General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.

- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.

- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.

- This document is paginated as submitted by the original source.

- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

ARCHITECTURE OF DERMATOPHYTE CELL WALLS:
ELECTRON MICROSCOPIC AND BIOCHEMICAL ANALYSIS

Yasuo Kitajima and Yoshinori Nozawa

1. Report No. NASA TM-77441	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle ARCHITECTURE OF DERMATOPHYTE CELL WALLS: ELECTRON MICROSCOPIC AND BIOCHEMICAL ANALYSIS		5. Report Date July, 1984
		6. Performing Organization Code
7. Author(s) Yoshinori Nozawa Yasuo Kitajima		8. Performing Organization Report No.
		10. Work Unit No.
9. Performing Organization Name and Address SCITRAN Box 5456 Santa Barbara, CA 93108		11. Contract or Grant No. NASw 3542
		12. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Byogensei hifukeijokin no saiboheki kochiku koso: Denken oyobi seikagakuteki kaiseki", Japan. J. Med. Mycol., Vol. 18, (No. 1), pages 3-15, 1977.

16. Abstract

A review with 83 references on the cell wall structure of dermatophytes.

17. Key Words (Selected by Author(s))		18. Distribution Statement	
		Unclassified and Unlimited	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 29	22. Price

ARCHITECTURE OF DEROMATOPHYTE CELL WALLS: ELECTRON MICROSCOPIC AND BIOCHEMICAL ANALYSIS

Yasuo Kitajima and Yoshinori Nozawa*

INTRODUCTION

The cell walls of true fungi not only physically protect the structure and function of the cells from the outside environment, but also have biological functions and properties with regard to the presence of specific antigens, the strength of pathogens, and dimorphism of the fungus. Consequently, clarification of the structure and functions of these complex cell walls is said to be one effective approach in explaining therapeutic problems with regard to current mycoses. A molecular structural model of cell walls has been seen in the field of bacteriology in biochemical and morphological research, particularly in research using electron microscopes. Various antibodies have been developed and their mechanisms have been clarified with this extensive research [1-3]. The importance of research on cell structures, including cell walls, is noted. On the other hand, research on the cell walls of true fungi has been considerably delayed in comparison to that of bacteria. Nevertheless, recent interest has been noted with regard to the cell walls of pathogenic true fungi and important information on dimorphism [4-5] and pathogenicity [16-22] is being accumulated.

Morphologically, the cell walls of true fungi are constructed from a mesh-lie structure made of microfibriles and an amorphous matrix, in which there are crevices between the fibriles

---

*Department of Biochemistry, Gifu University School of Medicine, Gifu, Japan
**Numbers in margin indicate foreign pagination.

(amorphous matrix). These two structures appear to give the cell walls a layered structure. Moreover, chemically, the cell walls are made from polysaccharides, such as mannan, various glucans, etc., and chitins containing small amounts of peptides [23]. At the heart of these biochemical analyses are the summaries of taxonomical and morphological data of Bartnicki-Garcia [24]. There have recently been stru tural models of the cell walls of dermatophyties [25] and scientific progress in this field is anticipated in the future.

Here we will attempt to clarify the structure of cell walls of dermatophytes by the following items, centering on research results on the cell walls of Trichophyton mentagrophytes [26, 28-30] and Epidermophyton floccosum [27, 31-33], which have been studied for the past 10 years in our laboratories.
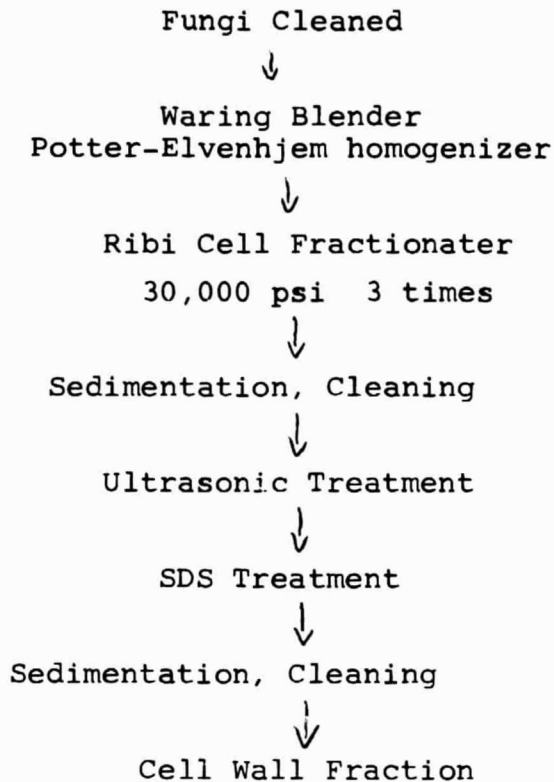
1. Separation and preparation of cell walls
2. Micro.tructure of cell walls by electron microscopy
3. Chemical composition of cell walls

    1) sugar component

    2) protein component

    3) fat component

4. Structural model of cell walls

    1) hypha cell walls

    2) septate cell walls

5. Morphological structure of cell walls

1.   Separation and Preparation of Cell Walls

The first requirement in the study of cell walls is to pre-
pare cell wall fractions that are of a high purity and are as
similar as possible to the native. One reason for the considerable
delay in the study of cell walls of dermatophytes in comparison
to that of enzyme-type true fungi is that these cell walls are
usually very difficult to prepare. Moreover, preparation is
further complicated by the fact that it is difficult to completely
decompose the cells because the fungi have many septae. Conse-
quently, in order to remove the cells that have not been decom-
posed because of the septae, preparation must be carried out by
density gradient-type centrifugation [29]. Moreover, cell wall
fractions with the necessary purity are not obtained and therefore,
the entire cell must first be completely destroyed in order to
separate cell wall fractions of a high purity.

Tests on various methods have been reported with regard to
decomposition of true fungi cells. For instance, ultrasonic
treatment, Sorvall ominmixer [35-37], French press 38-39, Braun
homogenizers [40-42], Waring blenders [42], or combinations of
these have been used. Although results that are just as good
as those seen with the Braun homogenizer are obtained [26], the
Ribi cell fractionator method gives the most efficient results
[29,21] By means of the Ribi cell fractionator (Ivan Sorvall,
Inc., Norwalk, Connecticut, U.S.A.), a high pressure is exerted
on the solution continuing fungi, which is placed in a copper
cylinder (approximately 150 ml). The solution is obtained with a
needle valve and cells are destroyed by exposure to a suddenly
reduced pressure.

Figure 1   Method for Preparing Cell Walls of Dermatophytes [31]

```
                    Fungi Cleaned
                         ↓
                  Waring Blender
            Potter-Elvenhjem homogenizer
                         ↓
               Ribi Cell Fractionater
                30,000 psi   3 times
                         ↓
              Sedimentation, Cleaning
                         ↓
               Ultrasonic Treatment
                         ↓
                  SDS Treatment
                         ↓
            Sedimentation, Cleaning
                         ↓
                Cell Wall Fraction
```

The most efficient method for preparation of fungus cell walls
is shown in the figure (Figure 1). First of all, the cleaned
fungi are sufficiently treated with a Waring blender to obtain a
fungi solution. Furthermore, a uniform solution is then obtained
by homogenzation with the glass Potter-Elvehjem homogenizer. The
fungi that have been thus treated can then be easily passed through
the needle valve of the Ribi cell fractionater. The efficiency
of fungi destruction is thereby improved. Next,
decomposition of the cells in a Ribi cell fractionater is performed
three times under a high pressure of 30,000 pounds/inch^3. The
dermatophytes of Trichophyton, Epidermophyton, and Microsporum
are all destroyed. After these products have been rinsed several
times, the membrane component that adheres to the inside of the
cell walls is removed by ultrasonic treatment. The final solution
is incubated for 1 hour at room temperature in 1% SDS (sodium dodecyl
sulfate)and is then rinsed and separated. According to electron
microscopic studies, the cell wall fraction of E. Floccosum prepared
in this way is very pure and 280 nm absorption by proteins in the
supernatant liquid is not noted (Figure 2a, b, c). This method was
4 also used by Barran 44 for Fusarium sulphureum and it has recently

been reported that good results were obtained. In addition, the protein decomposing enzyme method [29,45], the RNase method [29], and the density gradient centifugation method [29] have been reported. Although there are not problems with cell wall purity, these methods do have disadvantages from the points of the yield rate, operation complexity, etc.

Therefore, with regard to mechanical fractionation as seen with the Ribi cell fractionater, the difference in resistivity between different types of fungi is considerable, even when the same dermatophytes are used, and should be noted. For instance, in contrast to the fact that all cells were fractionated by treatment of E. Floccosum one time under 30,000 pounds/inch^2, the same extent of fractionation of T. mentagrophytes is seen only after 3 treatments. This fact shows that there is a difference in the structure of cell walls.

Next, the purity of the cell wall fractions differed with the cells, nucleus, and other membrane systems. In many reports the results of differential phase microscopes [45, 46] and of methylene blue staining tests [5, 47, 48] are sited. Although optical microscopes are sufficient for determining the presence of mixtures of unfractionated cells, it is also necessary to use the results of electron microscopic studies, that is, thin film methods, shadowing methods, and scanning electron microscopic methods to determine whether there are mixtures of cell substances. On the other hand, there are also methods that determine the amount of proteins [49,50] and nucleic acids [49,51] by absorption of the supernatant liquid at 280 nm and 260 nm. In addition, lymph fat substances can also be determined [31]. In any case, it appears that both electron microscopic methods and biochemical methods are essential for the determination of the purity of the cell wall preparation.

2. Microcell Structure by Electronmicroscopic Study of Cell Walls

Thin slice electron microscopy (electron microscopy), shadowing, scanning electron microscopy, and etching microscopy are widely used to observe the microstructure of cell walls of fungi.

In general, when the cells of true fungi are observed by thin specimen microscopy, a layered structure with a thickness of

5

approximately 0.1-0.5 is obtained. Dermatophytes appear
to have the same structure and reports have been seen on <u>Tricho-
phyton</u> [52-54] <u>Epidermophyton</u> [55-56], and <u>Microsporum</u> [57-58].
When the cell walls of E. Floccosum were observed after being
fixed with potassium permanganate and stained with uranium sulfate,
it was noted that the outer layer of the cell walls was very thin
at 0.01-0.05μ. . There was also a layer with a low
electron density of 0.1-0.2μand a layer with a somehwat higher
electron density of 0.1-0.2 μ was found inside the cell wall (Figure 2b) [31].
The boundary of the two inside layers was not clear. However, it
was very obvious that the outside layer was a very thin layer .
These findings are in agreement with the results of cell wall observations
on untreated cells [55,56]. As a rule, this 3-layered cell structure
is also noted in thin speciments of cell walls of Trichophyton [52-54]
and Microsporum [57-58].

On the other hand, according to the shadowing method, the
cell walls of dermatophytes are also free from microfibriles and an amorphous
matrix with crevices. In general, the inside surface is a clear
net-like structure because of these microfibriles and the outer
surface does not have a clear fibrile structure. Figure 3 shows
the shadowing micrographs of the inside surface of <u>E. Floccosum</u> [31].
A net-like structure where the microfibriles are arrranged irregularly
is seen and an area where fibrile crevices are noted is clear.
This structure is also seen in the cell walls of <u>T. rubrum</u>, <u>T.
mentagrophytes</u> [20], and <u>M. Coocki</u>. On the other hand, Figure 4a
shows the outer surface of the cell walls of <u>E. floccosum</u>.
A fibrile structure is not obvious. However, in examples that were SDS
treated the structure mainly consists of proteins (mentioned
later), as shown in Figure 4b. Therefore, it can be said that a
fibrile structure is seen in the bottom layer. These fibers
run parallel to the hyphae axis and form bundles. A fibrile structure
that differs from that of the inside surface is therefore formed [31].

Thereupon, the nonfibrile structure of the outside surface
appears to be the same as the outer surface with a high electron
density of the 3-layered structure that was seen with thin specimens [33].
This layer is physiologically different from the fibrile section
of the 2nd inner layer when freeze-fracture replicas are used. Figure 5
shows an electron micrograph of the replicas. A relatively smooth
surface without fibrile structures is seen. This inside layer
has a particle-like structure. The particle structure is reported to

6

be different depending on the type of fungus that is studied [50].
Furthermore, with regard to freeze fracture of the outside l.yer,
the cell walls of Geotrichum candidum [60], Penicillium urticae[61],
etc. is reported to have a "rodlet pattern." The outer layer
of these cell walls is structurally different from the inner layer in
thin speciment micrographs, shadowing micrographs, and freeze
fracture micrographs. This is a significant point from a chemical
sense and, as will be mentioned later, we were successful in preparing
only the outer layer and explaining its chemistry [33].

As was mentioned above, as a result of morphological studies,
it appears that the cell walls of the dermatophytes of T. mentagrophytes
and E. floccusum have a 3-layered structure. The outside layer
is very thin, but there is a structure where there are no fibers
and the electron stainability is very high. The 2nd layer is
made from microfibriles and an amorphous matrix.

3. Chemical Composition of Cell Walls

In order to determine the chemical structure of the cell
walls of true fungi, it is necessary to separate the high
molecular weight components, such as polysaccharades, peptides, etc,
which are one structural component. In order to do this, the
cells must be decomposed with a strong acid or alkaline water and
enzyme treated [51]. Therefore, tests on the
properties of the isolated polymers are necessary[51]. We have
carried out detailed studies on the hydrolysis and gas chromatography
conditions necessary for analysis of the polysaccharides in the cell
walls of dermatophytes [29,62].
Chemical analysis of the cell walls of dermatophytes has first
been reported by the X-ray diffraction studies of acid and alkali-
treated samples prepared by Blank (1953) [63].
Chitin was first discovered. Then Bishop[64], Shah and Knight [36]
and the authors of this text [26-33]   reported on analytical results
of prepared cell walls. However, it cannot be said that any of
the results were satisfactory. The chemical composition of the
cells walls of T. mentagrophytes, E. floccosum,   M. Canis, and M. gypseum
are shown in Table 1.

1) Sugar Component

As Shown in Table 1, approximately 80% of the cell walls of dermatophytes is sugar, that is, neutral sugar (glucose and mannose) and amino sugars (N-acetylglucosamine).

The first report of neutral sugars in the cell walls of dermatophytes was from studies on T. mentagrophytes. and E. fluccosum. However, Cummins and Harris (1968) [65] also detected traces in M. gypseum. Then Bishop (1962)[64] reported that both glucose and mannose were present as components of cell walls of all dermatophytes. However, according to Shah and Knight in 1968, the main component in analyses by paper chromatography was glucose. Only 10% of mannose was detected [36]. Furthermore, in the same report it is metnioned that although traces of galactose were found in M. gypseum, none was detected in the cell walls of T. mentagrophytes [29]and E. floccosum [31]. The amount of galactose in both of these cell wall types is less than 1%. However, the ethanol sediment of soluble fractions that were extracted for 5 hours with 1-N NaOH at $100^{o}C$ was a polysaccharide, probably galactomannone [30]. Consequently, although only 1% or less of galactose is seen from the cell walls themselves, by isolation, this is noted as a specific high molecular weight compound. Polysaccharides whose main components are galactomannone have been studied as components that have important specific antigen activity from an immunological standpoint [16-19].

Furthermore, the amino sugars contained in the cell walls of dermatophytes are assumed to be chitin because of the fact that glucosamine is eluted by hydrolytic decomposition of the cell walls, the fact that N-acetylglucosamine is eluted from the cell walls with chitinase decomposition, and the fact that by S-ray diffraction of the residue of the cell fraction after acid and alkali decomposition, the same pattern as seen with standard chitin is obtained [29 ,21, 36, 63] (figure 6) . The sugar compositions vary with the type of fungus and can be used in taxonomical classifications [54].

Table 1 Chemical Composition of Dermatophyte Cell Walls

	T. mentagrophytes*1)	E. floccosum*2)	M. canis*3)	M. gypseum 3)
a. 総 中 性 糖	51.9%	42	48.3	56.6
b. グ ル コ ー ス	36.2	35	37.5	45.8
c. マ ン ノ ー ス	11.7	7	11.4	6.7
d. ガ ラ ク ト ー ス	+	+	—	—
e. N-アセチルグルコサミン	30.4	34	26.6	29.7
f. タ ン パ ク	7.8	10	6.8	3.5
g. 脂 質	6.6	6	4.0	3.3

*1) 文献29   *2) 文献31   *3) 文献36

*1) reference 29   *2) reference 31   *3) reference 36

Key: a. total neutral sugars
      b. glucose
      c. mannose
      d. galactose
      e. N-acetylglucosamine
      f. protein
      g. fat

2) Protein Component

Although research on proteins contained in dermatophyte cell
walls indicates that 7-10% of the cell wall is occurpied by protein,
analyses are limited to the structure of amino acids in the total
cell wall. Shah and Knight [36]have carried out amino acid analyses
on cell walls of T. mentagrophytes, M. canis, M. gypseum and E. fluccosum
and have reported that although a large amount of methionin is found
in M. canis and M. gypseum, almost none is seen in T. mentagrophytes
and that the opposite correlation is noted with regard to cystein.
Moreover, a large amount of leucine is seen in T. mentagrophytes
and M. canis, but little is noted in E. fluccosum and M. gypseum.
The same results were obtained in our studies on T. mentagrophytes
and E. floccosum [29,30]. In general, a large amount of
amino acids that appear to participate in bonding with sugars is found
in these dermatophytes (glycine, alanine, glutamic acid, aspergillic
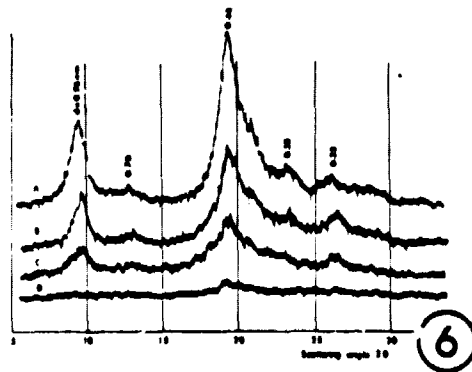acid, seronin, serine, and leucine) [66].

Figure 6 X-Ray Diffraction

A: standard chitin, B: prepared T. mentagrophytes cell wall acids,
alkali-treated specimen, B: E. floccosum fraction of the same type,
C: prepared cell walls of
both types

With regard to the presence of proteins with the above-mentioned
features (peptides) in the dermatophyte cell walls, almost no
knowledge is now available. The same is true of other true fungi.
Analyses by peptide mapping of proteins in the cell walls of
Aspergillus niger and Chaetomium globosum have been carried out, but no-
thing definite has been determined [67].

However, we have studied E. floccosum by isolation of the outer
layer of a 3-layered cell walls. It has been discovered that this
layer is mainly made from peptides (63%) [33]. When this fraction
was reacted with Helicase (digestive enzyme of Helix pomatia),
the 2 inner layers were easily dissolved. However, the outside
layer was not decomposed. Figure 7 shows an isolated outer layer
(exolayer) thin specimen image. It is clear that the structure is
a film-like structure with a thickness of 200-500 Å. Figure 8
is an image by staining. A roughnet-like structure is seen.
As was previously mentioned (Figure 5), freeze-fractured images are
observed as a smooth structure. This structure is that of a glycopeptide
containing only mannose. Furthermore, aspergillic acid and glutamic
acid [66], which are thought to participate in bonding of cerine,
seronin, and N-glycosyl, which seem to be the mannose or O-mannose
bonds, occupy 41% of the entire amino acid and glycine and alanine,
which are important component of peptide glycanes of bacteria,
occupy 27%. This fact appears to show that an

10

irregular peptide glycan-like layer is present in the outermost layer of the walls of cells of true fungi, as is the case with bacteria cell walls. The outermost layer of cell walls of T. mentagrophytes was recently isolated and it appears that the main component is protein (80%), while the walls also contain glucomannone (7%).

In any case, there are still many unclear points with regard to the protein content of cell walls of dermatophytes. Gander[69] has reported in detail on the glucoproteins of the cell walls of other true fungi.

3) Fat Components

The method of extraction of bound-lipids and easily extracted lipids by Bartnicki-Garcia [4,70] and the method of Bligh and Dyer [71] can be mentioned as methods for extraction of fats from the cell walls of true fungi. The bound lipids in the former method contain many other lipids and the latter is a useful method for analysis of fats. The amount of fat in dermatophyte cell walls is 2-7 % (Table 1). However, only the fat composition of E. floccosum has been analyzed and reported and the presence of triclycerides and liberated fatty acids has been clarified[31]. Traces of sterols, lymph fats, etc. have been detected. However, these appear to be mixed with the membrane components. The presence of ribopoly- saccharides, such as those seen with gram negative fungi, has not been confirmed.
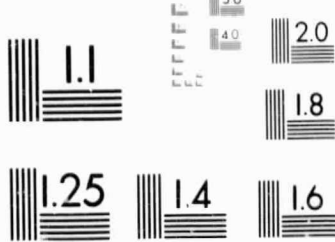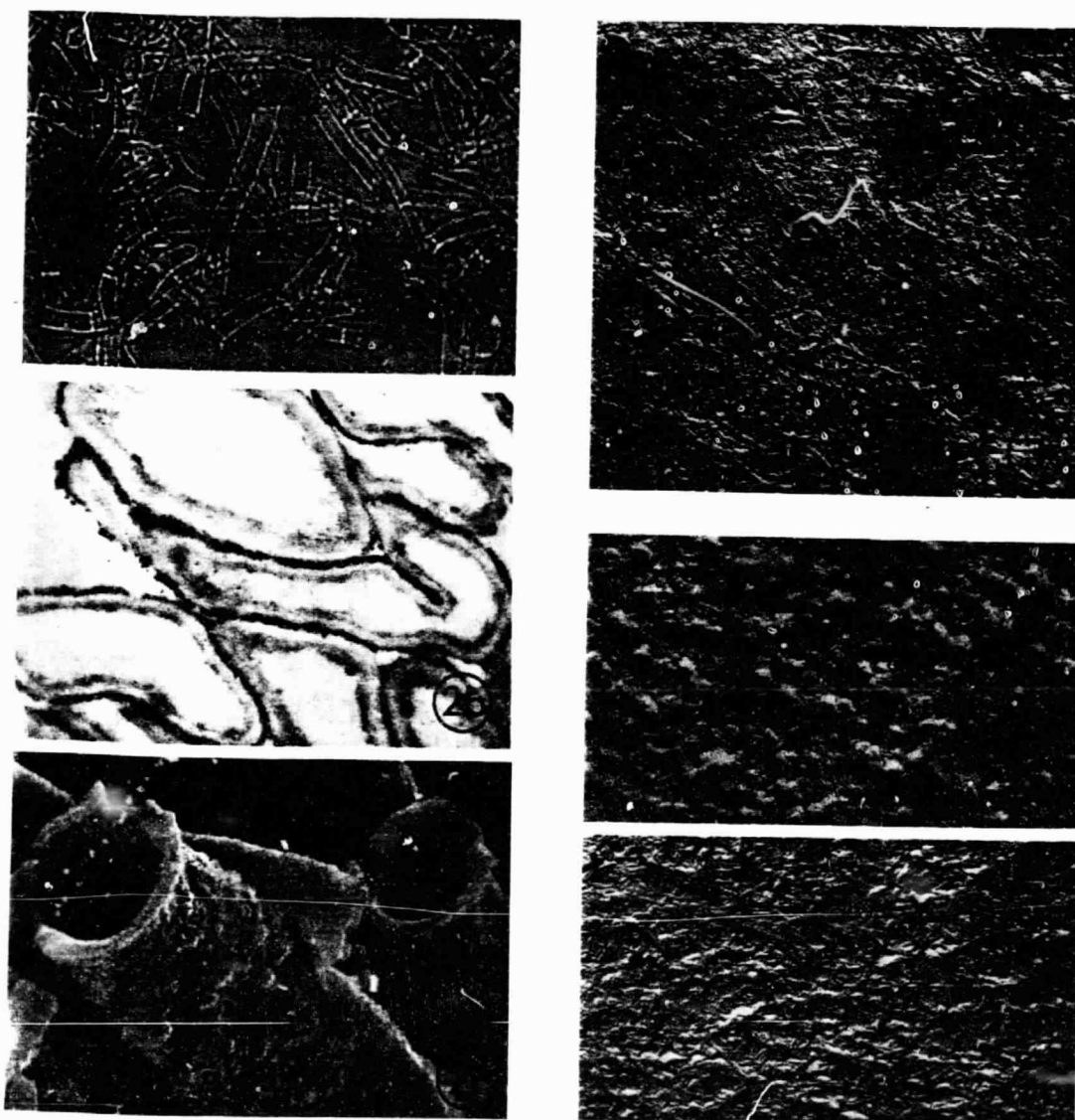
Figure 2a   Phase Differential Micrograph of Cell Wall Fraction of E. floccosum   X 400

      2b   Same thin layer micrograph; 3-layered structure noted.   x 11,000

      2c   Same scanning electron micrograph; x 5,000

Figure 3   E. floccosum   inner layer shadowing micrograph; fibrile and amorphous matrix structure, x 36,000

Figure 4a   Shadowing micrograph of outermost layer of E. flocossum; fibrile structure not noted; x 36,000

      4b   Shadowing micrograph of outermost layer of E. floccosum isolated by SDS treatment; different structure from inner layer; x 36,000

13
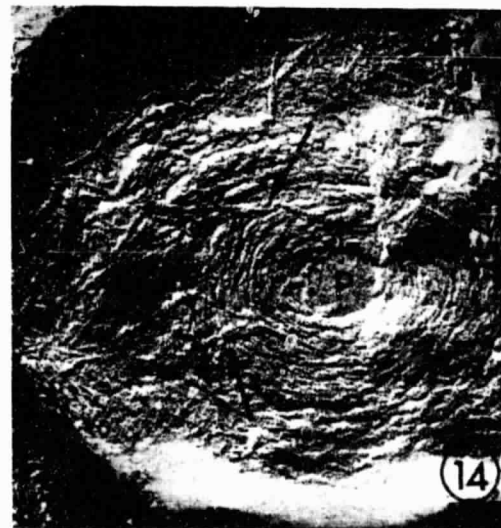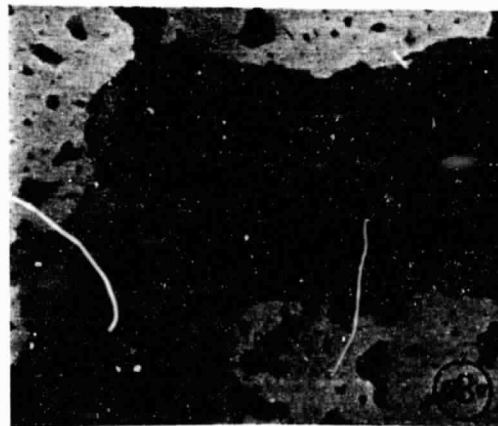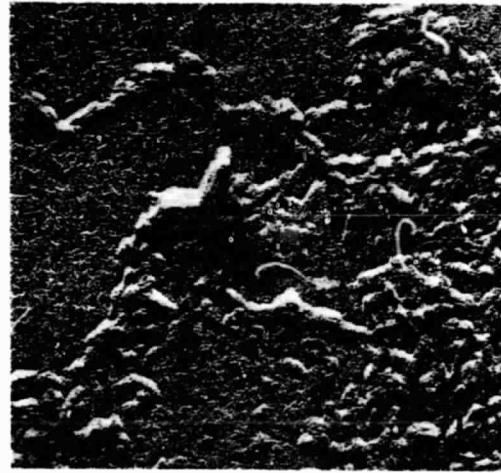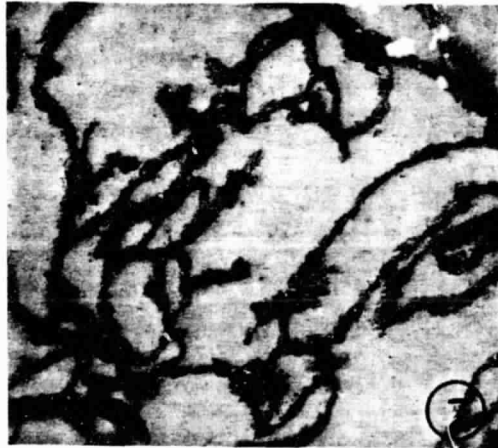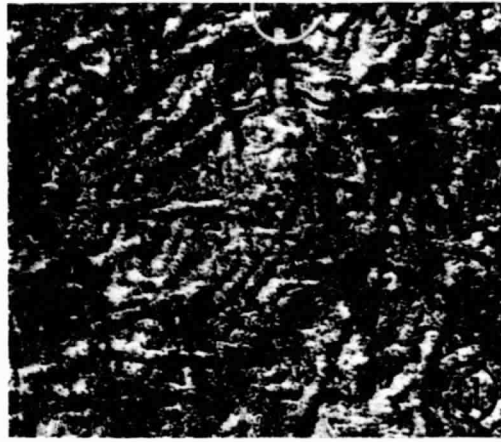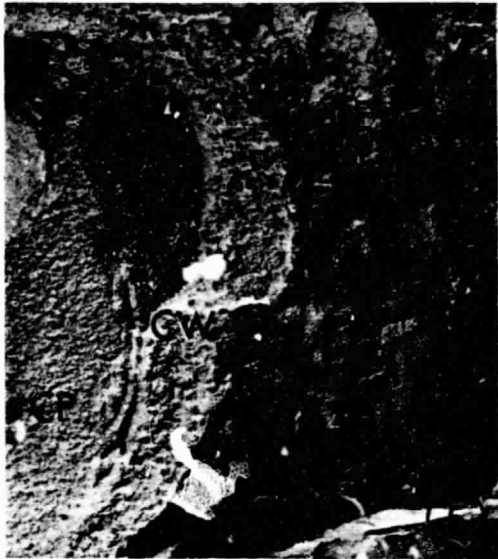
Figure 5 Freeze fracture of E. floccosum; E: tear along outer layer of cell wall; C: cross section of cell wall; PM tear of cell membrane (surface A); CP: cell cross section, x 36,000

Figure 7 Outer layer (ex-layer) of walls of cells isolated from E. floccosum, x 36,000

Figure 8 Negative stained speciment of outer layer of E. floccosum, x 10,000

Figure 11 Shadowing micrograph of alkali-treated fraction of T. mentagrophytes; diameter of approximately 200 A, x 100,000

Figure 12 Shadowing micrograph of fraction treated with acid (Figure 12); Fraction with which chitin is identified in X-ray diffraction, x 100,000

Figure 14 septa of T. mentagrophytes; middle layer noted in electron transmission of thin slice (mainly chitin; microfibrile structure from inside of hyphae cell walls (arrows)), x 50,000

4. Structural Model of Cell Walls

1) Structure of Hypha Cell Walls

The micromorphology and chemical structure of cell walls was discussed in above-mentioned item 2. However, here we will discuss the structural models of cell walls based on this data.

The two methods of electron micrographic cell chemistry and chemical analysis of the soluble and residue portions of alkali and acid enzyme treatment of cell walls will be considered for determinations of the chemical properties of the cell structure of cell walls. Poulain showed a model for the cell walls of T. mentagrophytes with the former method in 1976 (Figure 9). Researchers have used the latter method. However, only T. mentagrophytes [26,30] and E. floccosum [27] have been used.

Furthermore, one method for fractionation of the cell walls of T. mentagrophytes is shown in Figure 10. Moreover, the chemical structure of this fraction is shown in Tables 2, 3, and 4. First of all, fraction Sup-I that has been made soluble by initial alkali treatment has an amorphous matrix structure with fiber crevices. It is made from a polysaccharide whose main component is galactomannone having a 1,6 bond, 1,2 bond and 1,3 bond and a glucane whose main chain is a β-1, 6 and β-1, 3 bond. The residues that are obtained after these fractions have been extracted are made from only microfibriles, as shown in Figure 11. With regard to these microfibriles, the fibrile structure is lost by removal of the Sup-II of the acid-soluble

fraction. The structure changes to a crystal chitin structure,
as shown in Fgiure 12. Consequently, the microfibriles appear to
be made into fibriles by the glucomannone peptide complex (Sup-II)
which is soluble in acids and nonsoluble in alkalis [26]. The same
findings are obtained for E. Floccosum.

     The model of the cell walls is shown in Figure 13 in order to
clarify the above-mentioned points. Of the 3-layered
structure observed by observation of thin slices with electron
microscopes, the outermost layer (exolayer) is made mainly from
a sugar-protein layer whose main component is protein and which is
200-500 A thick. The 0.1-0.2 middle layer is a dense layer of microfibriles
made mainly from chitin and glucane. The inner layer is 0.1-0.2 thick
and has a rough microfibrile structure.   Amorphous
galactomannone-glucane-peptide complexes are found in the crevices.

     2) Septum Structure

     The septa that were observed by microscopic analysis of thin
specimens were made from 3 layers, of which one layer
in between two other layers had a low electron density [26,56].
This structure is the same structure as that of true fungi,
including dermatophytes. In the case of T. mentagrophytes,
the surface that makes contact with the cell membrane has
the same fibrile structure as the inner layer of the cell walls of
hypha [26]. However, this layer is peeled off by papain treatment
and presents a structure where the microfibriles in the middle
layer are arranged concentrically (Figure 14). This microfibrile
structure appears to be made mainly of chitin [26]. Figure 15
is a model of this structure [26].

Table 2 Sugar Composition of Each Sup Fraction of T. mentagrophytes
Cell Walls 30

	α 細 胞 壁	Sup- I	Sup- II	Sup- III
b 糖 含 量 (%)	47.9	67.5	58.0	47.0
c 構 成 糖 比	man : glc 1 : 3.1	man : glc : gal 7.9 : 1.1 : 1	man : glc 1 : 1.4	glc 1
d ペプチド含量 (%)	7.8	7.5	23.8	0

man: mannose, glc: glucose, gal: galactose

Key: a. cell walls   b. sugar content (%)   c. structural sugar ratio

d. peptide content (%)

Table 3 Sugar Composition of Each Res Fraction of <u>T. mentagrophytes</u> Cell Walls 30

b	a 細胞壁	Res- I	Res- II	Res- III
糖 含 量 (%)	47.9	22.9	12.9	3.3
c 構 成 糖 比	man : glc 1 : 3.1	man : glc 1 : 5.3	glc 1	glc 1
d N.アセチルグルコサミン (%)	37.4	50.4	62.6	77.1

man: mannose, glc: glucose

Key: a. cell walls b. sugar content (%) c. structure sugar ratio
d. N-acetylglucosamine (%)

Figure 4 Sugar Composition of Each Pt Fraction of <u>T. mentagrophytes</u> Cell Walls

a	Sup- I	Pt- I	Pt- II	Sup- I'
糖 含 量 (%)	67.5	83.9	68.8	23.9
b 構 成 糖 比	man : gal : glc 7.9 : 1.1 : 1	man : gal 25.7 : 1	man : gal : glc 16.4 : 1 : 2.8	man : glc 1 : 1.3
c ペプチド含量 (%)	7.5	12.5	6.8	0

man: mannose, glc: glucose, gal: galactose

Key: a. sugar content (%) b. structure sugar ratio c. peptide content (%)
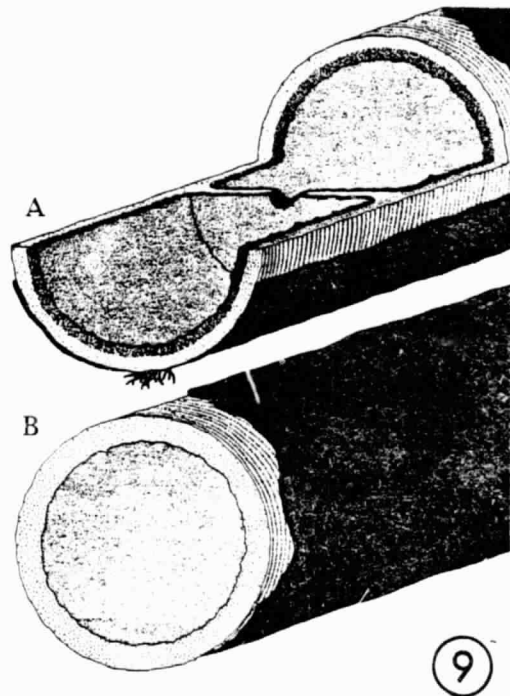
Figure 9 Model of Cell Walls of T. mentagrophytes Observed by
          Electron Microscopic Cell Chemical Staining [25]
A: 3 layered structure of hypha cell walls, B: inner layer lost
     in mature hypha. The inner layer is mainly composed of galactomannane
     and the middle layer is composed mainly of chitin. The outer
     layer is a layer with a microfibrile structure.

Figure 10   Fractionation of Polysaccharides in Cell
Walls of T. mentagrophytes [30]

Key: a. prepared cell walls   b. centrifugation for 5 hours in 1 N NaOH at
                                     ˙00°C

       c. supernatant fluid   d. cold ethanol

       e. sedimentation   f. felling liquid

       g. dialysis   h. ethanol chloride
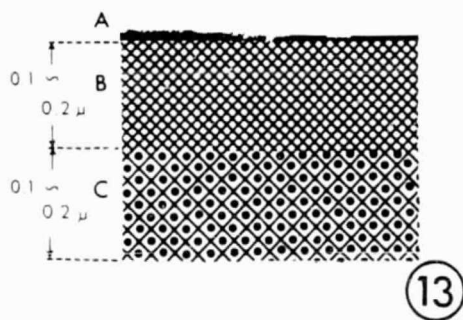
       i. freeze-drying

Figure 13 Cell Wall Models for T. mentagrophytes and E. floccosum
A: outer layer (exolayer) made mainly from sugar proteins, B:
layers made mainly from chitin-blucan fibriles, C: layer made from
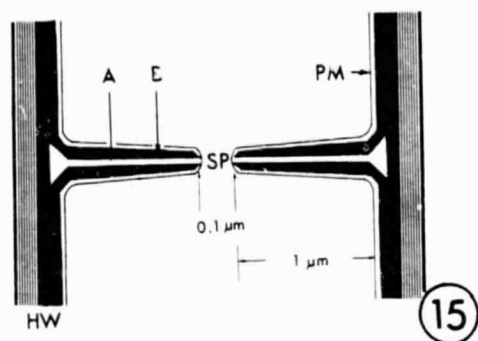fibriles in B and polysaccharide proteins soluble in an alkali

Figure 15 Cell Wall Model for T. mentagrophytes
A; chitin layer, B: layer made connected to inner layer of cell wall,
made from microfibriles and amorphous matrix, PM: cellular membrane,
HW: hypha wall

5. Morphology of Cell Walls
    The following method will be considered for the synthesis of
polysaccharide protein complexes, which are the basic
microfibrile and matrix substances arising from chitin, which is found
in cell walls. That is, 1. oligosufats synthesized from rzibosomes [72]
or membrane-like structures[73] and small amounts of peptides are
first excreted to outside the cell membrane and
self-assembly then occurs [74-76]. 2. polysacchride-synthesizing
enzymes, such as chitin-synthesizing enzymes, are excreted to
outside the cell or to the cell membrane [77-79]. Synthesis
therefore occurs outside the cell in this method.
    In contrast to the fact that a yeast protoplast
is not noted in cell cultures and the formation of cell wall microfibriles
is not seen, precursors that are excreted to outside the cell are
seen in gel cultures and therefore, self-assembly is possible.
Necas has studied this theory in detail [74]. Furthermore, there
are other mentions of polysaccharide synthesizing enzymes in the
bibliography.
    The former method involves an N-acetylgluocsamine -GDP and
chitin-synthesizing enzyme. When these are incubated, chitin
fibers are made from the enzyme particles [80] That is, synthesis of

19

chitin particles occurs. Furthermore, there have recently been
reports that the chitin-synthesizing enzyme chimogen is present
in the cell structure of the yeats [79]. On the other hand,
observations of freeze fractures have resulted in
discovering that the synthesis of cellulose microfibriles
in plant cells occurs from particles that appears to be enzymes on
the cell membrane [81-83].

Nevertheless, in dermatophytes there is little information on
chitin-synthesizing enzymes and polysaccharide-synthesizing enzymes,
in contrast to the large amount of information of the above-mentioned
yeasts. The two above-mentioned hypothesis should be used if
it were concluded that the cell walls of dermatophytes could
also have the same structure as that of yeast-type true fungi cell
walls.

Conclusion

As was previously mentioned, the presence of a microfibrile
structure whose main component is chitin and a sugar protein
complex that is soluble in an alkali (amoprhous matrix) was confirmed
in studies on the cell walls of dermatophytes. However, the molecular
structure of the sugars and the bonding mechanism with proteins
(heptides) was not clarified. This will be studied in the future.
It will be important in solving the many problems of
bacteriology.

Bibliography

REFERENCES

[1]   Aronson, A.I. and Fitz-James, P.: Structure and morpho-
        genesis of the bacterial spore coat. Bacteriol.
        Rev. Vol. 40: 360-402, 1976.

[2]   Schnaitman, C.A. Effect of ethylenediaminetetraacetic
        acid, Triton X-100, and lysozyme on the morphology and
        chemical composition of isolated cell walls of
        Escherichia coli. J. Bacteriol., Vol. 108, 553-563,
        1971.

[3]   Schaeffer, D. Sporulation and the production of anti-
        biotics, enzyme, and exotoxins. Bacteriol. Rev. Vol.
        33, 48-71, 1969.

[4] thru [13] illegible.

[14]   Mendonza, L., Gorin, P.A.J., Loyd, K.O. and Travassos, L.R.
        Polymorphism of Sporothrix schenckii surface plysaccharides
        as a function of morphological differentiation. Bio-
        chemistry, Vol. 15, 2423-2431, 1976.

[15]   San-Blas, F., San-Blas, G. and Cova, L.J. A morphological
        mutant of Paracoccidioides brasiliensis strain IVIC pb9,
        isolation and wall characterization. J. Gen. Microbiol.,
        Vol. 93: 209-218, 1976.

[16]   Grappel, S.F., Blank, F. and Bishop, C.T. Immunological
        studies of dermatophytes, IV. Serological reactivities
        of polysaccharides from Microsporun praecox, Trichophyton
        ferrugineum, Trichophyton sabouraudii and Trichophyton
        tonsurans. J. Bacteriol, Vol. 97, 23-26, 1969.

[17]   Grappel, S.D., Buscavage, C.A. Blank, F. and Bishop, C.T.
        Comparative serological reactivities of twenty-seven poly-
        saccharides from nine species of dermatophytes.
        Sabouraudia, Vol. 8, 116-125, 1970.

[18]   Grappel, S.G., Blank, F. and Bishop, C.T. Circulating
        antibodies in dermatophytosis, Dermatologica, Vol. 44,
        1-11, 1972.

[19]   Shimonaka, H., Noguchi, T., Kawai, K., Hasegawa, I.,
        Nozawa, Y. and Ito, Y. Immunochemical studies on the
        human pathogen Sporothrix schenkii; Effects of chemical
        and enzymatic modification of the antigenic compound
        upon immediate and delayed reactions. Infect. Immun.,
        Vol. 11, 1187-1194, 1975.

[20] Nozawa, Y., Noguchi, T, Ito, Y., Sudo, N. and Watanabe, S. Immunochemical studies on the Trichophyton mentagrophytes. Sabouraudia, Vol. 9: 129-138, 1971.

[21] Nozawa, Y., Noguchi, T., Uesaka, H. and Ito, Y. Studies on the immunologically active substances of the dermatophytes. Japan. J. Med. Mycol, Vol. 11, 159-165, 1970.

[22] Dova: Immunological problems of yeasts. Shinkinshi, 10, 179-190, 1969.

[23] Aronson, J.M. The cell wall, In: The fungi, (ed. Ainsworth, C.G. & Sussman, A.S.), Vol. pp. 49_76, Academic Press, New York, 1965.

[24] Bartnicki-Garcia, S. Cell wall chemistry, morphogenesis and taxonomy of fungi. Ann. Rev. Microbiol., Vol. 22, 87-108, 1968.

[25] Poulain, D., Biguet, J., Deblock, S. and Vernes, A. La paroi mycelienne de Trichophyton mentagrophytes: Etude ultrastructurale desescouches constitutives, apres application d'une methode de mise en evidence des polysaccharides. Sabouraudia, Vol. 13, pp 244-254, 1975.

[26] Kitajima, Y., Sekiya, T. Nozawa, Y. and Ito, Y.: Ultrastructure of the Trichopyton metagrophytes septum: Visualization of the lamellar structure. Japan. J. Med. Mycol., Vol. 17, 31-36, 1976.

[27] Kitajima, Y., Sekiya, Y., Nozawa, Y. and Ito, Y. Ultrastructural and chemical investigations on the cell wall architecture of Epidermophyton floccosum: a proposal of a cell wall model. Japan, J. Med. Mycol., Vol. 17, 92-101, 1976.

[28] Ito, Y., Setoguchi, T. and Nozawa, Y.: Cell walls of Trichophyton mentagrophytes: Isolation and fine structure. Japan. J. Med.Mycol., Vol. 9, 171-173, 1969.

[29] Noguchi, T., Kitajima, Y., Nozawa, Y. and Ito, Y.: Isolation, composition and structure of cell walls of Trichophyton mentagrophytes. Arch. Biochem. Biophys, Vol. 146, pp. 506-512 (1971).

[30] Kitajima, Y., Banno, Y., Noguchi, T., Nozawa, Y. and
      Ito, Y.: Effects of chemical modification of structural
      polymer upon the cell wall integrity of Trichophyton,
      Arch. Biochem. Biophys., Vol. 152, pp. 811-820, 1972.

[31] Nozawa, Y., Kitajima, Y. and Ito, Y. Chemical and ultra-
      structural studies of isolated cell walls of
      Epidermophyton floccosum. Biochem. Biophys. Acta,
      Vol. 307: pp. 92-103, 1973.

[32] Kitajiima, Y., Nozawa, Y. and Ito, Y. Ultrastructure and
      chemistry of Epidermophyton floccosum cell walls,
      Proc. of First Intersectional Congress of IAMS (ed.
      Hasegawa, T.) Vol. 2, pp. 149-153, Science Council of
      Japan, Tokyo, 1974.

[33] Kitajima, Y. and Nozawa, Y.: Isolation, ultrastructure
      and chemical composition of the outermost layer "exo-layer".
      of the Epidermophyton floccosum cell wall. Biochim,
      Biophys Acta, Vol. 394; pp. 558-568, 1975.

[34] Noguchi, T., Banno, Y., Watanabe, T., Nozawa, Y. and
      Ito, Y.: Carbohydrate composition of the isolated
      cell walls of dermatophytes. Mycopatholgia, Vol. 55,
      pp. 71-76, 1975.

[35] Hamilton, D.B. and Knight, S.G.: An analysis of the
      cell wall of Penicillium chrysogenum. Arch. Biochem,
      Biophys., Vol. 99: pp. 282-287, 1962.

[36] Shah, V.K. and Knight, S.G. Chemical composition of
      hyphal walls of dermatophytes. Arch. Biochem. Biophys.,
      Vol. 127: pp. 229-234, 1968.

[37] Szanszlo, D.J. Cooper, B.H. and Voges, H.S.: Chemical
      compositions of the hyphal walls of three chromomycosis
      agents. Sabouraudia, Vol. 10, pp. 94-102, 1972.

[38] Domer, J.E. Monosaccharide and chitin content of cell
      walls of Histoplasma capsulatum and Blastomyces dermatidis.
      J. Bacteriol., Vol. 107: ;; 870-877, 1971.

[39] Yamada, M. and Miyazaki, T. Ultrastructure and chemical
      analysis of the cell wall of Pythium debar _num, Japan.
      J. Mictobiol, Vol. 20 ; pp. 83-92, 1976.

[40] Research on Fungicide Components of Pathogenic True Fungi,
      particularly with regard to Contamination and Immunology.
      Shinkinshi, Vol. 3, pp. 157-179, 1963.

[41]   Manocha, M.S. and Colvin, J.R.   Structure and composi-
        tion of the cell wall of Neurospora crassa.
        J. Bacteriol, Vol. 94, pp. 202-212, 1967.

[42]   Mitchell, A.D. and Taylor, I.E.P.:   Cell-wall proteins of
        Aspergillus niger and Chaetomium globosum.   J. Gen.
        Microbiol. Vol. 59, pp. 103-109, 1969.

[43]   Buck, K.W. and Obaidah, M.A.:   The composition of the
        cell wall of Fusicoccur amygdali.   Biochem. J.,
        Vol. 125: pp. 461-471, 1971.

[44]   Barran, L.R. Schneider, E.F., Wood, P.J., Madnodihgh, C.
        and Miller, R.W.:   Cell wall of Fusarium sulphureum.
        Biochim. Biophys. Acta, Vol. 392, pp. 148-158, 1975.

[45]   Applegarth, D.A.   The cell wall of Penicillium notatum.
        Arch. Biochem. Biophys. Vol. 120, pp. 471-478, 1967.

[46]   Pao, V.M. and Aronson, J.M.   Cell wall structure of
        Sapromyces elongatus.   Mycologia, Vol. 62, pp. 531-541,
        1970.

[47]   Ruiz-Herrera, J.   Chemical components of the cell wall
        of Aspergillus species.   Arch. Biochem Biophys.,
        Vol. 122, pp. 118-125, 1967.

[48]   Zonneveld, B.J.M.   Biochemical analysis of the cell wall
        of Aspergillus nidulans.   Biochim. Biophys. Act,
        Vol. 244, pp. 506-512, 1971.

[49]   Reuvers, T., Tacoronte, E., Garcia-Mendoza, C. and
        Novaes-ledieu, M.   Chemical composition of cell walls
        of Saccharomyces fragilis.   Can J. Microbiol. Vol. 15,
        pp. 989-993, 1969.

[49]   Reuvers, T., Tacoronte, E., Garcia-Mendoza, C. and
        Novaes-Ledieu, M.   Chemical composition and cell walls
        of Saccharomyces fragilis, Can. J. Microbiol. Vol. 15,
        pp. 989-993, 1969.

[50]   Bulman, R.A. and Chittenden, G.J.F.   The mycelial cell
        wall of Penicillium chilesii G. Smith NRRL 1887,
        Biochim, Biophys, Acta, Vol. 444, pp. 202-211, 1976.

[51]   Taylor, I.E.P. and Cameron, D.S.:   Preparation and quanti-
        tative analysis of fungal cell walls: strategy and
        tactis.   Ann. Rev. Microbiol. Vol. 27, pp. 243-259, 1973.

[52]   Taplin, D. and Blank, H.   Microscopic morphology of
        Trichophyton rubrum.   J. Invest. Dermatol. Vol. 7,
        pp. 523 - 528, 1961.

[53] Pock-Steen, B. and Kobayashi, T. Ultrastructure of the hyphal wall and septum of Trichophyton metagrophytes. J. Invest. Dermatol. Vol. 55: pp. 489-496, 1965 '

[54] Urabe, H. and Izu, T. The ultrastructure of Trichophyton and a double cell wall in the hypha. J. Invest. Dermatol, Vol 52, pp. 508-513, 1969.

[55] Laden, E.L. and Erickson, J.O. Electron microscope study of Epidermophyton floccosum, J. Invest. Dermatol, Vol. 31, pp. 55-58, 1958.

[56] Werner, H.J., Jolly, H.W. and Hunter, L.J. Electron microscopic observations of Epidermophyton floccosum. J. Invest. Dermatol, Vol. 43, pp. 139-143, 1964.

[57] Werner, H.J., Jolly, H.W. and Spurlock, B.O. Electron microscope observations of the fine structure of Microsporum canis. J. Invest. Dermatol, Vol. 46, pp. 130-134, 1966.

[58] Werner, H.J., Jolly, H.W. and Carpenter, C.L. Observations of the fine structure of Microsporum dudouini. J. Invest. Dermatol, pp. 276-279, 1969.

[59] Takeo, K., Uesaka, I. and Nishiura, M. The wall particles of the genus Cryptococcus: Large size and characteristic distribution. J. Gen. Microbiol. Vol. 84, pp. 233-225, 1974.

[60] Cole, G.T. A correlation between rodlet orientation and conidiogenesis in Hyphomycetes. Can. J. Botany, Vol. 51, pp. 2413-2422, 1973.

[61] Sekiguchi, J., Gaucher, G.M. and Costerton, J.W. Microcycle conidation in Penicillium uriticae: An ultrastructural investigation of spherical spore growth. Can. J. Microbiol, Vol. 21, pp. 2048-2058, 1975.

[62] Nozawa, Y., Hiraguiri, Y. and Ito: Studies of the acid stability of monosaccharides by gas chromatography, with reference to the analysis of sugar components in the polysaccharides. J. Chromatog, Vol. 45, pp. 244-249, 1969.

[63] Blank, F. The chemical composition of the cell walls of dermatophytes. Biochim. Biophys. Acta, Vol. 10, pp. 110-113, 1953.

[64] Bishop, C.T., Blank, F. and Hranisavljevic-Jakovljevic, M. The water soluble polysaccharides of dermatophytes, galactomannans from Trichophyton. Can. J. Chem, Vol. 40, pp. 1816-1825, 1962.

[65] Cummins, C.S. and Harris, H. Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. J. Gen. Microbiol. Vol. 18, pp. 173-189, 1958.

[66]   Sentandreu, R. and Northcote, D.H.   The structure of a
       glycopeptide isolated from the yeast cell wall.
       Biochem, J., Vol. 109, pp. 419-432, 1968.

[67]   Miohell, A.D. and Taylor, I.E.P.   Cell wall-proteins of
       Aspergillus niger and Chaetomium glubosum. J. Gen.
       Microbiol., Vol. 59, pp. 103-109, 1969.

[68]   Hashimoto, T., Wu-Yuan, C.D. and Blumenthal, H.J.:
       Isolation and characterization of the radlet layer
       of Trichophyton mentagrophytes microconidial wall.
       J. Bacteriol. Vol. 127, pp. 1543-1549, 1976.

[69]   Gander, J.E.   Fungal cell wall glycoproteins and peptido-
       polysaccharides. Ann. Rev. Mictobiol. Vol. 49, pp. 103-
       119, 1974.

[70]   Domer, J.E. and Harmon, R.D.   Lipid, monosaccharide and
       chitin content of cell walls of Sepedonium. Sabouraudia,
       Vol. 10, pp. 56-62, 1972.

[71]   Bligh, E.G. and Dyer, W.J.   A rapid method of total
       lipid extraction and purification. Can. J. Biochem.
       Biophysiol, Vol. 37, pp. 911-917, 1959.

[72]   Marchant, R. and Moore, R.T.   Lomasomes and plasmalemmasomes
       in fungi. Protoplasma, Vol. 76, pp. 235-247, 1973.

[73]   Marchant, R. and Roberds, A.W.   Membrane systems associated
       with the plasmalemma of plant cells. Ann. Bot. N.S.
       Vol. 32, pp. 453-471, 1968.

[74]   Necas, O.   Cell wall synthesis in yeast protoplasts.
       Bacteriol. Rev., Vol. 35, pp. 149-170, 1971

[75]   Roberts, K., Gruney-Smith, M. and Hills, G.J.   Structure,
       composition and morphogenesis of the cell wall of
       Chlamidomonas reinhardi. J. Ultrastr. Res., Vol.40,
       pp. 599=613, 1972.

[76]   Necas, O.   Physiolosical conditions as important factors
       for the regeneration of naked yeast protoplast. Nature,
       Vol. 192, pp. 580-581, 1961.

[77]   Moor, H. and Muhlethaler, K.   Fine structure in frozen
       etched yeast cells. J. Cell Biol., Vol. 17,
       pp. 609-628, 1963.

[78]   Matile, P., Moor, H. and Muhlethaler, K.
       Isolation and properties of plasmalemma in yeast.
       Arch. Mikrobiol., Vol. 58, pp. 201-211, 1967.

[79]   Duran, A., Bowers, B. and Cabib, E.   Chitin synthetase
       zymogen is attached to the yeast plasma membrane.
       Proc. Nat. Acad. Sci. USA, Vol. 72, pp. 3952-3955,
       1975.

[80]   Garcia-Mendoza, C. and Ledieu, M.N. Chitin in the new wall
        of regenerating protoplasts of Candida utilis. Nature
        Vol. 220, p. 1035, 1966.

[81]   Ruiz-Herrera, J., Sing, V.O. va Der Woude, W.J. and Bartnicki-
        Garcia, S. Microfibril assembly by granules of
        china synthetase. Proc. Nat. Acad. Sci. USA, Vol. 72,
        pp. 2706-2170, 1975.

[82]   Brown, R.M. Jr. and Montezinos, D. Cellulose microfibrils:
        visualization of biosynthetic and orienting complexes in
        association with the plasma membrane. Proc. Nat. Acad.
        Sci. U.S A., Vol. 73, pp. 143-147, 1976.

[83]   Willison, J.H.M. An examination of the relationship between
        freeze-fractured plasma-lemma and cell wall microfibrils.
        Protoplasma, Vol. 88, pp. 187-200, 1976.