Production of Bacteriolytic Enzymes by *Streptomyces globisporus* Regulated by Exogenous Bacterial Cell Walls

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Mutanolysin biosynthesis and pigment production in *Streptomyces globisporus* ATCC 21553 were stimulated by adding bacterial cell walls to the medium. The increased bacteriolytic activity in the supernatant correlated with an increased de novo synthesis of mutanolysin and was between 4- and 20-fold higher than in cultures grown without bacterial cell walls. The increase in mutanolysin synthesis was brought about by enhanced transcription of the mutanolysin gene. The stimulation was only observed in medium which contained dextrin or starch as the carbon source. Glucose abolished the stimulation and also inhibited the low constitutive synthesis of mutanolysin. The induction of lytic activity was observed to require minimally 0.4 mg of bacterial cell walls per ml, whereas 0.6 mg of bacterial cell walls per ml yielded maximal lytic activity. Further supplements of bacterial cell walls did not result in enhanced lytic activity. The stimulation could be achieved independently of the phase of growth of the *Streptomyces* strain. Cultures grown in the presence of bacterial cell walls exhibited a higher growth yield. However, the accelerated growth was not the reason for the increased amount of mutanolysin produced. The growth of cultures with peptidoglycan monomers added to the medium instead of cell walls was similarly increased, but an effect on the biosynthesis of mutanolysin was not observed. All bacterial cell walls tested were capable of eliciting the stimulation of lytic activity, including cell walls of archaea, which contained pseudomurein.

Plants employ several strategies to protect themselves against fungal infections. Besides mechanical defense mechanisms which are always present, plants possess inducible chemical defense mechanisms (1). Induction of the chemical mechanisms is mediated by elicitors, which may consist of cell wall fragments of an invading pathogen (9). The elicitor reaction includes the biosynthesis of defense molecules which are toxic to the pathogen, e.g., induction of phytoalexins in *Glycine max* by cell wall fragments from *Phytophthora megasperma* (11), and also the biosynthesis of hydrolyses such as chitinases which lyse the invading fungus (3). A similar elicitor reaction was found by adding bacterial cell walls to plant cell cultures, e.g., to *Escherichia coli* (21). Because of this observation, we decided to investigate whether bacterial cell walls are also capable of stimulating secondary metabolism in procaryotes. We focused our investigations on streptomycetes, which produce a great number of different secondary metabolites such as antibiotics, enzyme inhibitors, and extracellular enzymes (31). In *Streptomyces globisporus* ATCC 21553, three extracellular bacteriolytic enzymes have been described: N-acetilmuramidases M1 and M2 (27, 40), and an l-alanine amidase (26). The M2 enzyme is a β,1,4-N-acetilmuramidase with an *M* of 10,400. It resembles hen egg white lysozyme in its inability to lyse O-acetylated or otherwise substituted peptidoglycan (27). The M1 enzyme (mutanolysin; Sigma Chemical Co., Deisenhofen, Germany) is a β,1,4-N,6-Diacetilmuramidase with an *M* of 20,300 (27). The amino acid sequence and crystallographic studies indicate that enzyme M1 resembles an enzyme synthesized by the fungus *Chalaraopsis* sp. (22, 30). The gene for the M1 lysozyme was cloned and sequenced from *S. globisporus* ATCC 21553 and *S. coelicolor* Müller DSM 3030 (4, 30).

Sequence comparison revealed that these genes were identical in the two organisms.

In this work, we report on the biosynthesis of mutanolysin in *S. globisporus* ATCC 21553, which was found to be induced by cell wall preparations from bacteria and archaea. This phenomenon is similar to the elicitation of plant cells by fungal and bacterial cell walls (9, 11, 21).

**MATERIALS AND METHODS**

**Bacterial strains and media.** For most experiments *S. globisporus* subsp. *globisporus* ATCC 21553 was used, which is identical to *S. globisporus* 1829. Mutanolysin was first purified and characterized from this strain (40). Other bacteria used were *S. globisporus* subsp. *caucasicus* DSM 40814 and *S. coelicolor* DSM 40233. Strains for preparation of cell walls are listed in Table 1. *S. globisporus* was grown in medium described by Yokogawa et al. (40), with dextrin as carbon source unless otherwise stated.

**Growth conditions.** Production of bacteriolytic enzymes was carried out in 100-ml Erlenmeyer flasks with baffles containing 10 ml of medium. Autoclaved cell walls were added to the medium prior to inoculation if not otherwise described. The medium was inoculated with either spores or mycelium. Cultures were grown in a water-cooled rotary shaker at 120 rpm and 30°C for 96 h. Cells were removed by centrifugation at 2,000 × *g*, using a microcentrifuge. Supernatant was removed, and pellets were lyophilized for measuring dry weight.

**Preparation of cell walls from gram-positive bacteria and archaea.** Bacteria and archaea were grown to stationary phase. Cells were harvested by centrifugation (20,000 × *g*, 20 min, 4°C) and resuspended in 10 mM Tris-Cl, pH 7.5. Cells were mechanically disintegrated with glass beads (34). Cell disruption was monitored by phase-contrast microscopy. Adherent fragments of cytoplasmic membrane and proteins were removed by boiling cell walls in 4% (wt/vol) sodium dodecyl...
sulfate (SDS) solution (5). The purity of the cell wall preparations was analyzed by quantitative determination of amino acids and amino sugars in hydrolysates of cell walls (4 N HCl, 100°C, 16 h), using a Biotronic LC 6001 amino acid analyzer (Biotronic, Mainz, Germany). If no non-cell wall amino acids could be detected, cell walls were regarded as pure.

**Preparation of Escherichia coli peptidoglycan-sacculi.** E. coli peptidoglycan-sacculi were prepared as described by Glauner et al. (19).

**Determination of lytic activity.** Two different assays were used to measure the lytic activity. For the optical assay, mycelia were removed from the culture broth by centrifugation. SDS-treated cell walls of *Micrococcus luteus* were suspended in 0.1 M potassium phosphate buffer (pH 6.8) and sonicated to give a suspension with an optical density at 580 nm of approximately 7. Cell wall suspension (100 μl) and 900 μl of culture supernatant were mixed in a cuvette. The decrease in optical density at 580 nm resulting from the degradation of cell walls was monitored with a spectrophotometer after incubation at 30°C for 5 min. The maximal linear decrease was used for determining lytic activity. One unit of lytic activity per milliliter is defined as producing a change in optical density at 580 nm of 0.01 per min at 30°C (pH 6.8), using a suspension of *M. luteus* cell walls as substrate. For the agarose diffusion assay, SDS-treated cell walls or heat-inactivated cells of *M. luteus* were mixed with 0.8% (wt/vol) agarose and cast into a petri dish. A 20-μl portion of the centrifuged culture supernatant fluid was applied to wells, which were incubated at 30°C overnight. The diameters (millimeters) of zones of lysis were measured after incubation.

**Preparation of antiserum.** An antiserum against gel-purified mutanolysin (Sigma) was raised in a female rabbit. Mutanolysin was separated from impurities by SDS-polyacrylamide gel electrophoresis (PAGE) (15%, wt/vol, polyacrylamide) and visualized with 1 M KCl (4°C, 16 h). The protein was recovered from excised bands by electroelution, using the Biotrap electroelution system (Schleicher & Schull, Dassel, Germany). The isolated protein was emulsified for the primary injection in complete Freund's adjuvant (Sigma). Subsequent booster injections were carried out with incomplete Freund's adjuvant (Sigma). Prior to immunization, rabbit serum was examined by immunoblotting for the absence of antibodies reacting with mutanolysin.

**SDS-PAGE and immunoblotting.** SDS-PAGE was performed by the method of Laemmli (29), using stacking gels and separating gels with 4% (wt/vol) and 12% (wt/vol) polyacrylamide, respectively. Extracellular protein was analyzed by precipitating the protein from 1 ml of fermentation broth with 7% (wt/vol; final concentration) trichloroacetic acid. After incubation for 1 h on ice, the suspension was centrifuged (8,000 × g, 10 min). The pellet was resuspended in 80 μl of buffer, consisting of 60 μl of SDS sample buffer and 20 μl of a saturated solution of Tris-Cl, pH 7.5 (28). Cellular protein was analyzed by disrupting the cells in a Sonifier (three pulses of 20 s each). Cell debris was removed by centrifugation (8,000 × g, 10 min). Supernatant was mixed with SDS sample buffer and applied to SDS-PAGE. Electrophoretic transfer of proteins to nitrocellulose membranes was carried out essentially as described by Eckerstorn et al. (13). Protein reacting with the anti-mutanolysin antiserum was visualized by subsequent incubation of the filter with 125I-protein A (Amersham Buchler, Braunschweig, Germany) and autoradiography on a Kodak X-ray film. Quantification was achieved by cutting out radioactive spots from immunoblots. Filter pieces were placed in Rotszint-11 (Roth, Karlsruhe, Germany) and measured with a liquid scintillation counter (Kontron MR 300).

**Analysis of RNA.** Total RNA was isolated from mycelia grown for 4 days in medium described by Yokogawa et al. (40). The isolation was performed according to the method of Hopwood et al. (23). Northern (RNA) blotting experiments were carried out as described by Sambrook et al. (33). RNA was separated in a 1% (wt/vol) agarose gel and transferred to a Hybond N membrane (Amersham). Hybridization was performed with 50% (wt/vol) formamide at a temperature of 32°C, using a 50-mer 32P-labeled oligonucleotide. The labeled oligonucleotide was prepared by using a 50-mer (GGCTGTCGTCGCCGACCAACCGGACCTGCGGGGCCTGCTGGA CATCGA)G and a decamer (GCTGATGTC) priming to the 50-mer. The complementary strand was synthesized by the method of Feinberg and Vogelstein (14). Filters were washed once at room temperature and twice at 35°C for 20 min and autoradiographed, using a Kodak X-ray film.
RESULTS

Bacterial cell walls increased the amount of bacteriolytic enzyme produced by *S. globisporus*. *S. globisporus* ATCC 21553 mycelia were grown with dextrin as a carbon source at 30°C. During a fermentation run, mycelia synthesized small quantities of bacteriolytic enzymes (Fig. 1A). Although the production of lytic activity is low under these conditions, the synthesis clearly started in the stationary phase (48 h, Fig. 1B); i.e., the dry weight of the cultures did not increase further. Hence, a clear separation of trophophase and idiophase was observed. The addition of cell walls of *Brevibacterium flavum* (1 mg/ml) to the medium increased the lytic activity about 10- to 20-fold compared with a culture without added cell walls (Fig. 1A). In cultures with cell walls, production of bacteriolytic enzymes started after about 24 h, when cells were still in the phase of maximal growth. Under these conditions, a clear separation of trophophase and idiophase was no longer feasible.

To investigate whether the higher lytic activity correlated with a higher amount of lytic enzymes or the activation of existing proteins, Western blot (immunoblot) analysis was carried out. As shown in Fig. 2, in cultures grown with bacterial cell walls, a higher quantity of mutanolysin was detected.

Since *S. globisporus* grew at a higher rate in the presence of bacterial cell walls (Fig. 1B), the greater amount of enzyme produced under this condition might be due to this observation. To exclude this possibility, a culture was grown with 6.5 nmol of the monomers of the peptidoglycan (N-acetylg glucosamine:N-acetylmuramic acid:ala:glu:meso-diaminopimelic acid in a ratio of 1:2:1:1:1; 6.5 nmol of peptidoglycan corresponds to about 1 mg of cell walls) per ml. As shown in Fig. 1B, cultures supplemented with the monomers grew better, suggesting that there was a nutritional effect caused by peptidoglycan monomers. However, there was only a slight increase in the amount of lytic enzymes (1.5- to 2-fold) compared with the control (Fig. 1A). Consequently, the nutritional effect cannot account for the stimulation of lytic activity of 10- to 20-fold in cultures grown with polymeric cell wall preparations.

To investigate whether the production of mutanolysin only was stimulated, culture fluid was mixed with antiserum raised against mutanolysin. After incubation overnight, about 40% of the lytic activity disappeared compared with the experiment without antibodies (data not shown). In a control, 12 U of purified mutanolysin (Sigma), which corresponded to the lytic activity level of a stimulated culture of *S. globisporus*, was inhibited completely by the antibodies. This indicated that, in addition to mutanolysin, the synthesis of at least one other bacteriolytic enzyme is stimulated by bacterial cell walls.

Effects of carbon source on production of mutanolysin. The medium of Yokogawa et al. (40), in which stimulation takes place, contains dextrin as the carbon source. When dextrin was replaced by glucose, stimulation of the syntheses of lytic enzymes was abolished. In addition, the constitutive formation of the enzymes, as shown in Fig. 1A, was no longer observed (data not shown). Replacing the carbon source (dextrin) with starch proved to be neither inhibitory nor stimulatory to the yield of bacteriolytic activity in the culture medium. Furthermore, the stimulation by cell walls could be accomplished similarly to that with the use of dextrin. Glucose might inhibit the activity of mutanolysin. To exclude this possibility, supernatant fluid of grown cultures was supplemented with purified mutanolysin (Sigma). Under these conditions, enzyme activity was fully measurable and thus not inhibited by glucose (data not shown).

Effect of different amounts of bacterial cell walls on production of lytic activity. *S. globisporus* cultures were grown with different amounts of *B. flavum* cell walls. After 96 h of incubation, mycelia were harvested and culture fluids were analyzed for lytic activity. The synthesis of lytic activity increased when 0.4 mg of bacterial cell walls per ml was added. With 0.1 and 0.2 mg of bacterial cell walls per ml, no significant effect was measured. A 0.6-mg/ml portion of bacterial cell walls gave maximal lytic activity. A further increase in the amount of cell walls did not result in increased lytic activity. The growth yield of *S. globisporus* was approximately the same with all amounts of cell walls tested.

Stimulation was independent of the growth phase of mycelia. To test whether a certain growth phase is essential to make cells competent for stimulation, cell walls of *B. flavum* (1...
mg/ml) were added to cultures of S. globisporus after 24, 48, and 72 h. At 72 h after addition of the stimulator, half of all cultures were harvested. The remaining cultures were grown for a total of 168 h. Lytic activity was measured in the fermentation fluid of all cultures. Results are presented in Table 2. There was no significant difference in the extent of stimulation. The growth of the cultures was only affected to the extent seen in Fig. 1B. Hence, stimulation was independent of the growth phase of mycelia.

**All types of bacterial cell walls tested led to induction of lytic activity.** Cell walls of B. flavum, which led to stimulation of lytic activity, contain peptidoglycan of the A1γ type (35). To investigate whether only certain types of peptidoglycan are capable of increasing the lytic activity, cell walls from organisms with different peptidoglycan types were prepared and used as stimulators (Table 3). Cultures were grown for 96 h, and 1 mg of cell walls per ml was added. Lytic activity of culture broth was measured by optical and agarose diffusion assays. There was no difference in lysis detectable when inactivated cells or cell walls of M. luteus were used as the substrate. All cell walls investigated led to a stimulation of lytic activity (Table 3), including that corresponding to mutanolysin, as indicated by immunoblot analysis (data not shown). Counting the radioactive spots of immunoblot experiments showed that the amount of mutanolysin had increased from about 12- to 50-fold (data not shown). Murein-sacculi of E. coli and cell walls of M. luteus and Lactobacillus plantarum induced the mutanolysin synthesis less efficiently than cell walls of the other organisms. However, the total lytic activity stimulated by cell walls of M. luteus reached the same level as when stimulated by other cell walls.

**Stimulation by cell wall preparations led to a de novo synthesis of mutanolysin.** To investigate whether the increased amount of mutanolysin in the supernatant resulted from a more efficient secretion or from de novo synthesis, Western blot experiments of total cell proteins were performed. Protein reacting with antiserum against mutanolysin was not detected in crude extracts of stimulated or unstimulated cells (data not shown). This indicated that mutanolysin is efficiently secreted; furthermore, the enzyme is obviously synthesised de novo after stimulation.

**Mycelia of S. globisporus grown with bacterial cell walls contained more mRNA coding for mutanolysin.** Higher mutanolysin production in cultures of S. globisporus grown with bacterial cell walls of B. flavum could be due to improved transcription of the mutanolysin gene or to the better translation of mutanolysin mRNA. To differentiate between these possibilities, Northern blot analysis was carried out. Total RNA from S. globisporus mycelia grown with cell walls of B. flavum (1 mg/ml) and without cell walls was isolated. Identical amounts of these RNAs were separated in an agarose gel, transferred to a membrane, and hybridized to the probe. In comparison to RNA from unstimulated cultures, the RNA from cultures grown with cell walls gave a signal corresponding to about 1,100 bp (Fig. 3). This indicated that in cells grown

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**TABLE 2. Effects of addition of B. flavum cell walls (1 mg/ml) at different times during fermentation on production of lytic activity**

<table>
<thead>
<tr>
<th>Time (h) of addition of stimulator</th>
<th>Lytic activity (U)</th>
<th>72 h after stimulation</th>
<th>After 168 h of fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>9.75</td>
<td>8.20</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>9.25</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>7.30</td>
<td>9.95</td>
<td></td>
</tr>
</tbody>
</table>

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**TABLE 3. Effects of bacterial cell walls of different peptidoglycan types on formation of lytic activity**

<table>
<thead>
<tr>
<th>Source of cell wall</th>
<th>Peptidoglycan type⁶</th>
<th>Lytic activity (U)</th>
<th>Stimulation (fold)</th>
<th>Zone of lysis (diam, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brevibacterium flavum</td>
<td>A1γ</td>
<td>7.50</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Curtobacterium insectillum</td>
<td>B2β</td>
<td>5.85</td>
<td>13</td>
<td>ND¹</td>
</tr>
<tr>
<td>Arthrobacter oxydans</td>
<td>A3α</td>
<td>5.95</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Arthrobacter atrocyaneus</td>
<td>A3α³</td>
<td>9.90</td>
<td>22</td>
<td>ND</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>A1γ</td>
<td>7.85</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Clavibacter michiganense subs. insidiosum</td>
<td>B2γ</td>
<td>6.75</td>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>A2</td>
<td>7.60</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>A3γ</td>
<td>8.15</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Streptomyces globisporus</td>
<td>A3γ</td>
<td>7.78</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>A1γ</td>
<td>3.62</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli wild type</td>
<td>A1γ</td>
<td>2.68</td>
<td>6</td>
<td>ND</td>
</tr>
<tr>
<td>Methanobrevibacter smithii</td>
<td>Pseudomurein</td>
<td>9.35</td>
<td>21</td>
<td>ND</td>
</tr>
<tr>
<td>Culture fluid without cells walls</td>
<td>0.45</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Culture fluid without cell walls (5 x)⁷</td>
<td>2.50</td>
<td>1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutanolysin</td>
<td>10 µg</td>
<td>5.40</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 µg</td>
<td>10.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:

- S. globisporus mycelia were grown for 96 h in medium described by Yokogawa et al. (40). Lytic activity was measured by optical assay (column 3) and by agar diffusion assay (column 4), as described in Materials and Methods. As controls, lytic activity in culture fluid after growth without added cell walls, of water, medium, and mutanolysin was measured.
- According to Schleifer and Kandler (35).
- ND, not determined.
- α-Carboxyl group of D-Glu substituted by D-Ala amide.
- Fivefold amount of culture fluid was used for measuring lytic activity.

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with bacterial cell walls more mRNA coding for mutanolysin existed.

**Stimulation of the production of lytic activity is not restricted to single Streptomyces strains.** The related strains *S. globisporus* subsp. *globisporus* ATCC 21553 and *S. coelicolor* DSM 40233 were tested to investigate whether only *S. globisporus* ATCC 21553 produces increased amounts of lytic enzymes in response to added bacterial cell walls. The amount of lytic activity produced by the strains clearly showed (Table 4) that these strains also produced more lytic activity in the presence of bacterial cell walls. The kind of lytic activity made by these strains, however, was not investigated.

**Bacterial cell walls act not only on lytic enzyme biosynthesis but also on the synthesis of pigment.** *S. globisporus* ATCC 21553 produces a yellow-brown pigment, which is secreted into the medium. This pigment is not yet chemically defined (31). By adding bacterial cell walls of *B. flavum* (1 mg/ml) to the culture, both the biosynthesis of lytic enzymes and pigment production were stimulated. The absorbance of the culture fluid in which stimulated cells were grown was significantly increased compared with a control culture. This clearly indicated that more pigment was secreted into the medium when bacterial cell walls were added. In addition, the formation of aerial mycelium was advanced in cultures grown with bacterial cell walls compared with control cultures without added cell walls. Similar behavior was observed when *S. globisporus* subsp. *caucasicus* 40814 and *S. coelicolor* DSM 40233 were grown with bacterial cell walls.

**TABLE 4. Effect of *B. flavum* cell walls on lytic activity of various Streptomyces strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lytic activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With cell walls</td>
</tr>
<tr>
<td><em>S. globisporus</em> subsp. <em>globisporus</em> ATCC 21553</td>
<td>10.65</td>
</tr>
<tr>
<td><em>S. globisporus</em> subsp. <em>caucasicus</em> DSM 40814</td>
<td>11.2</td>
</tr>
<tr>
<td><em>S. coelicolor</em> DSM 40233</td>
<td>9.75</td>
</tr>
</tbody>
</table>

* Mycelia were grown in medium described by Yokogawa et al. (40) for 96 h without or with (1 mg/ml) cell walls. Lytic activity was measured by the optical assay.

**DISCUSSION**

In this report, we demonstrate that the biosynthesis of an extracellular bacteriolytic enzyme in *S. globisporus* is induced by bacterial cell surface components. This observation exhibits similarities to elicitor phenomena so far only described in eucaryotes. In plants, elicitors derived from cell walls of an invading fungus have been described (9). Elicitors bind to specific receptors (7, 8) and mediate, via specific signal cascades, the specific expression of genes coding for defense mechanisms (11). As reported here, in *S. globisporus*, the stimulating surface component is part of a bacterial cell wall. Stimulation only occurred upon addition of cell wall preparations and not with the addition of monomer components of the peptidoglycan. Additional experiments indicate that a certain size of glycan moiety of peptidoglycan is essential for accomplishing stimulation (unpublished data). The average length of glycan chains in peptidoglycan was analyzed to be between 100 and 180 disaccharide units (32). A chain length of 140 disaccharide units corresponds to a chain width of 180,000. A molecule of this size is certainly too big to pass across the cytoplasmic membrane. Therefore, we assume that the molecule binds to a receptor similarly to elicitors in plant systems. As shown, a product of this elicitor reaction is mutanolysin. Hence, this enzyme exhibiting bacteriolytic activity might be synthesized by streptomycetes to gain a selective advantage in the natural environment.

Pseudomurein, which is found in distinct archaea, contains N-acetyl-L-talosaminuronic acid in the glycan strand instead of N-acetylmuramic acid. The anomic connection of the single saccharide units is β(1-3), compared with β(1-4) in bacteria (20). However, in all of these procaryotes the glycan strands form long stretches of structurally identical units. In our experiments, pseudomurein also led to an increased production of mutanolysin. This implies that, besides certain chemical features, mainly the three-dimensional structure of the stimulator is essential for accomplishing the elicitor reaction.

Recently, a similar effect has been found in *S. lividans*, in which the transcription of a chitinase was induced by chitin and repressed by glucose (18). An apparently similar effect has been observed in insects. In *Manduca sexta*, synthesis of lysozyme by fat bodies and other tissues was found to be stimulated by peptidoglycan (12). Things look different in *Clostridium thermocellum* and in the fungus *Trichoderma reesei*. Here, small molecules act as inducers for cellulase, sophorose in the case of *T. reesei* and cellulobiase in the case of *C. thermocellum* (2). However, the mechanism for this induction is not yet known.

From the findings presented, the question of whether elicitation in streptomycetes is a more general phenomenon arises. Additional experiments showed that in response to bacterial cell walls increased amounts of bacteriolytic enzymes were also produced by the related strains *S. globisporus* subsp. *caucasicus* DSM 40814 and *S. coelicolor* DSM 40233. In all of these strains, basal lytic activity was detected. This leads to the conclusion that the elicitation by bacterial cell walls is not restricted to single *Streptomyces* species. Further investigations are necessary to answer this important question.

A striking feature of the stimulation reaction in *S. globisporus* is the observation that the addition of bacterial cell walls to the medium not only influenced the biosynthesis of mutanolysin but also stimulated the synthesis of pigment and induced formation of aerial mycelium. So far, no data exist about the regulation of secondary metabolism in *S. globisporus*. In *S. coelicolor*, the best-investigated streptomycete, regulation of antibiotic formation has been found to be very complex.
Besides regulators which act on the biosynthesis of one specific antibiotic, e.g., ActII ORF4, which activates the biosynthesis of actinorhodin, at least 11 more regulators are known to act on the antibiotic formation globally. Some of these regulators are involved in both antibiotic formation and morphological differentiation. Provided the regulation of secondary metabolism is similar in S. globisporus, we assume that the elicitor acts on such a regulator. This is supported by the observation that both biosynthesis of mutanolyis and morphological differentiation were induced. How this regulation is mediated is the subject of further investigations.

The finding of elicitation by bacterial cell walls could lead to an application in biotechnology. By this means, higher quantities of extracellular lytic enzymes and eventually secondary metabolites could be produced simply and effectively with streptomycetes without the need for altering the producer genetically.

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nucleotide sequence of the N-acetylmuramidase M1-encoding gene from Streptomyces globisporus. Gene 88:81-86.