Production of Bacteriolytic Enzymes and Degradation of Bacterial Cell Walls During Growth of *Agaricus bisporus* on *Bacillus subtilis*

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(Received 1 September 1983; revised 27 October 1983)

The mechanism by which the mycelium of *Agaricus bisporus* can use *Bacillus subtilis* as sole carbon and nitrogen source was investigated. During fungal growth more than 80% of the muramic acid and diaminopimelic acid residues in the bacterial walls disappeared from the microbial biomass and appeared as soluble glycopeptides. Culture supernatants dissolved purified walls of *B. subtilis*, and bacteriolytic enzymes were obtained by ion-exchange chromatography of these supernatants. The main bacteriolytic enzyme activity was found to be a β-N-acetylmuramidase. Soluble bacterial wall fragments were partially purified by chromatography of the culture fluids. The chemical properties of these fragments were consistent with their having been produced by β-N-acetylmuramidase action on the peptidoglycan of the *B. subtilis* cell wall.

**INTRODUCTION**

Growth of the edible fungus, *Agaricus bisporus*, on bacteria as sole carbon and nitrogen source has been established (Fermor & Wood, 1981), and the relative importance of this mode of nutrition for the growth of the fungal mycelium in composted wheat straw has been determined (Sparling et al., 1982). Light and electron microscopy of the mycelium of *A. bisporus* using killed *Bacillus subtilis* as sole nutrient source showed that progressive breakdown of the walls of the bacteria accompanied growth of the fungus, which suggested that bacteriolytic enzymes were being produced (Fermor & Wood, 1981). This was supported by the detection of cleared zones which extended beyond the colony perimeter of *A. bisporus* colonies growing on killed bacteria embedded in agar. However, although a β-N-acetylglucosaminidase was detected in culture supernatants of *A. bisporus* grown on *B. subtilis*, and suggested to be a possible wall-degrading enzyme, these supernatants were not able to lyse suspensions of *Micrococcus lysodeikticus* (Fermor & Wood, 1981).

The present work aimed to examine the mechanism by which *A. bisporus* brings about the disintegration of the walls of *B. subtilis* as part of the process of use of the bacteria as a nutrient source. The extent of bacterial wall degradation was estimated by measurement of the losses of the unique wall components, DAP and muramic acid, from the bacteria into the culture fluid. Bacteriolytic enzymes were partially purified from culture supernatants, and their modes of action were determined. These were confirmed by the chemical characteristics of soluble wall fragments extracted from the culture supernatants.

**METHODS**

Organisms. The strains of *A. bisporus* (D621) and *B. subtilis* (168, derivative S2861) were described by Fermor & Wood (1981).

Cultures of *A. bisporus* on *B. subtilis* cells. *Bacillus subtilis* to be used as a growth substrate for *A. bisporus* was grown and extracted as described previously (Fermor & Wood, 1981). Liquid cultures of *A. bisporus* were grown at

**Abbreviations**: DAP, α,γ-diaminopimelic acid; FDNB, 1-fluoro-2,4-dinitrobenzene.
25 °C, without shaking, in the basal salts medium of Treschow (1944) containing thiamin and biotin and supplemented with freeze-dried *B. subtilis* (500 mg in 25 ml medium in 250 ml Erlenmeyer flasks) as sole C and N source. Flasks were inoculated with mycelial suspensions as described by Fermor & Wood (1981). Cultures were harvested by centrifugation at 10000 g for 30 min. Biomasses (fungal mycelium plus residual bacteria) were washed once with distilled water (15 ml) at 0 °C, recovered by centrifuging, freeze-dried and weighed. Water washes were combined with the original supernatants, filtered through 0.2 μm membrane filters, then frozen.

**Preparation of bacterial walls and assay for wall-degrading (‘bacteriolytic’) enzymes.** Walls of *B. subtilis* 168 were prepared from mid-exponential phase cultures by breakage of the cells with ballotini beads in a Braun MSK cell homogenizer, followed by an exhaustive washing procedure (Grant, 1979) or by the hot SDS purification method (Anderson *et al.*, 1978). Both methods included a heating step which inactivated autolytic enzymes present in the walls. Wall suspensions (2.5 mg ml⁻¹) in 20 mM-sodium acetate/acetic acid buffer, pH 5.0, containing, 1⁻¹, 0.2 g KCl, 0.2 g MgSO₄·7H₂O and 0.2 g CaCl₂, were used as substrate for the assay of bacteriolytic enzymes. Enzyme preparation (1 vol.) was added to this suspension (2 vol.), and the rate of change in turbidity (ΔOD₆0₀ h⁻¹) at 25 °C was measured.

**Preparation of bacteriolytic enzymes.** Cultures possessing bacteriolytic activity were centrifuged at 10 000 g and the biomass was washed with the assay buffer. Combined supernatants and washes were concentrated by ultrafiltration (Amicon Diaflow YM-10 membrane) then applied to a column (22 × 2.2 cm) of carboxymethylcellulose (Whatman microcrystalline CM-32) prepared as described by Himmelhoch (1971) and equilibrated with the assay buffer. Fractions (10 ml) were collected at a flow rate of 34 ml h⁻¹ with a linear gradient of 0-0.2 M NaCl in the same buffer (800 ml).

**Extraction of bacterial wall fragments from culture supernatants.** Protein was precipitated from culture supernatant (40 ml) with 10% (w/v) trichloroacetic acid. After ether extraction of the trichloroacetic acid, the supernatant was concentrated to 10 ml by rotary evaporation, then fractionated on a column (85 × 2.5 cm) of Sephadex G-25 (medium), equilibrated and eluted with distilled water at 96 ml h⁻¹, 58 × 10 ml fractions being collected. Pooled fractions were concentrated then applied to a column (70 × 2.0 cm) of Sephadex G-75 which was equilibrated and eluted with distilled water at 30 ml h⁻¹, 56 × 5 ml fractions being collected. Both columns were calibrated with a solution of Blue Dextran 2000 (Pharmacia) (0.2%, w/v) and CuSO₄·5H₂O (0.5%, w/v).

Phosphate-containing fractions from the Sephadex G-75 column were further fractionated by ion-exchange chromatography on a column (22 × 1.0 cm) of Bio-Rad AG50W-X4 (H⁺ form) which was washed with water (100 ml) to elute the negatively charged, phosphate-containing substances, followed by gradient elution with 0–4 M HCl (400 ml) to separate phosphate-free peptides (Mauck *et al.*, 1971). The flow rate was 35–40 ml h⁻¹, and 50 ml fractions were collected.

**Analytical methods.** Samples for amino acid analysis were hydrolysed in 6 M HCl at 105 °C for 16 h. Humin was removed by centrifugation, washed with water, and the combined hydrolysate and wash were deacidified by repeated evaporation from aqueous solution in vacuo over P₂O₅ and NaOH. As this evaporation procedure can cause losses of muramic acid (Ghuysen *et al.*, 1966), hydrolysates for hexosamine analysis (4 M HCl at 100 °C for 4 h in a N₂ atmosphere) were neutralized by extraction with N,N-diocetylaminomethane (10%, v/v in CHCl₃) (Smith & Page, 1948) after adjustment to about pH 2 with Bio-Rad AG2-X8 resin (HCO₃⁻ form).

DAP was determined by paper chromatography of replicate samples, with a range of standards, on Whatman 3MM paper in solvent system I (butan-1-ol/pyridine/glacial acetic acid/water; 60:40:3:30 by vol.) followed by staining the dried papers with the ninhydrin/cadmium acetate mixture of Heilmann *et al.* (1957). Spots were cut out, eluted with methanol (5:0 ml), and the absorbance values measured at 500 nm. Blank regions on each paper, parallel in position and equal in area to the DAP spots, were eluted to correct for background colour. Beer’s Law was consistently obeyed over the range 10–300 nmol DAP.

This method was also used to measure amino acids and hexosamines in hydrolysates of wall fragment preparations, except that two-dimensional paper chromatography was required in solvents II (butan-1-ol/glacial acetic acid/water; 3:1:1, by vol.) and III (pyridine/water; 4:1, v/v) (Ghuysen *et al.*, 1966). Satisfactory resolution of muramic acid in hydrolysates of culture supernatants and biomasses required the two-dimensional system of solvent IV (butan-2-ol/formic acid/water; 7:1:2, by vol.) followed by solvent I. In each assay aliquots of standard mixtures of amino acids or hexosamines were chromatographed on separate sheets.

Free amino groups were measured and N-terminal amino acids were identified using the FDNB methods of Ghuysen *et al.* (1966). Free reducing groups were estimated by the procedure of Thompson & Shockman (1968). Organic phosphate, as the difference between total and inorganic phosphate, was determined as described by Ames (1966). Total hexosamines in pooled column fractions were measured by the Elson-Morgan assay of Gatt & Berman (1966). Reducing terminal residues were identified and estimated by two-dimensional paper chromatography after NaBH₄ reduction and acid hydrolysis (Ghuysen *et al.*, 1966). Alanine and glutamic acid configurations were determined with d-aminoo acid oxidase (Wicken & Baddiley, 1963) and L-glutamic acid dehydrogenase (Work, 1971), respectively. Phosphorous-containing wall fragment preparations were analysed for teichoic acid constituents as described previously (Grant, 1979).
RESULTS

Degradation of bacterial walls during growth of A. bisporus on B. subtilis

When A. bisporus was grown on B. subtilis cells, the bacterial wall constituents DAP and muramic acid were rapidly lost from the biomass into the culture fluid (Fig. 1), whereas in uninoculated controls incubated for the full eight week period, no changes were observed in biomass DAP and muramic acid, and only traces of these substances were detected in the supernatants. In another experiment, in which the lag phase was approximately a week longer than that shown in Fig. 1, the rapid loss of DAP from the biomass was similarly delayed. Muramic acid was not measured in this experiment.

Bacteriolytic enzymes of A. bisporus

Supernatants of A. bisporus cultures grown on B. subtilis cells at an initial concentration of 20 mg ml\(^{-1}\) dissolved purified B. subtilis cell walls in buffered suspensions at pH 4-5. Consistently higher bacteriolytic activities were obtained in cultures grown on lower concentrations of bacteria. At the optimum concentration (5 mg ml\(^{-1}\)) the enzyme activity increased to a maximum at 2-3 weeks after inoculation, and then decreased. No activity was detected in supernatants of A. bisporus cultures grown in media without bacterial cells present.

Owing to high levels of amino and reducing substances in the culture supernatants, partial purification of the bacteriolytic enzymes was necessary before their modes of action could be investigated. This was carried out by carboxymethylcellulose chromatography of concentrated supernatants from 2-3-week-old cultures (Fig. 2). The major enzyme activity, \(E_1\), did not bind to the column, and was eluted with the first 80 ml of eluant. The minor activities, \(E_2\) and \(E_3\), were eluted with the gradient between 0.10 and 0.11 M-NaCl. In another preparation, \(E_1\) was again the major activity, but only a single peak was found in the \(E_2/E_3\) region of the chromatogram.

Fractions \(E_1\), \(E_2\) and \(E_3\) were dialysed to remove NaCl, concentrated by ultrafiltration to 50 ml (\(E_1\)) or 10 ml (\(E_2\) and \(E_3\)), then analysed for bacteriolytic activity against standard suspensions of B. subtilis cell walls. Dissolution of the walls was accompanied by an increase in reducing substances in the wall–enzyme mixtures (Fig. 3). Analyses for free amino groups showed that no increases occurred over the period of wall dissolution, although there was a relatively high background of FDNB-reacting material in the wall preparation which could have masked a slight increase in amino groups over the long incubation times. The experiment was repeated using walls in which existing free amino groups had been destroyed by nitrous acid (Fordham & Gilvarg, 1974); the rates of dissolution of the walls by the enzyme preparations were unaffected by this treatment, and no measurable amino-reacting substances appeared as the walls were dissolved.
Fig. 2. Gradient elution (0-0.2 M-NaCl) carboxymethylcellulose chromatography of culture fluid of \textit{A. bisporus} grown on \textit{B. subtilis} cells. \textit{UV} transmission (280 nm) was measured continuously (----). Fractions (10 ml) were assayed for bacteriolytic activity against a suspension of \textit{B. subtilis} cell walls (○), and this activity was detected in fractions 4-7 (E₁), 39-41 (E₂), and 42-44 (E₃). — NaCl gradient.

Fig. 3. Lysis of \textit{B. subtilis} cell walls by bacteriolytic enzyme fractions from culture fluid of \textit{A. bisporus} grown on \textit{B. subtilis}. (a) Reduction in turbidity of cell wall suspension by enzyme fractions E₁ (○), E₂ (□) and E₃ (△). ▽, Control wall suspension without enzyme addition. (b) Increases in reducing substances in enzyme digests during cell wall dissolution by E₁ (●), E₂ (■) and E₃ (▲). Units are μmol (glucosamine equivalent) released per mg wall originally present in the standard assay (see Methods).

After wall digestion with enzyme fractions E₁, E₂ and E₃, the percentages of muramic acid residues bearing free reducing groups were, respectively, 79.2, 90.3 and 76.0%. The corresponding percentages of glucosamine residues present as reducing terminals were 30.3, 8.4 and 8.7%. The major activity of all three enzyme fractions was therefore that of a β-N-acetylmuramidase, although the relatively high level of reducing-terminal glucosamine residues in the E₁ digest indicated that this fraction may also have possessed β-N-acetylglucosaminidase activity.

\textit{Bacterial wall fragments in culture supernatants – extraction and analysis}

After precipitation of protein with trichloroacetic acid, the supernatant of an eight week culture grown on bacteria (20 mg ml⁻¹) was fractionated on a Sephadex G-25 column (Fig. 4).
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Fig. 4. Sephadex G-25 (medium) gel filtration of culture fluid from an eight week culture of *A. bisporus* on cells of *B. subtilis*. Fractions (10 ml) were assayed for free amino groups (○) and reducing substances (●). $V_0$, Void volume (Blue Dextran 2000); $V_i$, inclusion volume (CuSO$_4$).

Fig. 5. Sephadex G-75 gel filtration of pooled fractions 1–29 of Sephadex G-25 chromatography of *A. bisporus* culture fluid (Fig. 4). Fractions (5 ml) were assayed for free amino groups (○), reducing substances (●) and organic phosphate (□), then pooled as zone A (fractions 28–35), zone B (fractions 36–41) and zone C (fractions 42–48). $V_0$, Void volume (Blue Dextran 2000); $V_i$, inclusion volume (CuSO$_4$).

Pooled fractions 1–18, 19–29 and 30–44 contained, respectively, 1·1, 75·3 and 7·6% of the DAP in the original supernatant (total recovery, 84·0%). Muramic acid was not measured, but chromatography of hydrolysates showed that, as with DAP, almost all of the muramic acid eluted from the column was in bound form in fractions 19–29.

Fractions 30–44 were composed largely of free alanine and glutamic acid, and traces of other amino acids, which accounted for the high amino content of these fractions (Fig. 4). No free DAP was detected, and the alanine and glutamic acid were present solely as the L-isomers. Fractions 30–44 also contained a mixture of wall- and protein-derived peptides which was not resolved successfully because of the small quantities present.

Fractions 1–29, which accounted for 91% of the DAP recovered from the column, were further fractionated on Sephadex G-75 into zones A, B and C (Fig. 5), analyses of which are shown in Table 1.
Table 1. Analysis of zones from Sephadex G-75 chromatogram*

<table>
<thead>
<tr>
<th>Sample</th>
<th>DAP (µmol)</th>
<th>Organic phosphate</th>
<th>Hexosamines</th>
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<tr>
<td>Original material</td>
<td>32.8</td>
<td>94.1</td>
<td>30.9</td>
</tr>
<tr>
<td>Zone A</td>
<td>5.3</td>
<td>59.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Zone B</td>
<td>13.0</td>
<td>5.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Zone C</td>
<td>4.3</td>
<td>13.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Percentage recovery</td>
<td>...</td>
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</table>
* Fig. 5.

Table 2. Analysis of wall fragment preparations from supernatants of A. bisporus grown on B. subtilis

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Ala (µmol)</th>
<th>Glu (µmol)</th>
<th>DAP (µmol)</th>
<th>Glucosamine (µmol)</th>
<th>Muramic acid (µmol)</th>
<th>Organic phosphate (µmol)</th>
<th>Glycerol (µmol)</th>
<th>Glucose (µmol)</th>
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<tbody>
<tr>
<td>A1</td>
<td>2.20</td>
<td>1.58</td>
<td>1.30</td>
<td>3.59</td>
<td>0.66</td>
<td>49.40</td>
<td>38.03</td>
<td>37.05</td>
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<td></td>
<td></td>
<td></td>
<td>Molar*</td>
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<tr>
<td></td>
<td>1.69</td>
<td>1.22</td>
<td>1.00</td>
<td>2.76</td>
<td>0.51</td>
<td>38.00</td>
<td>29.26</td>
<td>28.50</td>
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<tr>
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<td></td>
<td></td>
<td>Molar</td>
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<tr>
<td></td>
<td>2.45</td>
<td>1.90</td>
<td>1.26</td>
<td>1.20</td>
<td>0.98</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A2</td>
<td>1.94</td>
<td>1.51</td>
<td>1.00</td>
<td>0.95</td>
<td>0.78</td>
<td>ND</td>
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<td></td>
<td>3.23</td>
<td>2.43</td>
<td>1.83</td>
<td>1.79</td>
<td>11.50</td>
<td>6.12</td>
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<tr>
<td>B</td>
<td>1.68</td>
<td>1.31</td>
<td>1.00</td>
<td>0.97</td>
<td>0.62</td>
<td>0.33</td>
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<tr>
<td></td>
<td>0.54</td>
<td>0.49</td>
<td>0.29</td>
<td>0.20</td>
<td>0.39</td>
<td>ND</td>
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<tr>
<td>C2</td>
<td>1.86</td>
<td>1.68</td>
<td>1.00</td>
<td>0.70</td>
<td>1.34</td>
<td>ND</td>
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<tr>
<td></td>
<td></td>
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<td>Molar</td>
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<tr>
<td></td>
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<td>1.68</td>
<td>1.00</td>
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ND, not determined
* DAP = 1.00.

Zone B, which contained the bulk of the amino and reducing substances, was not fractionated further. Ion-exchange chromatography of zones A and C yielded phosphate-containing fractions, A1 and C1, and two major phosphate-free peptide fractions, A2 and C2. Fraction C1 contained phosphate and protein-derived amino acids, but only traces of DAP and muramic acid. This fraction, and several minor DAP- and muramic acid-containing peptide fractions obtained in low yields from the ion-exchange columns, were not examined further. The extraction procedure thus produced four major cell wall fragment preparations, A1, A2, B and C2.

Analysis (Table 2) showed that the main components of A2, B and C2 were peptidoglycan fragments, while A1, as well as containing peptidoglycan material, was composed largely of phosphate, glycerol and glucose residues in proportions similar to those of the main wall teichoic acid B. subtilis of (Duckworth et al., 1972). The relatively small proportion of phosphate in preparation B probably arose from incomplete separation from zone A material on the Sephadex G-75 column (Fig. 5).

Small quantities of aspartic acid, glycine, isoleucine, leucine, lysine, phenylalanine, serine and valine were also found in hydrolysates of all four preparations. Although protein-derived peptides were therefore present, the sum of the molar contents of these amino acids was less than the total alanine content, except in C2 where it was almost twice the alanine figure.

Treatment with FDNB to label free amino groups yielded no ether-soluble DNP-amino acids in acid hydrolysates, except for a trace of DNP-alanine in C2. The yellow colour in all four hydrolysates remained in the water layer, was totally extractable into water-saturated butan-1-ol (Ghuysen et al., 1966) and co-chromatographed with authentic mono-DNP-DAP prepared by the method of Mirelman & Sharon (1968). Reducing terminal analysis showed that 36.4, 23.5,
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31.4 and 63.6% of the muramic residues in A₁, A₂, B and C₂, respectively, bore free reducing groups, whereas the corresponding percentages for glucosamine residues were 18.4, 9.6, 8.6 and 8.7%.

DISCUSSION

The electron microscopic evidence of bacterial wall breakdown in cultures of A. bisporus growing on B. subtilis cells (Fermor & Wood, 1981) has now been confirmed by measurement of the progressive losses of the unique peptidoglycan components, DAP and muramic acid, from the bacteria, and the concomitant appearance of these compounds in bacterial wall fragments in the culture fluids (Fig. 1). Although the results in Fig. 1 indicated that neither DAP nor muramic acid was being used by the fungus as nutrient sources, in other experiments an overall loss of 10–20% of the DAP had occurred by the end of the growth period. Assimilation by A. bisporus of DAP or other peptidoglycan components is therefore uncertain, although the absence of free DAP, D-alanine and D-glutamic acid from culture supernatants, and the lack of peptidoglycan-degrading peptidases (see below), suggest that this was unlikely.

Extracellular bacteriolytic enzyme activity was found in the cultures although this was low, or even undetectable (Fermor & Wood, 1981), when high concentrations of bacteria were used, possibly because of strong binding of the bacteriolytic enzymes to the bacterial walls (Pooley et al., 1970; Herbold & Glaser, 1975). Proteases, produced by A. bisporus under these conditions (Fermor & Wood, 1981) may have been responsible for the decreases in bacteriolytic activity observed as the cultures aged.

The release of reducing groups during hydrolysis of wall suspensions by the three bacteriolytic enzyme fractions, and the subsequent discovery that these groups accounted for most of the muramic acid residues present in the walls, established that the main bacteriolytic enzyme present was a β-N-acetylmuramidase (EC 3.2.1.17). As the synthesis of three separate N-acetylmuramidases by A. bisporus seems unlikely, it is possible that the separation into E₁, E₂ and E₃ could have been an artefact of the chromatographic procedure, especially as in another experiment E₂ and E₃ occurred as a single activity. During purification of muramidase from the fungus Chalaropsis (Hash, 1963), ion-exchange chromatography separated two active fractions which were later shown to contain the same enzyme (Hash & Rothlauf, 1967).

The results of the enzyme studies were supported by the separation and analysis of bacterial wall fragments from the culture fluids of A. bisporus grown on B. subtilis. These substances, although not totally purified from protein-derived peptide material, together represented most of the DAP and muramic acid initially present in the culture fluids. Their compositions (Table 2) and properties were characteristic of the type of fragments produced by β-N-acetylmuramidase digestion of Bacillus walls (Hughes, 1968). Thus muramic acid residues were the main reducing terminals in the four preparations, and the absence of N-terminal alanine from A₁, A₂ and B indicated that the muramic acid–alanine linkages were intact. Although there was a trace of N-terminal alanine in C₂, this could have arisen from the relatively high concentrations of protein-derived material in this preparation. The peptidoglycan material in A₁, a high molecular weight fraction as indicated by its gel filtration properties, was associated with teichoic acid, probably as a covalently linked complex (Hughes, 1968). The probable involvement of glucosamine in peptidoglycan–teichoic acid linkage units (Coley et al., 1978) may have contributed to the high levels of this amino sugar found in preparation A₁ (see Table 2).

The absence in the four preparations of free N-terminals other than those on the DAP residues was consistent with the lack of appearance of amino groups during wall digestion by the enzyme fractions, and indicated that N-acetylmuramyl-L-alanine amidase or peptidoglycan endopeptidase activities (Ghuysen et al., 1966; Ghuysen, 1968) were not present. Single free amino groups on the DAP residues could conceivably have resulted from specific endopeptidase hydrolysis of peptidoglycan cross-bridges between DAP and D-alanine (Hughes, 1970), but hydrolysates of FDNB-treated preparations always contained unlabelled DAP residues, which indicated that many cross-bridges remained intact.

The presence of intact cross-bridges, the relatively low reducing terminal figures, and the gel filtration characteristics of the preparations showed that the wall fragments, with the possible
exception of C₂, were oligomers of the basal disaccharide–peptide structure of the peptidoglycan. The presence of intact muramyl linkages in the fragments contrasted with the almost total hydrolysis of these linkages in isolated walls by the enzyme fractions E₁, E₂ and E₃. This incomplete hydrolysis may have been due in part to the enzyme binding effect of high bacterial concentrations, but also to the rapid loss of bacteriolytic activity in older cultures.

β-N-Acetylmuramidase has thus been established as the principal bacteriolytic enzyme produced by A. bisporus. Although bacteriolytic peptidases and amidases were probably absent, extracellular β-N-acetylglicosaminidase (EC 3.2.1.30) is known to be produced when A. bisporus is grown on B. subtilis (Fermor & Wood, 1981), and some evidence for this activity was provided by the detection of reducing terminal glucosamine residues in the A₄ fragment preparation and in the digests of isolated walls by the enzyme fraction E₁. As this fraction included all the substances which failed to bind to the ion-exchange column (Fig. 2) it is possible that it contained a glucosaminidase which either attacked the intact walls relatively slowly, or simply caused further degradation of wall fragments released by the muramidase. Both types of glucosaminidase activity are known (Rogers, 1979).

Predation of bacteria by protozoa and slime moulds, and by other bacteria such as Myxococcus species, has been known for many years, and the bacteriolytic enzyme systems from a number of these organisms have been investigated (e.g. Upadhyay et al., 1977; Braun et al., 1972; Sudo & Dworkin, 1972). In contrast, the use of the bacteria as a food source by filamentous fungi has received little attention, despite the generally acknowledged importance of these organisms in the decomposition of organic materials and in nutrient cycling. The discovery that several common filamentous fungi were capable of degrading and assimilating bacterial cells in culture (Fermor & Wood, 1981; Fermor, 1983) suggested the existence of an important link in microbial food chains which had hitherto been overlooked. The present finding that A. bisporus produces an extracellular β-N-acetylmuramidase during growth on bacterial cells provides strong support for this hypothesis, since the linkage hydrolysed by this enzyme occurs only in the peptidoglycan of bacterial walls and envelopes.

We thank Barbara Prosser, Steve Matcham and Sarah Molyneux for excellent technical assistance.

REFERENCES


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