Cell Wall Structure of the Marine Fungus, *Atkinsiella dubia*

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**Summary.** Cell walls of the marine Oomycete, *Atkinsiella dubia* were prepared and an analysis of the wall constituents was made. The walls contained approximately 80% polysaccharides and 14% proteins along with small quantities of lipid and ash. The carbohydrate fraction was composed primarily of glucan along with 1.8% glucosamine and a trace of galactosamine. An analysis of the amino acid composition of the protein fraction showed the presence of 18 identified amino acids including a surprisingly high (20% of total amino acids) hydroxyproline content. The polysaccharide fractions of the wall were mostly glucans with solubility properties similar to those reported for other Oomycetes. As anticipated, the glucans of mechanically isolated walls were virtually identical to those prepared from chemically isolated walls. The minor glucan component, cellulose, was found to occur in the form of poorly crystalline cellulose I. As expected, electron microscopy of wall specimens showed microfibrillar and amorphous regions. It was stressed that *Atkinsiella* walls, like those of other Oomycetes, contain large quantities of $\beta$-1→3 and $\beta$-1→6 linked glucan along with a smaller amount of cellulose.

Among the fungi known traditionally as Phycymycetes, the organisms in the Class Oomyeetes differ from other phycymycetous forms since their cell walls contain cellulose and chitin is absent (Frey, 1950; Bartnicki-Garcia, 1966; Aronson et al., 1967). At one time the Oomycetes were thought to be unique in this respect, but recent investigations indicated that cellulose, although not common throughout the fungi, does occur on other taxa (e.g. Aronson, 1965; Bartnicki-Garcia, 1968). However, recent studies of wall structure in the Oomycetes revealed that cellulose was not the major wall constituent (Parker et al., 1963) and focused attention on the noncellulosic polysaccharides which are $\beta$-(1→3) and $\beta$-(1→6) linked glucans (Bartnicki-Garcia, 1966; Aronson, et al. 1967; Cooper and Aronson, 1967).

*Atkinsiella dubia* (Atkins) Vishniae is a little known and rarely collected Oomycete (Fuller et al., 1964). Since it is of marine origin, our investigation yielded the opportunity to make comparisons of cell wall structure between *Atkinsiella* and related fresh water forms.
Materials and Methods

Culture Methods. Atkinsiella dubia was grown in culture medium of the following composition: NaCl, 20 g; KCl, 0.9 g; KH₂PO₄, 0.1 g; MgSO₄·7H₂O, 6.5 g; CaCl₂·2H₂O, 0.15 g; monosodium glutamate, 2.0 g; glucose, 5.0 g; TRIS buffer, 1.0 g; trace metals, according to Goldstein (1963); and double-distilled water to make 1 l of solution. To avoid precipitate formation the CaCl₂, MgSO₄, and glucose were autoclaved separately in a portion of the water. The rest of the medium was adjusted to pH 7.8 with 0.1 N HCl. The two portions of the medium were combined after autoclaving. A. dubia can be maintained for many generations on this medium and, hence, does not require an external source of any vitamins or amino acids. Although nitrate is not utilized as a source of nitrogen (NH₄)₂SO₄ can be substituted for the monosodium glutamate in the medium employed. Like a number of the other phycomycetous marine fungi (Goldstein, 1963), A. dubia will not grow when the amount of sodium (added here as NaCl) in the medium is reduced below a minimum level. Good growth of this fungus occurs when the range of NaCl is between 1.5 and 3.0%. Demonstration of an absolute requirement for sodium by A. dubia will require further study. After cultivation for 10 days on a shaker, the mycelia were harvested, washed with H₂O on a Buchner funnel, and subjected to gentle suction to remove excess moisture.

Cell Wall Isolation. Cell walls were isolated mechanically by homogenizing the mycelia in a Servall Omnimixer operated at maximum speed (ca. 16,000 rpm). The homogenizer cup was cooled in an ice bath through the entire procedure. Initially the hyphae were broken by suspending them in cold 0.03 M EDTA, pH 7.8 and homogenizing for 10 min. The homogenizer was stopped after each minute of treatment and 2—3 min were allowed for cooling the contents of the cup. The homogenate was centrifuged at 1600 g, the supernatant fluid was discarded, and the sediment of partially cleaned walls was washed five times with cold H₂O. The walls were then homogenized in cold H₂O for 15 min with cooling at 1 min intervals as described above. After each 5 min of homogenization, and at the completion of this treatment, the walls were washed twice in H₂O. A further 5 min of homogenizing was carried out in cold 0.03 M EDTA and followed by five washings in H₂O. A final 5 min of homogenization in H₂O was employed and the walls were then washed five times with H₂O and freeze-dried. Microscopic examination of the completed wall preparation showed that it was apparently free of cytoplasmic particles. Mechanically isolated walls, prepared as described, were used in all analytical procedures unless otherwise noted.

For X-ray diffraction and electron microscopic observations, cell walls were isolated chemically (Aronson et al., 1967). They were then treated according to the procedure of Roelofsen and Houwink (1953) which involved heating in a mixture of 30% H₂O₂ and glacial acetic acid (1:1 v/v) and then heating in 0.1 N HCl.

Cell Wall Hydrolysis. Complete acid hydrolysis of mechanically isolated walls was carried out by treating 100 mg of wall material with 3.0 ml of 27 N H₂SO₄ for 10 hours at 22°C. The acid was then diluted with H₂O to 3 N and the sample was heated at 90°C for 10 hours. The hydrolyzate was neutralized with BaCO₃ and the precipitated BaSO₄ was removed by filtration. The filtrate was freeze-dried yielding a salt contaminated hydrolyzate. This dried powder was suspended in hot 50% ethanol and the ethanolic fluid was filtered. The filtrate was concentrated to 20 ml by rotary evaporation at 40°C. This final concentrate was freeze-dried and subsequently utilized in the identification of the monosaccharide constituents of the cell walls.

For amino acid analyses, cell walls (20 mg) were hydrolyzed in 6 N HCl at 110 °C for 20 hours.