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Extraction and Precipitation of Chitosan from Cell Wall of Zygomycetes Fungi by Dilute Sulfuric Acid

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A new method was developed in this work for extraction of chitosan from the zygomycetes cell wall. It is based on the temperature-dependent solubility of chitosan in dilute sulfuric acid. Chitin is soluble in neither cold nor hot dilute sulfuric acid. Similarly chitosan is not soluble at room temperature but is dissolved in 1% H_2SO_4 at 121 °C within 20 min. The new method was developed to measure the chitosan content of the biomass and cell wall. The procedures were investigated by measuring phosphate, protein, ash, glucuronic acid, and degree of acetylation. The cell wall derivatives of fungus *Rhizomucor pusillus* were then examined by this new method. The results indicated 8% of the biomass as chitosan. After treatment with NaOH, the alkali-insoluble material (AIM) contained 45.3% chitosan. Treatment of AIM with acetic acid resulted in 16.5% acetic-acid-soluble material (AcSM) and 79.0% alkali- and acid-insoluble material (AAIM). AcSM is usually cited as pure chitosan, but the new method shows major impurities by, for example, phosphate. Furthermore, AAIM is usually considered to be the chitosan-free fraction, whereas the new method shows more than 76% of the chitosan present in AIM is found in AAIM. It might indicate the inability of acetic acid to separate chitosan from the cell wall.

Introduction

Chitosan, copolymer of glucosamine and *N*-acetyl glucosamine, is nowadays produced most often by deacetylation of chitin from shellfish wastes. The cell wall of zygomycetes is an alternative source for chitosan production.[1] Chitosan is traditionally produced from the cell wall of fungi by a two-step extraction process involving alkali and acid treatments. Proteins, lipids, and alkali-soluble carbohydrates are first dissolved in, for example, 2–4% NaOH at 90–121°C within 15–120 min, and the cell wall material containing chitosan is obtained as alkali-insoluble material (AIM). The AIM is then treated with an aqueous solution of an acid such as 2–10% acetic acid at 25–95 °C for 1–24 h to dissolve the acetic-acid-soluble material (AcSM) and separate it from the remaining material in the cell wall, which is called alkali- and acid-insoluble material (AAIM). AcSM is generally considered to be the "fungal chitosan" and can be precipitated by raising the pH to 9–10 followed by centrifugation.[2–4]

The cell wall of zygomycetes is considered to be composed mainly of chitin and chitosan (20–50%) and polymers of glucuronic acid in lower concentrations. In this class of fungi glucose is found only in spores.[5] Phosphates also occur in the cell walls of these fungi.[6] In the cell wall, chitosan may interact with other polysaccharides and phosphates in a complex such that common acidic treatments are not able to break and extract the chitosan from the cell wall completely. In these cases, a considerable amount of chitosan remains in the AAIM.

It is well-known that chitosan is soluble in several acids such as acetic, citric, lactic and hydrochloric acids. However, chitosan was reported as insoluble in sulfuric acid at room temperature with possible solubility in hot boiling solutions.[7] We have tested this fact in preliminary experiments, which became the basis of the current work.

The aims of this work are to develop a method to break the chitosan complex in the cell wall and separate chitosan as well as characterize different preparations of cell wall material with chitosan as the main focus. Dissolution of pure chitin and chitosan in hot and cold dilute sulfuric acid solutions and their recovery after cooling of the hot soluble extracts were examined. Different cell wall preparations were then characterized by measuring the chitosan, protein, phosphate, *N*-acetyl glucosamine, glucuronic acid, and ash content. The cell wall of *Rhizomucor pusillus* was finally characterized by the developed method as an example of the applications. An additional objective of this work was to find out the composition of the soluble portion of AIM, soluble in acetic acid, previously believed to be the main chitosan fraction.[8]

Experimental Section

Fungal Strain and Preparation of the Biomass. *Rhizomucor pusillus* CCUG 11292 obtained from the Culture Collection University of Göteborg (Göteborg, Sweden) was used in this study. It was grown on xylose-rich wastewater of an industrial ethanol plant, where *Saccharomyces cerevisiae* produced ethanol from spent sulfite liquor. The liquor was diluted to 25% of its original concentration and the initial sugar (all pentoses, some mannose and galactose) concentration was ca. 4.2%. The liquor was supplemented with 0.015 M $NH_4H_2PO_4$

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Table 1. Yields of Cell Wall Derivatives from Biomass of *R. pusillus*

cell wall derivative	yield (g/g of AIM)	yield (g/g of biomass)
AIM	1	0.210 ± 0.023
AcSM	0.165 ± 0.004	0.035 ± 0.001
AAIM	0.790 ± 0.007	0.166 ± 0.002
(AcSM+AAIM)	0.955 ± 0.011	0.201 ± 0.003

and 0.07 M ammonium hydroxide. Cultivation was performed in a 60 L air-lift fermentor at 36–38 °C and pH 5.7–6.2. Harvesting of 85% of the volume and replacement with fresh volume was done twice a day, and the yield of biomass was 0.7 g/g of sugars. The bioreactor produced 970 g of dry weight mycelium in 4 days of cultivation. The biomass was harvested on a screen, washed with water, and kept at −20 °C until use.

Preparation of Cell Wall Derivatives. Cell wall derivatives were prepared according to the method presented by Synowiecki et al.[4] with some modifications. Wet mycelium of *Rhizomucor pusillus* that contained 18.5% dry weight was treated with 0.5 M sodium hydroxide (30 mL of sodium hydroxide per g of mycelium dry weight) at 90 °C for 2 h. Alkali-insoluble material (AIM) was separated by centrifugation (10 min, 4000 g), washed 10 times with distilled water, and stored at 4°C until use. The yield of AIM from biomass was measured after drying the AIM at 50 °C until constant weight was achieved.

The AIM was treated with 10% acetic acid (100 mL per g of dry AIM) at 60 °C for 6 h. The alkali- and acid-insoluble material (AAIM) was separated by centrifugation (10 min, 4000 g), washed 5 times with distilled water, and stored at 4 °C until use. Acetic-acid-soluble materials (AcSM) present in supernatants were precipitated by adjusting the pH to 10 by adding 2 M sodium hydroxide to the acetic acid solution. The precipitated AcSM was then separated by centrifugation (10 min, 4000 g) and washed 5 times with distilled water until neutral pH was achieved and stored at 4°C. The yields of AAIM and AcSM were measured after drying at 50°C. The yields of AIM, AAIM, and AcSM from the biomass are presented in Table 1.

Hot Dilute Sulfuric Acid Treatment for Extraction of Chitosan. To develop optimum conditions for chitosan extraction from mycelium, we treated reference materials including two 75–85% DD shellfish chitosan of (a) low molecular weight with 20 cP viscosity (1% solution in 1% acetic acid), and (b) medium molecular weight with 200 cP viscosity (Sigma-Aldrich) and chitin (unbleached, MP biomedicals Europe, France) with hot sulfuric acid at 95 °C overnight and 121 °C for 20 min in an autoclave with different acid concentrations (0.5, 1.0, and 2.0% (v/v)) and acid/chitosan ratios (100, 150, and 200 mL/g). The solubility of the chitin and chitosan at these conditions were examined. They were then cooled on ice for 1–2 h to precipitate the soluble part, and the recovery of the material was measured. The best condition for chitosan solubility was used for extraction of chitosan from cellular or cell wall materials (Figure 1). Sulfuric acid (1% v/v) was mixed with biomass (20 ml/g) or AIM, AAIM, and AcSM (100 mL/g), and the procedure presented in the results was followed to extract the chitosan. Pure cellulose (Avicel PH-101, Biochemika, Fluka, Ireland) and starch (Merck, Germany) were also treated with the same conditions as chitosan, and their solubility was tested in both hot and cold sulfuric acid.

***N*-Acetyl Glucosamine Content and Degree of Deacetylation (DD).** *N*-Acetyl glucosamine content and DD of samples were measured by acid hydrolysis and HPLC analysis according to the method developed by Ng et al.[9] Dry materials (0.01–0.05 g) were mixed with 1.5 mL of 12 M sulfuric acid and 1.0 mL of 1.4 mM oxalic acid in tubes, which were closed tightly and placed in an autoclave for 20 min at 121 °C to liberate the acetyl groups of *N*-acetyl glucosamine residues in the form of acetic acid. After being cooled to room temperature, the hydrolyzates were diluted 25 times and filtered before analyses. Acetic acid concentration was measured by HPLC in an ion-exchange Aminex column (HPX-87H, Bio-Rad, Richmond, CA) at 60 °C with 0.6 mL/min eluent of 5 mM sulfuric acid with UV−Vis detector (Waters 2486, Waters, Ma). *N*-Acetyl glucosamine content ($F_{\text{NAc-GlcN}}$) in solid samples and DD were then calculated according to the following formulas

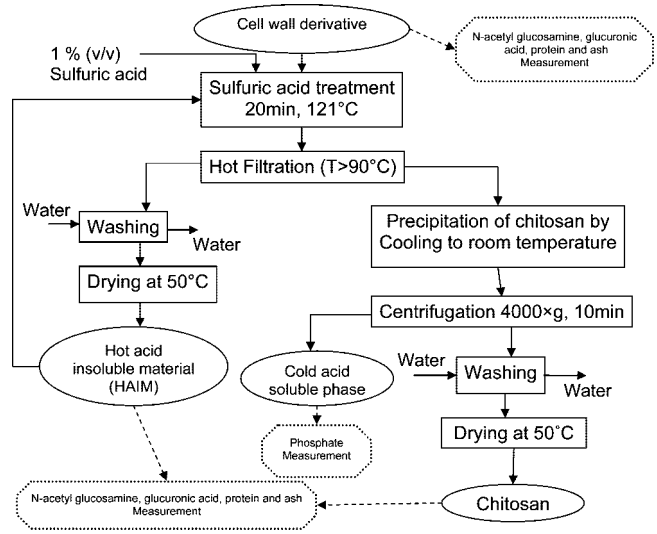

Figure 1. Procedure for extraction of chitosan by sulfuric acid from cell wall derivatives.

$$F_{\text{NAc-GlcN}} = \frac{204A}{W} \quad (1)$$

$$\text{DD} = \left(1 - \frac{A}{A + \frac{W - 204A}{161}}\right) \times 100 \quad (2)$$

where W is the dry weight of the sample and A is moles of acetic acid liberated during the hydrolysis reaction. Pure *N*-acetyl glucosamine was used as a reference for possible correction of the A factor.

Protein Content of Cell Wall Materials. The protein content of the cell walls was measured according to Biuret method.[10] Known amounts of AIM or its derivatives were mixed with 3 mL of 1 M NaOH in tubes. The samples were then boiled for 10 min and cooled in an ice bath. Subsequently, 1 mL of 2.5% $CuSO_4 \cdot 5H_2O$ was added to each tube and whirly-mixed for 5 min. The samples were then centrifuged and the absorbance was measured at 555 nm. Different concentrations of bovine serum albumin were used as standards for determination of protein in the samples.

Phosphate Contents of Cell Wall Materials. Phosphate content of cold acid soluble phase was measured by ammonium molybdate spectrometric method according to European standard ISO6878.[11] Cold acid-soluble phase was mixed with ascorbic acid and acid molybdate reagents, and the absorbance was measured at 880 nm. Different concentrations of potassium dihydrogen phosphate were used as standards for the determination of total phosphate in the samples.

Glucuronic Acid Content of Cell Wall Material. Glucuronic acid content of AIM and its derivatives were measured according to the method developed by Blumenkrantz and Asboe-Hansen.[12] Known amounts (5–10 mg) were mixed with 1.2 mL of sodium tetraborate solution (0.0125 M in 98% sulfuric acid). The resulting suspensions were heated at 100 °C for 5 min. After being cooled in an ice−water bath, 20 μL of *m*-hydroxydiphenyl reagent (0.15% in 0.5% NaOH) was added to each sample. The absorbance was measured at 520 nm against the control samples without reagent. Pure glucuronic acid was used as standard.

Infrared Spectroscopy. Samples of reference chitosans and chitosan derived from AIM were recovered after sulfuric acid treatment, dried at 50 °C, and subjected directly to FTIR using a DuraSample IR instrument (SensIR Technologies, Danburg, CT).

Themogravimetric Analysis. Thermogramimetric analysis (TGA) was performed with a TA instruments Q500 thermogravimetric analyzer

Table 2. Solubility of Pure Chitin, Chitosans (low and medium molecular weights), Cellulose, And Starch in Sulfuric Acid at 121°C within 20 min and Their Recovery after Being Cooled on Ice for 1–2 h

substrate	H_2SO_4 % (v/v)	acid:substrate (mL/g)	solubility	substrate recovery (%)[a]
chitosan-low	0.5	100	>60%	96.66 ± 4.60
	0.5	150	>75%	97.14 ± 0.47
	0.5	200	>80%	96.41 ± 4.94
	1	100	soluble	96.87 ± 0.04
	1	150	soluble	95.00 ± 2.93
	1	200	soluble	89.52 ± 1.92
	2	100	soluble	90.24 ± 0.73
	2	150	soluble	84.40 ± 1.81
	2	200	soluble	84.01 ± 0.50
chitosan-med	0.5	100	>50%	71.10 ± 1.32
	0.5	150	soluble	79.82 ± 0.80
	0.5	200	soluble	78.11 ± 1.00
	1	100	soluble	76.62 ± 1.33
	1	150	soluble	72.86 ± 1.03
	1	200	soluble	74.03 ± 0.96
	2	100	soluble	73.96 ± 0.30
	2	150	soluble	65.89 ± 1.10
	2	200	soluble	60.02 ± 2.30
chitin	1	100	<2%	97.7 ± 2.70
cellulose	1	100	<1%	>99%
starch	1	100	soluble	0.0%[b]

[a] The recovery is calculated on the basis of the amount of original substrate. [b] Starch was soluble in sulfuric acid after being cooled on ice.

(TA instruments-Waters LLC, Germany) from 25 to 500 °C in ambient nitrogen and from 500 to 900 °C in ambient air at a heating rate of 10 °C/min.

Degradability of Extracted Chitosan in Nitrous Acid Solution. Approximately 0.05 g of chitosan extracted by sulfuric acid was mixed with 1.5 mL of 2 M sodium nitrite solution and 0.5 mL of 2 M HCl was added to this mixture, and the degradability of the substrate was evaluated after 30 min. A similar procedure was tested for chitin and cellulose.

All experiments were performed at least in duplicates and results are presented as averages.

Results

Solubility of Chitin, Chitosan, Cellulose and Starch in Hot Sulfuric Acid. The solubility of pure chitosan and chitin in hot sulfuric acid (0.5–2%) was investigated and the results are summarized in Table 2. Two different chitosans with low and medium molecular weight were investigated. The effect of temperature, sulfuric acid concentration, and acid:chitosan ratio were studied. Chitin was practically not dissolved in either cold or hot sulfuric acid. We achieved no solubility of chitin in the acid at room temperature, whereas less than 3% of chitin disappeared from the solid phase within 20 min at 121 °C in 1% acid concentration (Table 2). Neither Chitosan was soluble in sulfuric acid at room temperature. However, increasing the temperature made it dissolve. Dissolution of chitosan in 1% H_2SO_4 at 95 °C was first investigated. The acid was able to dissolve it only partially within 2 h, even with complete mixing. However, the chitosan was dissolved completely when kept at this temperature overnight.

Both the low and medium molecular weights of chitosan were dissolved in sulfuric acid at 121°C, within 20 min without mixing, if the acid concentrations were higher than 0.5% (Table 2). The chitosan was only partially dissolved in 0.5% H_2SO_4 if it was mixed with 100, 150, or 200 mL/g acid, although more chitosan was dissolved at a higher acid:chitosan ratio (Table 2). In all cases, dissolution of the chitosan with medium molecular weight was faster than that with the low molecular weight.

The chitosan dissolved in hot sulfuric acid was precipitated after being cooled on ice and was recovered by filtration. In general, the solution of low-molecular-weight chitosan became turbid almost immediately after cooling, whereas the medium molecular weight chitosan took a longer time to precipitate. The recovery of these chitosans is presented in Table 2. No further precipitation of chitosan was observed after the solutions were kept at 4 °C for a month. The recovery of the low-molecular-weight chitosan dissolved in 0.5 and 1.0% acid was more than 89.5%, whereas using 2% sulfuric acid resulted in lower recovery of chitosan by a few percent (Table 2). A similar trend was found for recovery of the medium-molecular-weight chitosan. However, the recovery of this chitosan was less than 80% in all the experiments (Table 2).

Because using 0.5% sulfuric acid with a low amount of the acid solution resulted in only partial solubility and 2% acid in lower recovery of chitosan, we chose a 1% acid concentration for extraction of chitosan from cell and cell wall derivatives. In that condition, the solubility of pure cellulose was also measured. The results show that cellulose is not soluble in either cold nor hot sulfuric acid. On the other hand, starch became soluble in dilute sulfuric acid by raising the temperature and stayed soluble after the solution was cooled on ice (Table 2).

New Method for Cell Wall Characterization. The method used in this work for separation of chitin and chitosan by hot sulfuric acid was applied as a basis for developing a new method to characterize cell wall materials of fungi. We have examined this method in cell wall characterization of the zygomycete *R. pusillus*. This new method is schematically summarized in Figure 1. Chitosan, phosphate, glucuronic acid, total proteins, ash, and hot acid-insoluble material (HAIM) are the major components that are measured by the method described below.

Details of the Method for Cell Wall Derivatives. AIM, AAIM, and AcSM are the materials that can be characterized by this method. The total protein, ash, glucuronic acid, and *N*-acetyl glucosamine content of these materials can be first measured. The materials are then treated with 100 mL/g 1% H_2SO_4 in an autoclave at 121 °C for 20 min. In this step, chitosan and phosphates are dissolved in the acid, whereas hot acid-insoluble material (HAIM) including chitin, proteins, and some unknown materials remains in the particulate fraction. The hot suspension should be immediately filtered at high temperature, because chitosan tends to precipitate by cooling. The

Table 3. Content of Chitosan, HAIM, and Phosphate in the Cell Wall Derivatives of *R. pusillus* Grown on Sulfite Liquor

	yields in g/g of source (AIM, AAIM, or AcSM)			
source	chitosan	HAIM	phosphate	total
AIM	0.453 ± 0.004	0.290 ± 0.017	0.199 ± 0.024	0.942 ± 0.045
AAIM	0.437 ± 0.001	0.321 ± 0.030	0.193 ± 0.007	0.951 ± 0.038
AcSM	0.385 ± 0.076	0.061 ± 0.028	0.317 ± 0.006	0.763 ± 0.110

filtrate is then cooled on ice. Chitosan precipitates from the cold filtrate and can be separated from the cold acid soluble phase by centrifugation at 4000 g for 10 min, followed by washing with pure water and drying at 50 °C (Figure 1). The phosphate content of the soluble phase can then be measured. Because HAIM might contain some undissolved chitosan, the treatment with hot sulfuric acid is repeated on HAIM until no further chitosan is detected. *N*-Acetyl glucosamine content and the degree of deacetylation (DD) of the remaining HAIM and chitosan are then measured by hydrolysis of the acetyl groups of the *N*-acetyl glucosamine units into acetic acid by concentrated sulfuric acid followed by HPLC measurement (Figure 1).

Method for Chitosan Extraction from Cell Biomass. The above mentioned procedure with hot sulfuric acid can be applied for measuring chitosan directly from the biomass without alkali treatment. The only difference is the amount of acid solution, which can be 20 mL/g dry biomass. We have tested higher amounts of the acid solution up to 100 mL/g, but the difference between the chitosan yields was less than 5%.

Characterization of *R. pusillus* Cell Wall Derivatives. The newly developed method was applied to characterize the cell wall materials AIM, AAIM, and AcSM of *R. pusillus*. In the first treatment with hot sulfuric acid, 44.3% of AIM, 43.6% of AAIM, and 38.5% of AcSM were recovered as chitosan. A further similar treatment on the remaining HAIM derived from AIM and AAIM resulted in a 1.0 and 0.1% increase in the yield of chitosan obtained from those cell wall derivatives, respectively. No further chitosan was detected in the third treatment with hot sulfuric acid. The total chitosan extracted from the cell wall derivatives are presented in Table 3. The IR spectrum of this cell wall chitosan is very similar to the spectra of the references (Figure 2). The chitosans extracted by sulfuric acid from different sources and pure chitosans were treated with sodium nitrite and HCl solutions and disappeared completely in the solution. However, nitrous acid had almost no effect on pure chitin and cellulose.

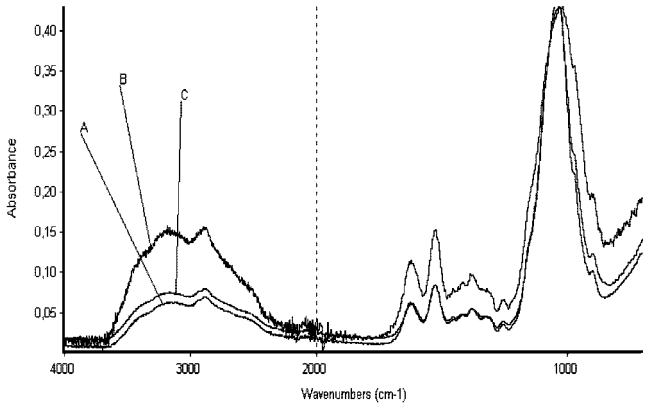

Figure 2. FTIR spectra of extracted fungal chitosan from AIM (C) in comparison with pure chitosans (medium (A) and low (B) molecular weights) after treatment by sulfuric acid.

After the filtrate was cooled to room temperature and the chitosan was recovered as cold acid-insoluble material, phosphate concentrations were measured in the sulfuric acid supernatants. Results show that during the three steps of sulfuric acid extraction from AIM and AAIM and one step from AcSM, 19.9% of AIM, 19.3% of AAIM, and 31.7% of AcSM were liberated in the form of phosphate in sulfuric acid (Table 3). Of this total phosphate, 89 and 93% from AIM and AAIM, respectively, were obtained in the first step and the remainder in the second step. No further phosphate was obtained in the third step. Protein analysis showed that 4.95% of AIM and 4.2% of HAIM (derived from AIM) were protein (Table 4).

Inorganic materials, which were stable at 900°C in ambient oxygen, made 19.5% of AIM, 0.3% of HAIM, and 1.3% of chitosan (derived from AIM). Glucuronic acid content of the cell wall material and its derivatives were less than 1.0% (Table 4).

Total *N*-acetyl glucosamine content of AIM and its derivatives (HAIM and chitosan) were measured and results are summarized in Table 4. *N*-Acetyl glucosamine makes up 8.5% of the AIM. Most of these acetylated groups were collected in HAIM with 34.6% *N*-acetyl glucosamine. Only 3.1% of the extracted chitosan derived from AIM was *N*-acetyl glucosamine, which leads to DD 97.5% for this fraction.

The sum of chitosan, phosphate, and HAIM corresponded to 94.2% of AIM, 95.1% of AAIM, and 76.3% of AcSM (Table 3).

Direct Chitosan Extraction from Biomass. Biomass of *R. pusillus* was treated directly with hot sulfuric acid, filtered, and cooled to precipitate chitosan. The results indicated 8% of biomass as chitosan, whereas 51.8% of the biomass was not soluble in hot sulfuric acid (HAIM). These results in comparison with the chitosan content of AIM indicate that alkali extraction doesn't have a significant effect on chitosan yield.

Discussion

The dependence on temperature of the chitosan solubility in dilute sulfuric acid can open up several new opportunities for extraction and characterization of this polymer. This property is not shared with other components of the fungal cell wall such as chitin, glucan (cellulose), glucuronic acid, proteins, and phosphates. We have used this characteristic to develop a simple method to extract chitosan and characterize the cell wall materials of zygomycetes and tested it with *R. pusillus*.

Previous works[2–4,8,13,14] refer to AcSM as a chitosan-rich fraction from cell wall materials. However, our results indicate that AcSM is not pure chitosan and that AAIM contains chitosan. Chitosan was previously reported to be 20–50.0% of the cell wall of zygomycetes.[5] We extracted 45.3% of the cell wall of *R. pusillus* as chitosan, which is in the line of other investigation. However, the presence of 43.7% chitosan in AAIM shows the inability of acetic acid to dissolve all chitosan present in the cell wall. On the other hand, phosphate was a major source of impurity of AcSM.

Table 4. *N*-Acetyl Glucosamine ($F_{NAc\text{-}GlcN}$), Protein, Ash, and Glucuronic Acid Content of AIM of *R. pusillus* and Its Derivatives after Hot-Dilute Sulfuric Acid Treatment (see Figure 1)

	$F_{NAc\text{-}GlcN}$ (g/g)	protein (g/g)	ash (g/g)	glucuronic acid (g/g)
AIM	0.085 ± 0.002	0.049 ± 0.016	0.194 ± 0.001	0.008 ± 0.002
HAIM	0.346 ± 0.010	0.042 ± 0.028	<0.005	0.004 ± 0.001
chitosan	0.031 ± 0.003	<0.001	0.013 ± 0.002	<0.001

The purity of chitosan extracted by sulfuric acid was investigated by its degradability in nitrous acid solution.[15] Complete digestion of extracted chitosan in nitrous acid and similarity of its IR spectra to spectra of reference chitosans might indicate the high purity of this cell wall derivative. On the other hand, inability of the method to recover all of the reference chitosans might be because of partial hydrolysis of chitosan chains that leads to the formation of cold acid-soluble chitosan oligosaccharides. However, because the hydrolysis occurs mostly on medium-molecular-weight chitosan, the mechanism of this process is not completely clear. As previously mentioned, medium-molecular-weight chitosan is dissolved faster and precipitates later than the low molecular weight. It might indicate higher interaction of hot sulfuric acid with chitosan with longer chains, which results in a higher degree of hydrolysis of longer chains than the shorter ones.

The phosphate measured was 19.9% of AIM and 19.3% of AAIM of *R. pusillus* in this work (Table 3). Normal phosphate concentrations in the cell wall of fungi are in the range of 0.1–2.0% of the wall dry weight, but the cell walls from several Mucorales contain much higher amounts, 16% for *Mucor mucedo* and 23% for *Mucor rouxii* hyphae.[5] Hot acid-insoluble material (HAIM) was 29% of AIM and 32.1% of AAIM. Total *N*-acetyl glucosamine content of HAIM (derived from AIM) was 34.6%, and because the yield of HAIM is 29% of AIM, this value is in agreement with *N*-acetyl glucosamine content of AIM, 0.085 g/g. *N*-Acetyl glucosamine could be present in the form of chitin or other sources such as glycoproteins. HAIM may also contain polyglucuronic acid and polyphosphate that have not been hydrolyzed. It is therefore difficult to judge the more detailed composition of HAIM. Further methods should be developed to be able to analyze this material.

Direct extraction of chitosan from biomass showed that alkali extraction doesn't have major impact on chitosan recovery.

Conclusion

Chitosan can be extracted from its complex in the cell wall of fungi by hot dilute sulfuric acid treatment with high purity and yield. This method is able to break the chitosan complex and liberate polyphosphates in the form of soluble phosphates. Chitin is recovered as hot acid-insoluble material.

Nomenclature

AIM	alkali insoluble material
AAIM	alkali and acid insoluble material
AcSM	acetic acid soluble material
HAIM	hot acid insoluble material

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