70 spots, respectively. The electrophoretic patterns of protein from strains N2 and N4 cultured in the P^+ medium showed large differences in the relative abundance of proteins secreted from mycelia of the two strains of the same *P. nameko*. Comparison of the electrophoretograms of the P^+ and P^- cultures revealed that Pi deficiency influences the kind and quantity of proteins secreted from the mycelia. In strain N2, 18 spots appeared only in the P^- culture and 5 spots appearing in both cultures showed higher relative intensity in the P^- culture. In strain N4, the corresponding figures were 17 spots and 10 spots. Eleven of these spots were common to both strains.

Effect of Pi deficiency on synthesis and secretion of acid phosphatase in the three strains Changes in acid phosphatase activity on a mycelial dry weight basis in the mycelia and the culture supernatant during the P^+ and P^- cultures were examined. As shown in Fig. 5A–C, the activity in the mycelia of the three strains cultured in the P^+ medium remained low throughout the cultivation, while the activity in the P^- medium increased remarkably after 15 d. The rate of increase was highest in strain N2, and its activity at 25 d was 2.2 and 2.9 times those of strains N4 and N301, respectively. The activities in the P^- culture of strains N2, N4 and N301 at 25 d were 32, 20 and 18 times those in the P^+ culture, respectively.

In the culture supernatants, the activity on a mycelial dry weight basis of the three strains cultured in the P^+ medium was very low throughout the cultivation, while that in the P^- medium remarkably increased after 7 d (Fig. 5D–F). The rate of increase was highest in strain N2, and its activity at 25 d was 4.2 and 3.2 times those of strains N4 and N301, respectively. Compared with the P^+ cultures, the activities in the P^- cultures of strains N2, N4 and N301 at 25 d were 1274, 360 and 260 times higher, respectively.

SDS-PAGE analysis of acid phosphatases induced and secreted by Pi deficiency Some acid phosphatases

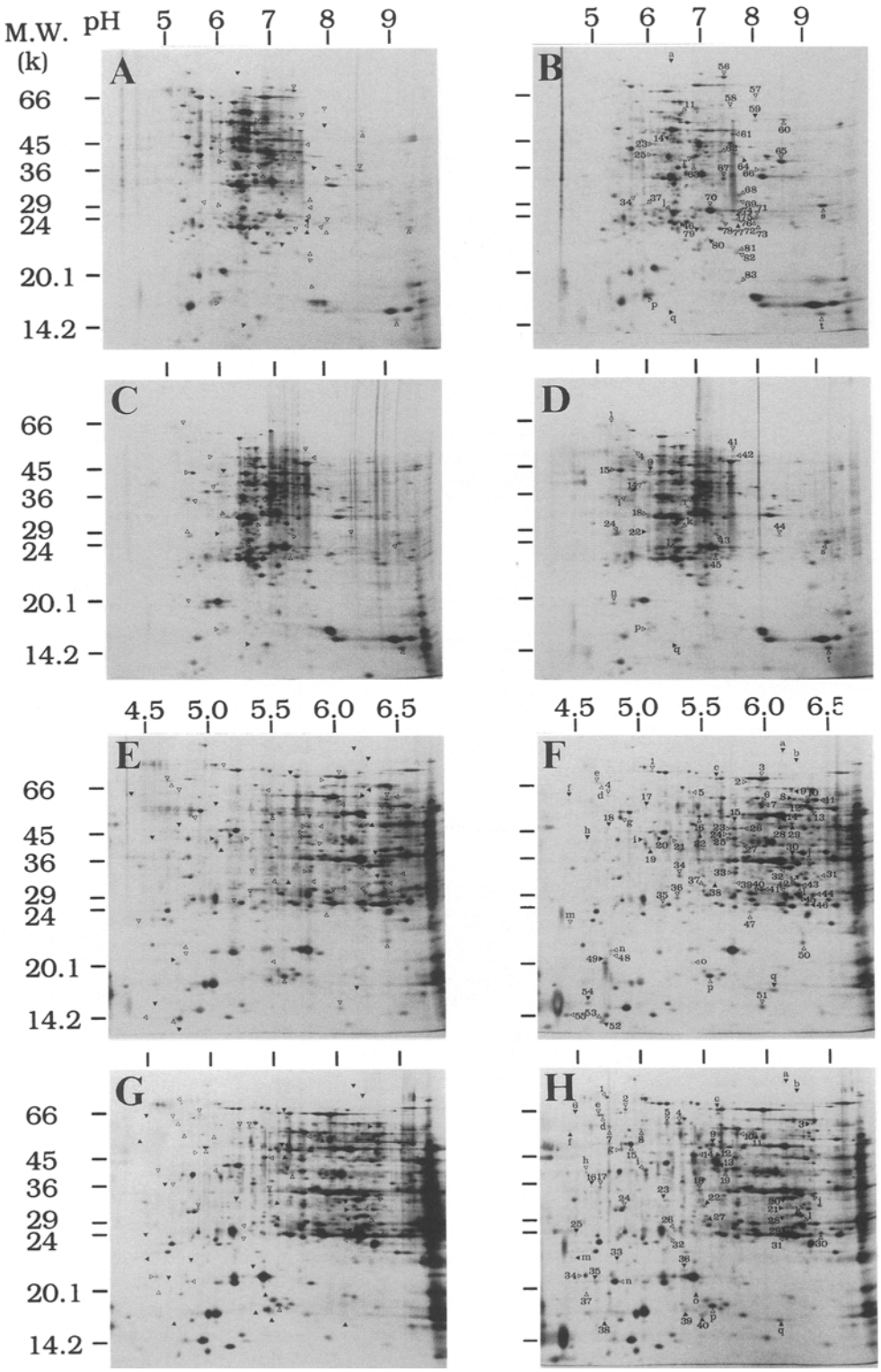

Fig. 3. 2D-PAGE analysis of total protein from mycelia of strains N2 (A, B, E, F) and N4 (C, D, G, H) cultured in P^+ (A, C, E, G) and P^- (B, D, F, H) media for 20 d. About 20 and 40 μg of total proteins were separated by 2D-PAGE with pH gradients of 3–10 (A–D) and 4–7 (E–H), respectively, and visualized by silver staining. Polypeptides that were increased in relative amount and induced by Pi deficiency are indicated by open and closed arrowheads, respectively. Polypeptides that were specific to one strain or common to both strains are indicated by numbers or letters, respectively. The molecular weights of marker proteins are indicated on the left.

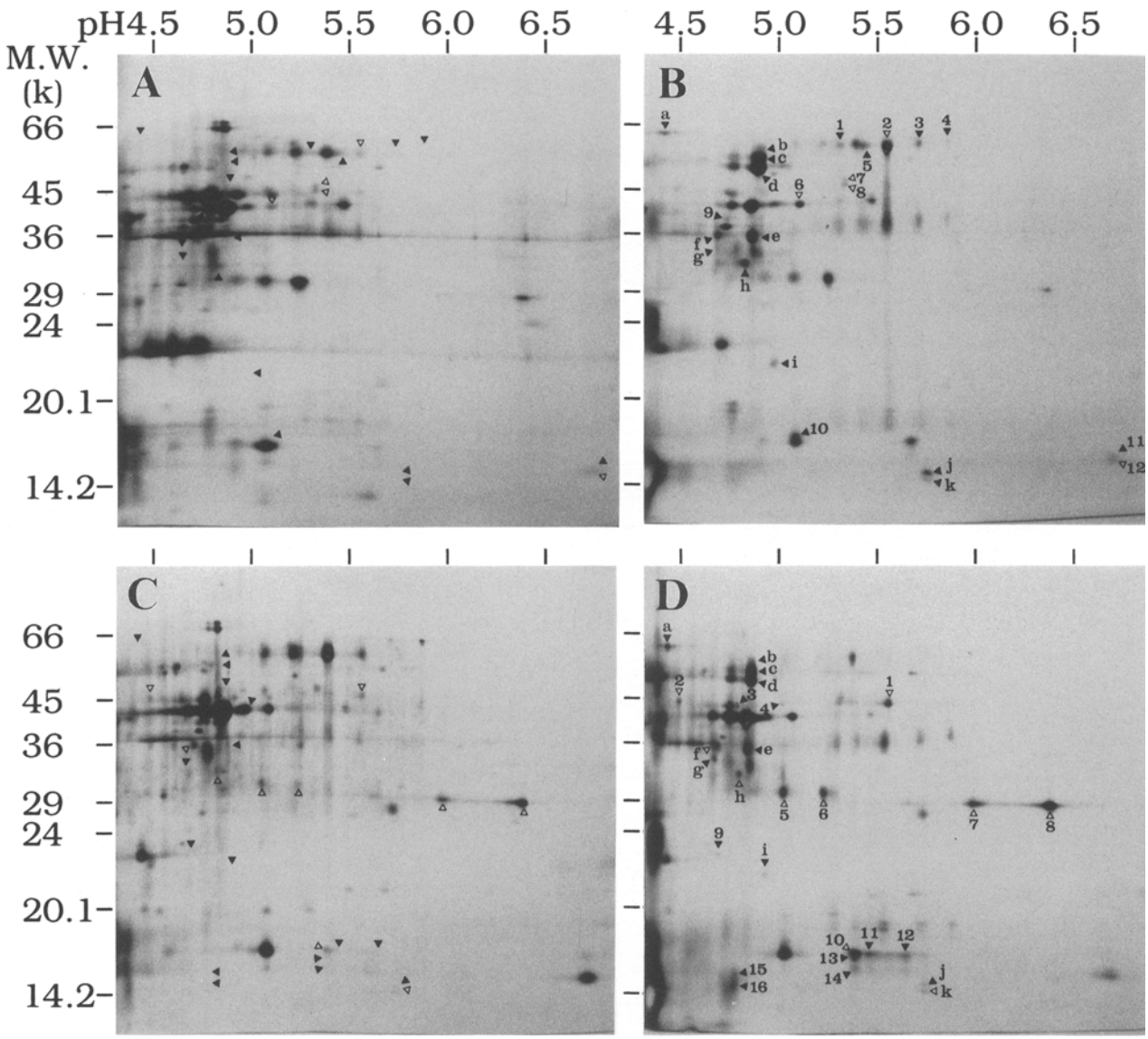

Fig. 4. 2D-PAGE analysis of total protein from culture supernatants of P^+ (A, C) and P^- (B, D) cultures of strains N2 (A, B) and N4 (C, D) for 20 d. About 20 μg of proteins were separated by 2D-PAGE with a pH gradient of pH 4–7 and visualized by silver staining. Polypeptides that were increased in relative amount and induced by Pi deficiency are indicated by open and closed arrowheads, respectively. Polypeptides that were specific to one strain or common to both strains are indicated by numbers or letters, respectively.

regain their activity upon removing SDS after SDS-PAGE. In mycelium of strain N114 of *P. nameko*, two of three acid phoshatase isozymes induced by Pi deficiency have been reported to be detectable by activity staining after SDS-PAGE (Yazaki et al., 1997). Therefore, the induction and secretion of acid phosphatase in the three strains by Pi deficiency were examined by this method. Electrophoretograms of soluble proteins and activity from the mycelia and the culture supernatants of the P^+ and P^- cultures at 20 d of cultivation are shown in Fig. 6. In the mycelia of the three strains, two active bands were detected only in the P^- culture at positions corresponding to molecular weights of 47,000 and 48,000 (Fig. 6A). However, no increased polypeptide band was found in the neighborhood of the active bands.

In the culture supernatants of the three strains, two active bands with molecular weights of 47,000 and 48,000 were also detected only in the P^- culture (Fig. 6B). Two polypeptide bands were found at the same positions as the active bands, and their densities agreed relatively well with the concentrations of the active bands of the three strains.

Discussion

To investigate differences in the adaptive abilities of three strains of *P. nameko* to Pi deficiency, we analyzed their mycelial growth, acid phosphatase activity, and

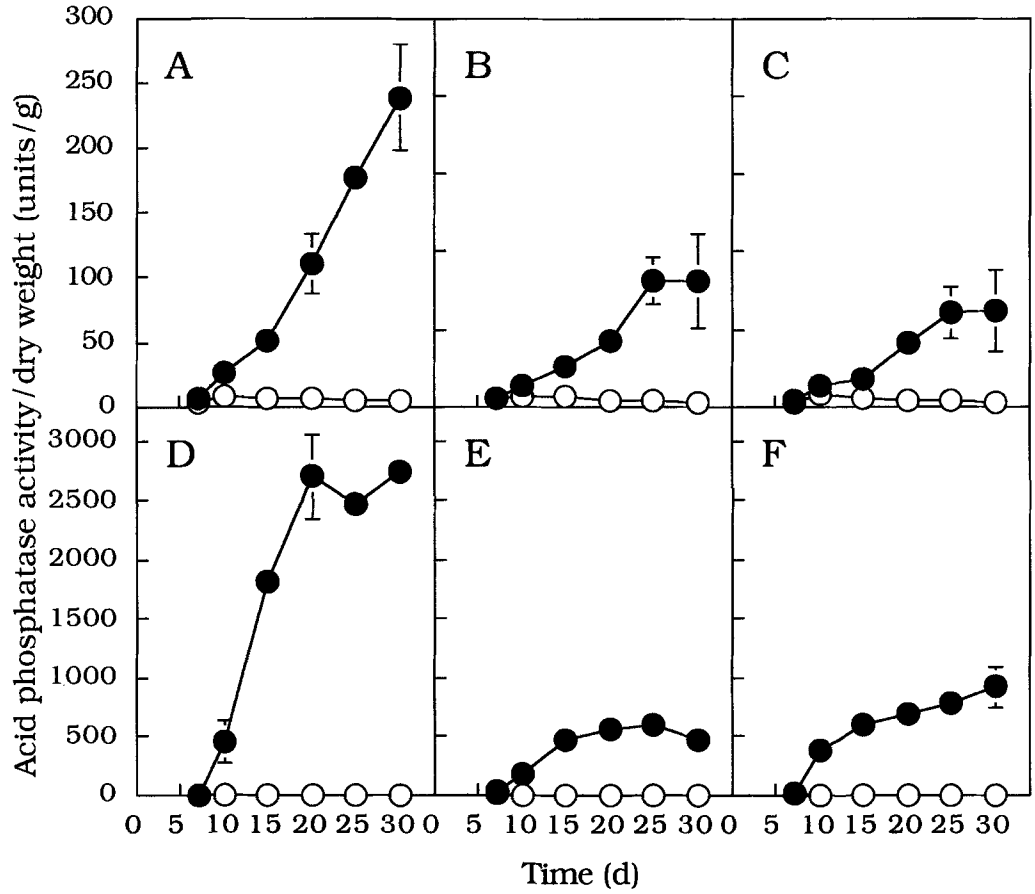

Fig. 5. Changes in activity of acid phosphatase on a mycelial dry weight basis in mycelia (A–C) and culture supernatants (D–F) of strains N2 (A, D), N4 (B, E) and N301 (C, F) during P^+ (○) and P^- (●) cultures. Date are means and SD of three replicates. SD bars are not shown when they are smaller than the symbols.

protein.

The mycelial growth of the three strains was depressed by Pi deficiency (Fig. 1). We have obtained the same result in strain N114 of *P. nameko* (Joh et al., 1996b). Therefore, Pi deficiency is considered to inhibit the mycelial growth of *P. nameko* irrespective of strain. However, the extent of inhibition differed among the three strains, increasing in order of the strain N4, N301, and N2, which suggests differences in adaptive ability among the three strains.

The effect of Pi deficiency on the mycelial soluble protein content was different among three strains (Fig. 2). However, in all strains, soluble protein content tended to be higher in the P^- culture than in the P^+ culture. Thus, Pi deficiency resulted in increase of soluble protein content in the mycelia of the three strains. It also increased soluble protein amount in the culture supernatant. These findings show that the mycelia of *P. nameko* adapted to Pi deficiency by increasing the synthesis and secretion of protein. The same results have been obtained in strain N114 (Joh et al., 1996b). Since it has been reported that the amounts of intracellular and extracellular proteins were decreased by Pi deficiency in various organisms (Bostian et al., 1983; Fife et al., 1990; Nagano and Ashihara, 1993; Theodorou et al., 1991), these results indicate the high adaptive ability of *P. nameko* to Pi deficiency.

Responses to Pi deficiency differed among the three strains. The increases of intracellular and extracellular soluble protein amounts and acid phosphatase activities under Pi deficiency were largest in strain N2. In particular, the extracellular soluble protein amount and acid phosphatase activity increased remarkably (Fig. 2D, 5D). However, the depression of growth by Pi deficiency was the greatest in strain N2. These findings suggest that the mycelium of strain N2 tries to acquire Pi from the external environment rather than to enhance the availability of internal Pi under Pi deficiency and actively secretes proteins, including acid phosphatase; but growth was depressed because there was little available phosphate in the medium. On the other hand, the mycelium of strain N4 grew relatively well under Pi deficiency, though the increases in intracellular and extracellular soluble protein amounts and acid phosphatase activities were smaller than those of strain N2. The increase in extracellular acid phosphatase activity in strain N4 was the smallest among the three strains. Therefore, the mycelium of strain N4 seem to have a high natural ability to grow by utilizing a small quantity of internal P efficiently or to have an adaptive mechanism that can utilize P efficiently

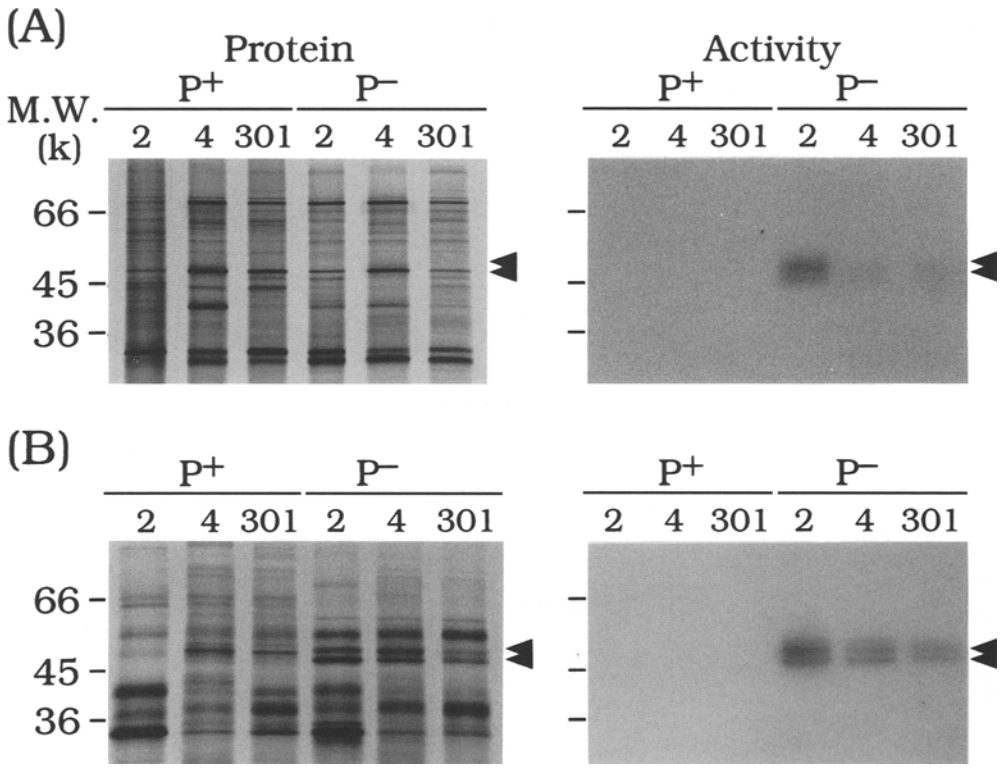

Fig. 6. Induction and secretion of acid phosphatases in mycelia (A) and culture supernatants (B) of strains N2, N4 and N301 at 20 d of cultivation in the P^- culture. About 1 and 3 μg of proteins for silver staining and activity staining were loaded onto the gel, respectively. Arrowheads on the right indicate bands of acid phosphatase.

without dynamic quantitative changes in protein. Strain N301 grew as slowly as strain N2 under Pi deficiency, the increase in intracellular acid phosphatase activity in strain N301 was the smallest among the three strains, and the increases in extracellular acid phosphatase activity and extracellular soluble protein amount were also small. Furthermore, no pronounced difference was found in the intracellular soluble protein content between the P^+ and P^- cultures. Accordingly, it is thought that strain N301 is inferior to the other strains in its ability to adapt to Pi deficiency. Thus, differences in the adaptive ability and mechanism of adaptation to Pi deficiency were found among the three strains.

Next, an attempt was made to explain the differences in adaptive abilities by investigating the qualitative changes in total protein caused by Pi deficiency. Two strains, N2 and N4, which exhibited large differences in adaptive ability, were selected and their total proteins were analyzed by 2D-PAGE. The analysis of mycelial total protein showed that 103 and 65 polypeptides were synthesized in relative abundance under Pi deficiency in strains N2 and N4, respectively (Fig. 3). Many of the polypeptides were specific to each strain, suggesting that metabolic changes in adaptation to Pi deficiency are considerably different between the two strains. Fewer polypeptides increased in strain N4 than in strain N2. Because the growth of strain N4 was little depressed by Pi deficiency, the mycelium of strain N4 is considered to utilize P efficiently with little change in its metabolism. On the other hand, more polypeptides increased in strain N2, even though the growth depression of strain N2 was larger than that of strain N4. Therefore, the complicated metabolic changes induced by Pi deficiency may cause such qualitative changes in proteins. Recently, changes in soluble proteins induced by Pi deficiency in various organisms have been examined by 2D-PAGE (Eymann et al., 1996; Fife et al., 1990; Osorio and Jerez, 1996; Usuda and Shimogawara, 1995). The results revealed that 4–20 polypeptides increase in abundance under Pi deficiency. Thus, more polypeptides were synthesized abundantly under Pi deficiency in *P. nameko* than in other organisms. This may represent the high adaptive ability of *P. nameko* to Pi deficiency.

Analysis of total protein in the culture supernatant showed that the number of polypeptides secreted decreased in response to Pi deficiency in the two strains (Fig. 4). The polypeptides that increased in relative abundance under Pi deficiency accounted for over one third of those detected ones in the two strains and are presumed to play important roles in utilization of external P. Although the mycelium of strain N2 secreted a larger amount of soluble proteins than that of strain N4, polypeptides that increased in response to Pi deficiency were fewer than in strain N4. This shows that the mycelium of strain N2 try to adapt to Pi deficiency by increasing the secretion of proteins involved in the adaptive mechanism, such as acid phosphatase.

2D-PAGE analysis revealed that many polypeptides

are changed quantitatively by Pi deficiency. Among the polypeptides that increased in relative abundance, 20 and 11 polypeptides in the mycelia and culture supernatant, respectively, were common to the two strains, suggesting that the two strains have partially the same adaptive mechanism for Pi deficiency. These polypeptides may be those related to fundamental metabolism, such as phosphoenolpyruvate carboxylase, glucose dehydrogenase, or ADP-glucose pyrophosphorylase described in plants (Duff et al., 1989; Johnson et al., 1996; Gyaneshwar et al., 1999; Ball et al., 1990). Since, especially in mycelial proteins of strain N4, these common polypeptides seemed to increase more markedly than those of strain N2 (e.g., spot i or n in Fig. 3), the increase of key enzymes in fundamental metabolism may allow the strain to utilize internal P efficiently and to grow relatively well under Pi deficiency. However, the specific functions of polypeptides other than acid phosphatase are unclear, and our attempts to elucidate them are currently underway.

The intracellular and extracellular acid phosphatase activities of all the strains increased remarkably in response to Pi deficiency (Fig. 5). As shown in Fig. 6, two isozymes of acid phosphatase with the molecular weights of 47,000 and 48,000 were induced and secreted in all the strains, suggesting that a common induction and secretion mechanism of acid phosphatase by Pi deficiency exists in *P. nameko*. Polypeptide bands could be detected clearly at the positions corresponding to active bands in the culture supernatants of all the strains, but not detected in the mycelia. The increase of acid phosphatase activity by Pi deficiency was also larger in the culture supernatants than in the mycelia. These findings suggest that acid phosphatase plays a central role in adaptation in the external environment, but that other inducible proteins may be functionally more important in the mycelia.

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