JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Phytase Activity from Cultivated Edible Mushrooms and Their Production Substrates

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Phytase is used commercially to maximize phytic acid degradation and to decrease phosphorus levels in poultry and swine manure. To determine phytase content in edible mushrooms, basidiomata of *Agaricus bisporus* and three specialty mushrooms (*Grifola frondosa*, *Lentinula edodes*, and *Pleurotus cornucopiae*) and spent mushroom substrate (SMS) were surveyed. Enzyme activity ranged from 0.046 to 0.074 unit/g of tissue for four *A. bisporus* types (closed and open whites and closed and open browns) grown at The Pennsylvania State University's Mushroom Test Demonstration Facility (MTDF). The addition of various nutrient supplements to phase II mushroom production substrate did not alter phytase activity in *A. bisporus*. Portabella mushrooms (open brown) obtained from a commercial farm had significantly higher levels of phytase activity (0.211 unit/g of tissue) compared to *A. bisporus* grown at the MTDF. Of the specialty mushrooms surveyed, maitake (*G. frondosa*) had 20% higher phytase activity (0.287 unit/g of tissue) than commercial portabella mushrooms. The yellow oyster mushroom (*P. cornucopiae*) ranked second in level of phytase activity (0.213 unit/g of tissue). Shiitake (*L. edodes*) contained the least amount of phytase in basidiomata (0.107 unit/g of tissue). Post-crop steam treatment (60 °C, 24 h) of SMS reduced phytase activity from 0.074 to 0.018 unit/g. Phytase was partially purified from commercially grown portabella basidiomata 314-fold with an estimated molecular mass of 531 kDa by gel filtration chromatography. The optimum pH for activity was 5.5, but appreciable phytase activity was observed over the range of pH 5.0−8.0. Partially purified *A. bisporus* phytase was inactivated following a 10-min incubation at ≥60 °C.

KEYWORDS: Phytate; phytic acid; spent mushroom substrate; mushroom compost; specialty mushrooms

INTRODUCTION

Commercially produced mushrooms are valued primarily as a culinary food. However, the bioactive components of mushrooms of nutritional, medicinal, and biological importance recently have received much more attention (*1*−*3*). Phytases (*myo*-inositol hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8), with known presence in *Agaricus* (*4*), belong to a family of enzymes (histidine acid phosphatases) that catalyze the hydrolysis of phytate (*myo*-inositol hexakisphosphate) and generate *myo*-inositol phosphates, *myo*-inositol, and inorganic phosphates (*5*−*7*). Currently, the major use for phytases is incorporation as an animal feed additive (*8*, *9*) to assist in the breakdown of phytate to compounds utilizable by monogastric animals (chickens and pigs). The usefulness of phytase supplementation to increase iron absorption in humans has been demonstrated (*10*, *11*), and the degradation of phytic acid from corn tortillas and refried beans by phytases in raw fruit and vegetable extracts was demonstrated in vitro (*12*).

Phytases may be purified from numerous sources including plants, animals, bacteria, and fungi. Most research has focused on the purification of phytases from microbial sources because these sources are more promising for phytase production on a commercial level. A variety of methods have been used to purify phytases including ammonium sulfate precipitation, ultrafiltration, ion exchange, and gel filtration chromatography. Studies involving the purification of phytases from different sources are usually performed to identify the unique properties, primarily the pH optimum and thermotolerance, of a particular phytase. Current phytase research efforts focus on improving characteristics of this enzyme. Increased thermotolerance to survive the heating period during animal feed pelletization is seen as an essential step in lowering the costs associated with using phytase. In addition, improvements in the pH optimum, enzyme substrate specificity, and enzyme stability may increase the efficacy of phytase, thereby reducing phosphorus pollution (*8*).

We present results of a survey to quantify phytase activity in several cultivated edible mushrooms and mushroom cultivation substrates and report a purification strategy for phytase from basidiomata of *Agaricus bisporus*.

MATERIALS AND METHODS

Samples. Mushrooms and mushroom cultivation substrates were collected from The Pennsylvania State University's (PSU) Mushroom Test Demonstration Facility (MTDF), The Mushroom Research Center

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10.1021/jf040220m CCC: $27.50 © 2004 American Chemical Society
Published on Web 11/12/2004

Table 1. Fungi and Spent Mushroom Substrates (SMS) Surveyed for Phytase Production

mushroom species	sample type	source
A. bisporus	white button (first, second, and third breaks)	Pennsylvania State University
	brown mushroom (crimini)	Pennsylvania State University
	portabella (port)	Pennsylvania State University
	portabella (port2)	commercial mushroom farm
	Breakfast Flat	Pennsylvania State University
	nonsteamed SMS (white button)	Pennsylvania State University
	nonsteamed SMS (portabella)	Pennsylvania State University
	steamed SMS (white button; 60 °C for 24 h)	Pennsylvania State University
L. edodes	basidioma	Pennsylvania State University
G. frondosa	basidioma	commercial mushroom farm
P. cornucopiae	basidioma (yellow)	Pennsylvania State University

(MRC), and a commercial mushroom farm (Kennett Square, PA) (**Table 1**). The production substrate used for the cultivation of *A. bisporus* was a phase II composted formulation of wheat straw bedded-horse manure (from PSU horse stables) supplemented with dried chicken manure and corn distillers gain (N content at spawning = 2.5%) and gypsum (a flocculating agent). Several "breaks" or "flushes" of mushrooms may be harvested from a single spawned (inoculated) *A. bisporus* production substrate every 7−8 days once mushroom production begins. First, second, and third break harvests from a single crop of a white hybrid *A. bisporus* were tested for phytase activity to determine if phytase levels varied from break to break. Other *A. bisporus* mushrooms included in this survey were crimini (brown closed mushrooms), portabella (mature brown mushrooms with exposed hymenia), and breakfast flats (mature white mushrooms with exposed hymenia). The crimini and portabella, as well as the white button mushrooms and breakfast flats, used for production at the MTDF were of the same *A. bisporus* strains, respectively. These mushrooms were included to determine if developmental stage had an effect on phytase levels. Steam-treated and nontreated spent mushroom substrates (SMS) collected after the harvest of three breaks of mushrooms also were assayed for phytase activity. SMS was steam-treated by increasing the temperature of the production room to 60 °C for 24 h.

The production substrate for *Lentinula edodes* and *Grifola frondosa* consisted of a sterilized basal medium of sawdust (*Quercus* spp.) supplemented with millet, rye, and bran at rates of 50% for *L. edodes* and 30% for *G. frondosa*. The primary ingredients used for *Pleurotus cornucopiae* production substrate was pasteurized chopped wheat straw. All mushroom and production substrate samples were stored at −80 °C until used.

Tissue Extractions. Crude protein extractions were performed as described by Phillippy (*4*). Mushroom samples were homogenized in five parts of ice-cold 20 mM sodium acetate buffer (pH 5.5) containing 0.1 mM phenylmethanesulfonyl fluoride (PMSF) in a Waring blender for 60 s. Samples were centrifuged for 30 min at 25000*g* at 4 °C, and the supernatants were filtered through 0.2 μm polyethersulfone (PES) membranes. Total protein of extracts and all steps of protein purification were routinely quantified using the Bradford method (*13*).

Partial Purification of Phytase. All chromatographic steps were performed using the ÄKTA FPLC high-performance liquid chromatography system controlled by UNICORN 3.0 (Amersham Pharmacia Biotech, Upssala, Sweden). Filtered crude protein extract from port2 was loaded onto a 25 mL bed volume Source 30Q FF ion-exchange column (Amersham Pharmacia Biotech) equilibrated with 20 mM piperazine buffer (pH 5.5). After elution of unbound phytase-inactive protein from the column with 1 column volume of equilibration buffer, 100 mM NaCl was added to the equilibration buffer for 1 column volume. Following elution with 100 mM NaCl, a linear gradient of NaCl from 0.1 to 1.0 M was applied to the column at a flow rate of 1.5 mL/min, and 5 mL fractions were collected. Fractions (5 mL) that displayed phytase activity were pooled and concentrated by ultrafiltration with a 30000 NMWL cutoff membrane (PL-30, Millipore Corp., Bedford, MA). Retentate and filtrate were retained for phytase activity assay. A final ultrafiltration retentate volume of ∼2 mL was loaded onto a Pharmacia XK 26/100 Sephacryl S-200 (Amersham Pharmacia Biotech) with a column volume of 368.1 mL that had been equilibrated with 20 mM piperazine (pH 5.5). Fractions of 4 mL were collected at a flow rate of 1.5 mL/min. The molecular weight of the obtained product was estimated using a gel filtration chromatography standard kit (Bio-Rad, Hercules, CA) run on the same column with identical parameters.

Enzyme Assay. Phytase activity was detected and quantified by measuring the liberation of inorganic phosphate released from phytic acid (dodecasodium salt) at 37 °C and pH 5.5 for 60 min. This reaction was terminated by the addition of a molybdate/vanadate reagent. This reagent reacted with phytase-liberated phosphate to produce a yellow complex that was measured spectrophotometrically at 415 nm and was directly proportional to the phytase concentration in the sample. One phytase unit is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37 °C and pH 5.5 under the conditions of the assay (*14*). All statistical analyses were performed using the SAS program JMP 5.1 (*15*). The Tukey−Kramer Honestly Significant Difference (HSD) was used to separate treatment means ($P < 0.05$).

Supplementation and Phytase Expression in *A. bisporus* Basidiomata. The effect of various supplements on phytase levels in mushrooms was explored by treating mushroom substrate with various combinations of phytic acid (Sigma P-3168, St. Louis, MO), commercial phytase Natuphos 5000G (BASF, Mount Olive, NJ), and commercial ProMycel Gold mushroom-growth supplement (Spawn Mate, New Albany, IN). Treatments were arranged in a 2 × 3 × 2 factorial design with four replicates. Supplements were added to 15 g of spawn at the following rates: 0 or 3 g of phytic acid; 0, 1, or 2 g of Natuphos 5000G; or 0 or 27 g of [4%] ProMycel Gold. Supplemented spawn was mixed with 2.27 kg (wet weight) of substrate, and mushrooms were grown in 25 cm diameter × 13 cm deep cylindrical plastic pots at the MRC. The spawned mushroom substrate was maintained at 24 °C for 13 days for mycelial colonization and then overlaid with a 3.5 cm deep layer of casing, a mixture of sphagnum peat and ground limestone. The compost was maintained at 24 °C for 10 days, at which time the temperature was lowered to 18 °C to induce formation of mushrooms. Throughout the cropping cycle, the air temperature was maintained at 18 °C and the casing was routinely watered to field capacity. Harvested mushrooms were assayed for phytase activity as described above.

Enzyme Characterization. Optimal pH curves were determined as previously described by Wyss et al. (*16*). Incubations were carried out with equal concentrations of phytic acid (8.4 g/L) in the following buffers: 0.25 M glycine−HCl, pH 2.5; 0.25 M sodium acetate, pH 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5; 0.25 M imidazole−HCl, pH 6.0, 6.5, and 7.0; and 0.25 M Tris-HCl, pH 7.5, 8.0, 8.5, and 9.0. The percent relative activity for each of these pH values was used to determine the optimal pH activity from *A. bisporus* and its substrate.

Using the determined optimal pH for enzymatic activity, thermotolerance was determined by incubating the partially purified enzyme for 10 min at temperatures as follows: 30, 40, 45, 50, 55, 60, 70, 80, and 90 °C (*17*). After incubation, samples were assayed for enzyme activity as described above.

RESULTS

Crude protein extracts from mushrooms and mushroom substrates showed phytase activity at pH 5.5 for all samples

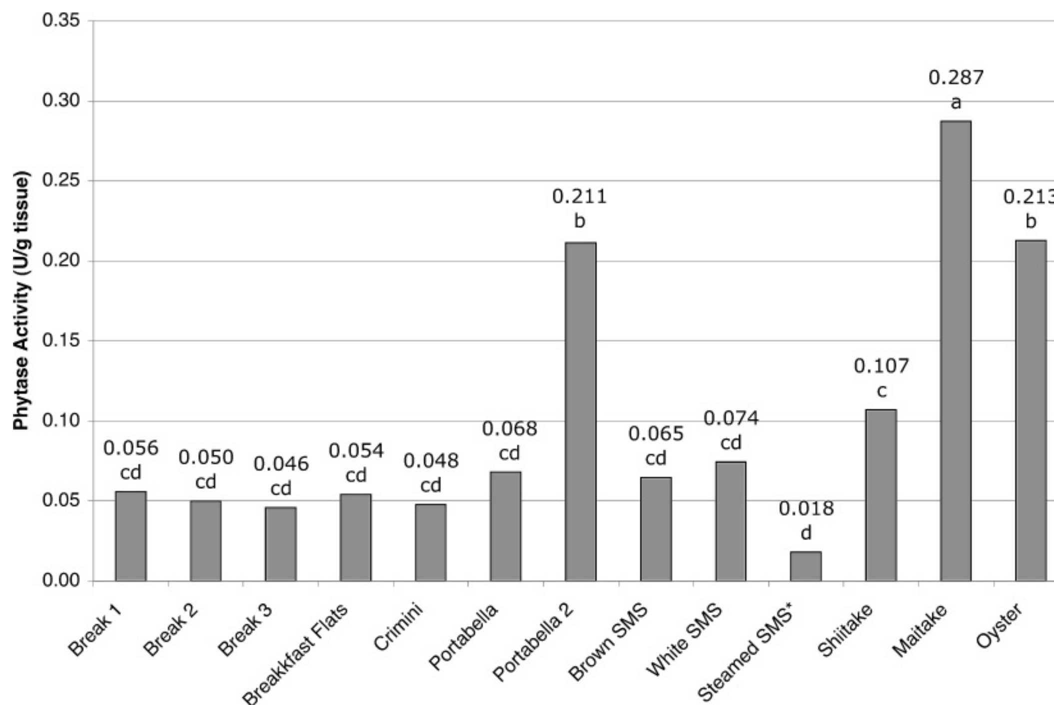

Figure 1. Mean ($n = 4$) phytase activity (units/g of fresh tissue) of crude protein extracts from various mushrooms and mushroom cultivation substrates. Samples were obtained from the Pennsylvania State University with the exception of portabella 2 and maitake (obtained from a commercial mushroom farm). Letters indicate significant differences between samples ($P < 0.05$, Tukey HSD). * Steamed SMS was pasteurized at 60 °C for 24 h.

tested (**Figure 1**). No significant differences were observed among any *A. bisporus* samples obtained from the MTDF. The portabella obtained from a commercial mushroom farm displayed significantly ($P < 0.05$) higher levels of phytase activity compared to other *A. bisporus* samples.

Of the specialty mushrooms, maitake had the highest levels of phytase activity (0.287 ± 0.067 unit/g of tissue). The yellow oyster mushroom (*P. cornucopiae*) showed the second highest level of phytase activity (0.213 ± 0.025 unit/g of tissue) and was comparable to the amount of phytase activity detected in the portabella 2 (0.211 ± 0.031 unit/g tissue) obtained from the commercial mushroom farm (**Figure 1**).

The addition of phytic acid (Sigma), commercial phytase Natuphos 5000G (BASF), and/or commercial ProMycel Gold mushroom-growth supplement (SpawnMate) had no significant effect on phytase activity levels (**Figure 2**). Mean phytase activity levels ($n = 4$) ranged from 0.0452 ± 0.0236 to 0.0907 ± 0.0385 unit/g of tissue (wet weight).

Phytase from crude protein extracts of *A. bisporus* basidiomata was partially purified in a three-step process involving anion-exchange chromatography, ultrafiltration, and gel filtration chromatography. A summary of the purification scheme is given in **Table 2**. The phytase enzyme was purified ~314-fold with a recovery of 1% and a specific activity of 14.7 units/mg of protein.

Anion-exchange chromatography yielded 5% of the total phytase activity detected in crude protein extracts. Ultrafiltration of pooled anion-exchange fractions yielded 2% of the total phytase activity with a specific activity of 0.39 unit/mL. Flow-through from anion-exchange chromatography and ultrafiltration membranes did not detect phytase activity. Gel filtration chromatography of the ultrafiltrate (2 mL) yielded 1% of the total phytase activity from crude protein extracts with a 341.3-fold purification. Phytase activity assays of fractions collected from gel filtration gave a single peak at fractions 41 and 42. On the basis of gel chromatography standards, the molecular weight of the protein eluting at this volume was estimated at 531 kDa (**Figure 3**).

The pH profile of the partially purified phytase was performed from pH 2.5 to 8.5. Phytase activity was detected within the range of pH 5.0−8.0 (**Figure 4**), and a single pH optimum of 5.5 was observed at 37 °C. At temperatures of 50 °C and above, phytase activity was not detectable (**Figure 5**). Partially purified phytase was inactivated after a 10 min exposure to temperatures of 60 °C and above (**Figure 5**). Phytase was stable when stored at 4 °C for at least 2 weeks.

DISCUSSION

This is the first survey of phytase activity in mushroom production substrates. Two previous studies demonstrated phytase activity in basidiomata of *A. bisporus* and *L. edodes* (*4*, *18*) but cultivation substrates were not tested. In our study, no significant differences were observed in phytase activity levels from basidiomata of *A. bisporus* harvested at the MTDF. All of these mushrooms were grown on the same cultivation substrate prepared under the same environmental conditions. The higher level of phytase activity observed in portabella mushrooms obtained from a commercial mushroom farm suggests that cultivation substrate, environmental growth conditions, mushroom strain, and/or postharvest age may influence phytase expression.

A previous study reported phytase activity of 0.078 unit/g of tissue in *A. bisporus* (*4*). Phytase activity observed in MTDF-grown portabella mushrooms was slightly lower (0.068 unit/g of tissue). One explanation for lower activity may be due to differences in cultivation substrate formulation between mushroom farms. It has been demonstrated that the availability of inorganic phosphorus in the growth medium may influence synthesis of phytase (*19*). Phytase production in *Candida krusei*, for example, may be controlled by phosphate concentration of the medium (*20*). Maximum phytase production occurred in a medium containing 0.5 mg of phosphate per 100 mL, but an

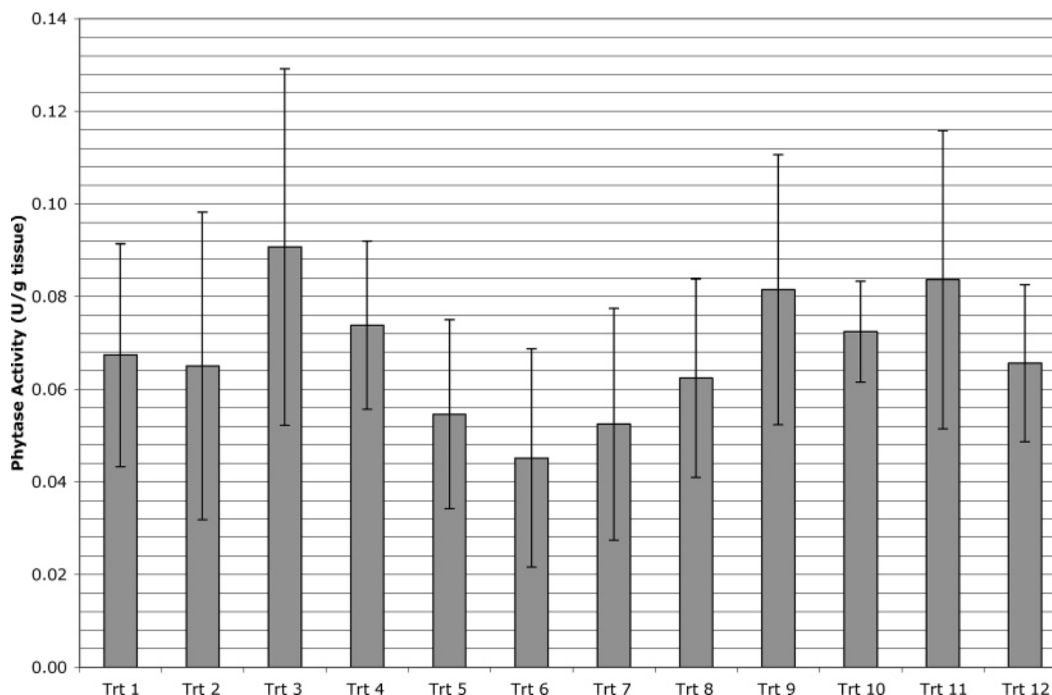

Figure 2. Mean ($n = 4$) phytase activity (units/g of fresh tissue) of crude protein extracts from samples of the phytase induction study. All samples were obtained from the Pennsylvania State University Mushroom Research Center. Supplementation rates for each treatment added to 2.27 kg (wet weight) of mushroom substrate and 15 mushroom spawn were as follows: Trt 1 = 1 g of phytase; Trt 2 = no supplementation; Trt 3 = 27 g of ProMycel Gold + 2 g of phytase; Trt 4 = 3 g of phytic acid; Trt 5 = 27 g of ProMycel Gold + 3 g of phytic Acid; Trt 6 = 27 g of ProMycel Gold + 1 g of phytase; Trt 7 = 2 g of phytase; Trt 8 = 27 g of ProMycel Gold + 3 g of phytic acid + 2 g of phytase; Trt 9 = 27 g of ProMycel Gold + 3 g of phytic acid + 1 g of phytase; Trt 10 = 3 g of phytic acid + 1 g of phytase; Trt 11 = 27 g of ProMycel Gold; Trt 12 = 3 g of phytic acid + 2 g of phytase. Error bars indicate that no significant differences among treatment means were observed ($P < 0.05$, Tukey HSD).

Table 2. Summary of Phytase Purification from Portabella 2 (*A. bisporus*) Basidioma

purification step	total activity (units)	total protein (mg)	specific activity (units/mg of protein)	purification (-fold)	% yield
crude extract	16.20	378.58	0.04	1.00	100
anion exchange	0.75	4.89	0.15	3.59	5
ultrafiltration	0.35	0.88	0.39	9.20	2
gel filtration	0.16	0.01	14.61	341.32	1

increase in the concentration to >5 mg per 100 mL caused inhibition of phytase synthesis.

Poultry manure is a common ingredient used in many mushroom production substrate formulations. Because chicken-feed formulations may vary between farms, it is likely that the phosphorus and phytic acid content of chicken manure sourced from different farms is variable depending on whether the farm supplements its chicken feed with phytase or inorganic rock phosphate. A survey of phosphorus levels in spent mushroom cultivation substrate in Ireland during 2002 found a reduction in phosphorus content by approximately one-third compared to levels in 1998 (*21*). This observed reduction in phosphorus levels was attributed to the increased use of phytase in chicken feed since 1998 (*21*). Although the addition of phytic acid and/or phytase to mushroom cultivation substrate in our study had no significant effects on phytase levels in mushrooms, it is possible that phytic acid from a source other than a purified form of phytic acid may induce higher levels of phytase activity in mushrooms. Because the feed formulation used by the source of the MTDF chicken manure was unknown, it may be desirable to compare the phytase levels of mushrooms grown with phytase-fed chicken manure to phytase levels of mushrooms grown with manure from chickens that did not receive phytase.

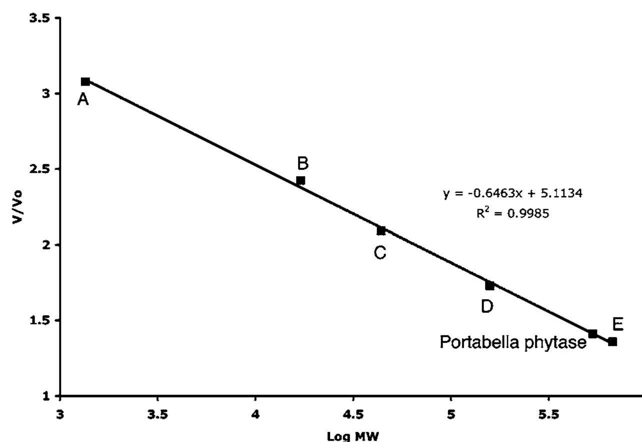

Figure 3. Gel filtration calibration performed on XK 26/100 Sephacryl S-200 column. Protein standards (Bio-Rad, Hercules, CA) used were (**A**) vitamin B_{12} (1.35 kDa), (**B**) myoglobin (17 kDa), (**C**) ovalbumin (44 kDa), (**D**) γ-globulin (158 kDa), and (**E**) thryoglobulin (670 kDa). Portabella phytase eluted with an estimated size of 531.2 kDa in 20 mM piperazine (pH 5.5).

Phytase was partially purified from the commercial portabella samples with relatively higher phytase activity. The purification strategy of this enzyme included anion-exchange, ultrafiltration, and gel filtration chromatography. A similar purification strategy has been used to purify phytase from *Schwanniomyces castellii* (*22*). Using gel filtration, Segueilha et al. (*22*) estimated the size of the *S. castellii* phytase at ~490 kDa. SDS-PAGE analysis of this phytase suggested that the enzyme was tetrameric and consisted of one subunit of 180 kDa and three subunits of 75 kDa. Estimation of the molecular mass of the partially purified phytase from *A. bisporus* by gel filtration indicates a protein of

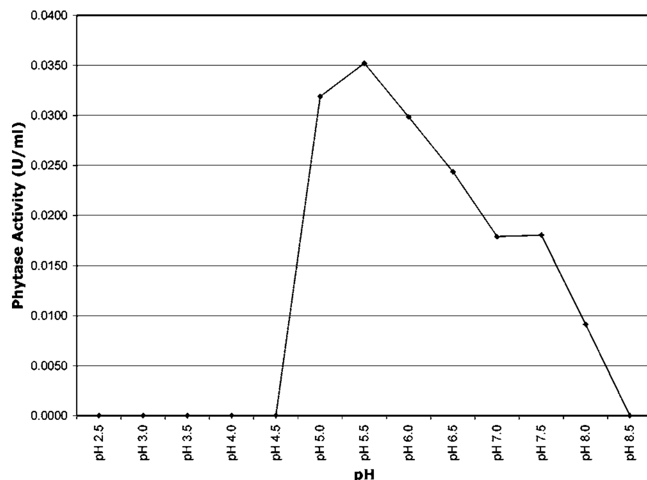

Figure 4. Effect of pH on partially purified phytase activity from portabella (*A. bisporus*). Samples were incubated for 1 h at 37 °C in the presence of phytic acid. Data represent the means of two assays.

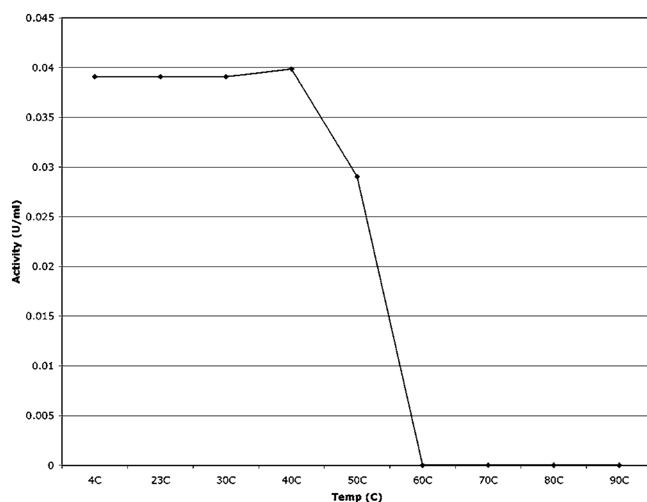

Figure 5. Thermotolerance of *A. bisporus* phytase activity. Partially purified phytase activity was assayed following a 10-min incubation at 4, 23, 30, 40, 50, 60, 70, 80, and 90 °C. Data represent the means of two assays.

531 kDa, a rather high value compared to the molecular weights of other reported phytases (commonly below 100 kDa) by SDS-PAGE (*23*, *24*). A phytase purified from *Klebsiella aerogenes* of ~700 kDa is the only reported phytase of higher molecular mass (*25*). The authors estimated the size of this phytase by gel filtration and provided SDS-PAGE evidence for an unusually small (10−13 kDa) enzymatically active peptide that was generated during the isolation process. They suggested that a small fragment of the native protein with an intact active site might exhibit enzyme activity.

The optimum pH for the *A. bisporus* phytase is ~5.5. This value is characteristic for most phytases isolated from bacteria and fungi (*26*). Stable phytase activity was observed for *A. bisporus* phytase in the pH range of 5.0−8.0. A similar range (pH 6.0−8.0) was observed for a phytase from an *Enterobacter* sp. that was isolated from soil near the roots of leguminous plants (*27*). However, many reported fungal phytases do not exhibit activity above pH 7.0 (*28*). The higher pH range for *A. bisporus* phytase is consistent with the acceptable pH range of mushroom compost to support mycelial growth at the time of spawning. pH values between 6.5 and 8.2 are acceptable at the time of spawning and, the pH of compost is known to drop from 7.5 to 6.0 after cropping (*29*).

The lowest levels of phytase activity were detected in steam-treated SMS. This finding indicates that phytase from *A. bisporus* is not thermotolerant, that is, did not remain active after the mushroom crop was terminated by steam treatment. The detection of low-level phytase activity in steam-treated SMS was unexpected because most characterized phytases are not heat-stable. It is possible that steam did not completely permeate the spent production substrate, allowing for pockets in the substrate where the phytase enzyme could remain active. Alternatively, the reduced levels in phytase activity of steam-treated SMS compared to nonsteamed SMS suggest that phytase from *A. bisporus* is the primary species of this enzyme present in mushroom compost. The residual phytase activity may be due to the presence of thermotolerant phytases produced by thermophillic microorganisms that develop during the composting process.

Thermotolerance studies on partially purified *A. bisporus* phytase showed complete inactivation following a 10-min incubation of 60 °C and above. Approximately 75% of its activity was retained following an incubation of 50 °C for 10 min. These findings are consistent with the results obtained from the survey of mushrooms and mushroom production substrates for phytase activity. Crude protein extracts from steam-treated SMS displayed extremely low phytase activity in comparison to SMS that had not been steam-treated.

The pH optima and thermotolerance for *A. bisporus* phytase are consistent with the range of values reported from other microbes for these characteristics. Given the unusually high molecular weight of the native form of this enzyme, further investigations into the composition and specificity of this enzyme are required. Studies conducted on native phytases from fungal sources have focused primarily on phytases from ascomycetes and deuteromycetes. Relatively little is known about native phytases purified from basidiomycetes. Previous studies on phytases from basidiomycetes have focused primarily on characterization of recombinant phytases (*30*) rather than native forms of this enzyme. Lassen et al. (*30*) characterized phytase-encoding cDNAs from four basidiomycete fungi (*Peniophora lycii, Agrocybe pediades*, *Ceriporia* sp., and *Trametes pubescens*). Analysis of these phytases indicated initial hydrolysis of the 6-phosphate group on phytic acid, in contrast to phytases from *Aspergillus* spp. that initially hydrolyze the 3-phosphate group on phytic acid. Findings such as these indicate the variability of this enzyme between different groups of fungi and suggest that further studies of native phytases from basidiomycetes are required to more fully reveal the range and activity of these enzymes.

A. bisporus is typically cultivated on a composted substrate of straw, hay, horse manure, corncobs, poultry manure, and gypsum. After several breaks from a given cultivation substrate, yield diminishes, and the substrate is declared "spent" (SMS) and then is replaced with fresh compost. Mushroom production in this manner results in the generation of $>10^6$ m^3 of SMS annually (*31*), which is typically disposed of by land application. The detection of phytase activity in nonsteamed SMS suggests the possibility of using SMS as a new source from which phytase could be extracted. However, SMS is steam-treated as a post-crop pest management strategy. The value obtained from nonsteamed SMS as a source of phytase would have to far outweigh the complications if the commercial mushroom industry were to no longer steam-treat SMS. In addition, ~6−8 kg of SMS would be required to yield the manufacturer's suggested 600 units of phytase that is typically supplemented to 1 kg of chicken feed. However, the purification strategy

developed in this study would not be cost-effective, nor would it give high enough recovery for such large quantities of sample.

This study represents the first survey of phytase activity in many popular edible mushrooms. Although phytase activity was detected in all mushrooms and mushroom production substrates, maitake displayed the highest levels of phytase activity. Although the usefulness of phytase supplementation has been demonstrated in increasing iron absorption in humans (*10*, *11*), it is still unknown if the detected phytase activity levels in edible mushrooms would have a similar effect in humans. Whereas Sandberg et al. (1996) used extremely high levels of phytase (2×10^5 units) to demonstrate the effectiveness of dietary phytase to increase iron absorption in humans, it has been demonstrated that the addition of phytase (285 units/kg) with citric acid (3.125 g/kg) to leavening bread can enhance total iron dialyzability 15-fold (*32*). This finding allows for much more cost-effective levels of phytase to be used and suggests the possibility that phytase in maitake (287 units/kg) might serve to help enhance total iron dialyzability in the human diet.

Although the phytase levels detected in mushrooms are relatively low, degradation of phytic acid from corn tortillas and refried beans by phytases in raw fruit and vegetable extracts has been shown in vitro (*12*). The phytase activities detected in this present study suggest that the levels in mushrooms could have a similar effect in vitro. However, *A. bisporus* phytase does not appear to be a good candidate for direct use in the human diet. The relatively low pH (1.2−3) of human gastric fluid would inactivate the *A. bisporus* phytase (inactive below pH 4.5), rendering it nonfunctional for phytate hydrolysis. Thus, claims for a functional role of *A. bisporus* phytase in the human diet are not possible due to low enzyme levels and a higher pH range of activity. However, the utilization of this enzyme to reduce phytic acid in vitro (i.e., leavening bread) should be further explored. Attempts should continue to find other functional compounds in *A. bisporus*. Such findings may increase the demand for mushrooms and increase profit margins for growers.

ACKNOWLEDGMENT

We thank Mark W. Signs for expertise and assistance with the protein purification steps and Vija Wilkinson for maintenance of cultures and preparation of spawn.

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Received for review April 29, 2004. Revised manuscript received September 9, 2004. Accepted September 24, 2004.

JF040220M