SALTON, M. R. J. (1955). *J. gen. Microbiol.* **12**, 25–30.

Isolation of *Streptomyces* spp. Capable of Decomposing Preparations of Cell Walls from various Micro-organisms and a Comparison of their Lytic Activities with those of certain Actinomycetes and Myxobacteria

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SUMMARY: Dispersion of isolated cell walls of *Streptococcus faecalis* in washed agar provided an opaque medium on which cell-wall decomposing micro-organisms were isolated from soil. All of the organisms isolated on *S. faecalis* cell-wall agar were *Streptomyces* spp. The lytic activities of seven isolates of *Streptomyces*, *S. albus*, two strains of *Micromonospora chalybeata*, *Micromonospora* sp., *Nocardia gardneri* and three strains of *N. corallina*, were studied on cell-wall agar media prepared from five Gram-positive and three Gram-negative bacteria and from the yeast *Candida pulcherrima*. The three strains of *Nocardia corallina* showed no lytic activity on any of the cell-wall substrates. All of the actinomycetes tested were without activity on the Gram-negative cell-wall agar media. Most of the actinomycetes produced lysed zones on Gram-positive cell-wall agar and the greatest lytic activities were observed with *Bacillus megaterium* and *Candida pulcherrima* cell-wall substrates. *Cytophaga johnsonae* and two strains of *Myxococcus fulvus* were lytic on *Candida pulcherrima* cell-wall agar but no lysis occurred on the bacterial cell-wall agars.

Although trypsin lyses certain heat-killed bacteria, in particular those belonging to the Gram-negative group, the cell walls constitute the principal components of the trypsin-resistant residues (Salton, 1953*a*). Thus dispersion of heat-killed bacteria in agar would not provide a suitable substrate for the detection of micro-organisms which produce cell-wall decomposing enzymes; organisms possessing proteolytic enzymes would also exhibit lysed zones due to digestion of protoplasmic constituents of heat-killed cells. With the methods available for the separation of bacterial cell walls from protoplasmic material (Salton & Horne, 1951), isolated cell walls may be used for the preparation of cell-wall agar media. Dispersion of cell walls in washed agar provided an opaque medium suitable for the isolation of cell-wall decomposing micro-organisms from soil; the organisms were detected by the production of lysed zones. This paper describes a method for the isolation of cell-wall decomposing micro-organisms and records the lytic activities of certain actinomycetes and myxobacteria on cell-wall agar media.

METHODS

Preparation of cell walls. Cell walls of the following organisms were prepared: *Streptococcus faecalis* (NCTC 6782); *S. agalactiae* Lancefield group B (NCTC 6175); *Micrococcus lysodeikticus* (NCTC 2665); *Staphylococcus aureus* strain Duncan; *Bacillus megaterium* strain KM; *Escherichia coli* strain B; *Pseudomonas aeruginosa*; *Rhodospirillum rubrum* and *Candida pulcherrima* (*Torulopsis*

pulcherrima). Cells were harvested from liquid media by centrifugation after growth under appropriate conditions. With the exception of *Pseudomonas aeruginosa*, cells were washed twice with distilled water on the centrifuge and suspended in distilled water. *P. aeruginosa* was washed with 1 % saline and suspended in 1 % saline. Cells were disintegrated with Ballotini (Chance Bros. grade 12) beads in the Mickle disintegrator with the exception of those of *Bacillus megaterium* and *Rhodospirillum rubrum* which were disintegrated in the Raytheon 9Kc. Magnetostriction Oscillator. The subsequent steps in the preparation of cell walls were those outlined by Salton & Horne (1951). The walls of *C. pulcherrima* were deposited from suspension by centrifuging for 15 min. at 3000 r.p.m.

Preparation of cell-wall agar plates. Cell-wall agar plates were prepared as follows: 10 ml. of medium consisting of 1.5 % washed agar, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4 \cdot 7H_2O$ were poured into Petri dishes and allowed to set as a bottom layer; c. 8 ml. melted 2 % washed agar was added to c. 2 ml. concentrated cell-wall suspension (containing 25–35 mg. dry weight cell wall/ml.) and poured as a top layer. Uniformly opaque cell-wall agar plates were obtained and used for the isolation of micro-organisms from soil or for testing the lytic activities of other micro-organisms. As cell walls were not prepared under aseptic conditions, heating cell-wall suspensions for 5 min. at 80° before dispersion in washed agar decreased the number of contaminants appearing on the cell-wall agar, especially when plates were to be incubated for periods of 7 days or more. Preliminary experiments comparing the lytic activities of the various micro-organisms on cell-wall agar prepared from untreated and heated (5 min. at 80°) cell-wall suspensions showed no detectable differences.

Heated-cell agar plates. In some experiments the lytic activities of the micro-organisms on heat-killed cells were studied. The plates were prepared as for the cell-wall agar plates, using instead of cell-wall suspensions, thoroughly washed suspensions of bacteria killed by heating for 5 min. at 100°.

RESULTS

Isolation of cell-wall decomposing micro-organisms from soil

For the isolation of cell-wall decomposing micro-organisms, small grains of soil were placed on the surface of the cell-wall agar, the plates were incubated and examined at intervals for the appearance of lysed zones around the soil grains. Cell-wall agar plates prepared with walls from *Streptococcus faecalis*, *Escherichia coli* or *Rhodospirillum rubrum* were inoculated with soil and incubated at 30°. Lysed zones appeared around the soil grains on *Streptococcus faecalis* cell-wall agar after incubation for several days. No lysed zones were observed even after prolonged incubation of *Escherichia coli* and *Rhodospirillum rubrum* cell-wall agar plates inoculated with soil.

The growth from a number of lysed zones on *Streptococcus faecalis* cell-wall agar was picked off and streaked out on *S. faecalis* cell-wall agar and on glycerol + asparagine agar (Conn, 1921). The latter medium was useful in the purification of the organisms isolated from the cell-wall agar as it enabled the

conservation of cell-wall material for the final testing of purified cultures. Several successive transfers on glycerol + asparagine agar were made and the growth on these plates tested for retention of lytic activity on *S. faecalis* cell-wall agar. All of the single-colony isolations were finally re-tested on *S. faecalis* cell-wall agar, after the organisms had been examined for the presence of contaminants on several different media.

The twelve isolates obtained from the soil-inoculated *Streptococcus faecalis* cell-wall agar plate were identified as *Streptomyces* spp.; seven strains were selected for more detailed studies of morphological and cultural characteristics. A comparison of the characteristics of the seven strains with the descriptions of the seventy-three species of *Streptomyces* given in *Bergey's Manual of Determinative Bacteriology* (1948) does not permit their assignment to any of the described species.

The organisms were maintained on a medium containing 1.5 g. (wet weight) washed, heat-killed *Streptococcus faecalis* cells; 0.1 g. K_2HPO_4 ; 0.05 g. $MgSO_4 \cdot 7H_2O$; 1.5 g. agar/100 ml. medium. All strains showed abundant spore formation on this medium within 48 hr. incubation at 30°, thus providing suitable spore inocula for subsequent experiments.

*A comparison of the lytic activities of Streptomyces spp.
and of some actinomycetes*

To test the lytic activities of the seven strains of *Streptomyces* and a number of related actinomycetes, cell-wall agar was prepared with the walls from a number of bacterial species and from the yeast, *Candida pulcherrima*. Cell-wall agar media were inoculated by streaking some of the growth from the stock cultures of the actinomycetes on the agar surface. The method of testing the various actinomycetes for their abilities to decompose the cell walls is illustrated in Pl. 1, figs. 1 and 2, where the growth and lytic activities of the organisms on *Streptococcus faecalis* cell-wall agar is shown (5 days incubation at 30°).

As shown in Pl. 1, fig. 3, the seven strains of *Streptomyces* isolated on *Streptococcus faecalis* cell-wall agar exhibited greater lytic activities on *Bacillus megaterium* cell-wall agar than that observed on the *Streptococcus faecalis* cell-wall agar. *Nocardia gardneri* and the two strains of *Micromonospora chalceae* also produced more extensive zones of lysis on *Bacillus megaterium* cell-wall agar (shown in Pl. 1, fig. 4) than that observed with *Streptococcus faecalis* cell-wall agar.

The seven strains of *Streptomyces*, *Streptomyces albus*, *Nocardia gardneri*, three strains of *Nocardia corallina*, two strains of *Micromonospora chalceae* and a *Micromonospora* sp. were tested for lytic activity on cell-wall agar media prepared with the walls from five Gram-positive bacteria and from the yeast *Candida pulcherrima*. The three strains of *Nocardia corallina* were devoid of lytic activity on all of the cell-wall agar media; the *Micromonospora* sp. showed no activity or only weak activity on the various cell-wall substrates. When tested on *Staphylococcus aureus* cell-wall agar, *Streptomyces* sp. strain 1B and *S. albus* exhibited weak lytic activity, *Nocardia gardneri* and the two

strains of *Micromonospora chalceae* produced more extensive zones of lysis, and the other actinomycetes were non-lytic. The results of the tests of the lytic activities of the actinomycetes on the cell-wall agar prepared from the other Gram-positive organisms are presented in Table 1.

Table 1. *The lytic activities of some actinomycetes on cell-wall agar media*

Test organism	Plates incubated for 5 days at 30°.				
	<i>S. faecalis</i>	<i>S. agalactiae</i>	<i>B. megaterium</i>	<i>M. lysodeikticus</i>	<i>C. pulcherrima</i>
<i>Streptomyces</i> sp. 1A	++	++	+++	++	++
<i>Streptomyces</i> sp. 1B	++	++	+++	++	++
<i>Streptomyces</i> sp. 2A	+	+	+	+	++
<i>Streptomyces</i> sp. 3·1	++	+	++	++	++
<i>Streptomyces</i> sp. 4A	++	+	++	++	+++
<i>Streptomyces</i> sp. 5A	++	++	+++	++	++
<i>Streptomyces</i> sp. 7·2	++	++	+++	++	++
<i>Streptomyces albus</i>	++	++	+	+	+
<i>Nocardia gardneri</i> NCTC 6531	++	++	+++	++	+++
<i>Micromonospora chalceae</i> (strains SG5 and G7)	++	++	+++	+++	+

+, lytic zone c. 1 mm.; ++, 1–4 mm.; +++, 4+ mm.

None of the actinomycetes showed evidence of lytic activity when tested on cell-wall agar prepared with walls of the Gram-negative organisms *Escherichia coli*, *Pseudomonas aeruginosa* or *Rhodospirillum rubrum*. The absence of lysed zones on *Escherichia coli* cell-wall agar inoculated with actinomycetes is shown in Pl. 1, fig. 5. This is in marked contrast with the extensive lysis observed with most of the actinomycetes (excluding the three non-proteolytic strains of *Nocardia corallina*) on agar containing heat-killed cells of *Escherichia coli* (Pl. 1, fig. 6). Similar results were obtained with heat-killed cells of *Pseudomonas aeruginosa*.

The diffusible nature of the lytic substances (presumably enzymes) produced by the actinomycetes was demonstrated by cutting out blocks of the cell-wall agar from the broad lysed zones and placing them on fresh cell-wall agar. Clearing of the cell-wall agar in the absence of any detectable growth was observed with *Streptococcus faecalis*, *Bacillus megaterium* and *Candida pulcherrima* cell-wall agar blocks.

The lytic activities of several myxobacteria

The chitin-decomposing myxobacterium *Cytophaga johnsonae* studied by Stanier (1947) and two strains of *Myxococcus fulvus* were tested on *Escherichia coli* and *Pseudomonas aeruginosa* cell-wall agar and on heated-cell agar. The two species of myxobacteria produced extensive lysis within several days incubation at 30° on heated-cell agar prepared from the two Gram-negative bacteria. Although *Escherichia coli* and *Pseudomonas aeruginosa* cell-wall agar media supported weak growth of the myxobacteria, there was no evidence of lysis even after 2 weeks incubation at 30°. When tested on *Streptococcus faecalis* and *Bacillus megaterium* cell-wall agar, the myxobacteria showed no lytic activities. However, *Cytophaga johnsonae* and the two strains of *Myxo-*

coccus fulvus had effected extensive lysis on *Candida pulcherrima* cell-wall agar plates incubated for 3 days at 30°; *Cytophaga johnsonae* showed greater lysis than the two strains of *Myxococcus fulvus*.

DISCUSSION

As a group, the actinomycetes possess marked bacteriolytic properties, and the lytic activities of these organisms have been studied extensively by Welsch (1947). It was not surprising that the organisms isolated on bacterial cell-wall agar belonged to the *Streptomyces* group. The bacteriolytic properties of the actinomycetes appear to be due to two groups of enzymes: (1) the proteolytic enzymes responsible for the dissolution of heat-killed bacteria (Tai & van Heyningen, 1951; Muggleton & Webb, 1952; Born, 1952); (2) enzymes capable of bringing about a dissolution of isolated cell walls of certain bacteria, e.g. lysis of group A streptococcus walls by *Streptomyces albus* enzymes (McCarty, 1952*a, b*; Salton, 1952*a*). The ability of *Streptomyces* spp. and other actinomycetes to effect a dissolution of bacterial cell walls is further illustrated in this paper. The cell walls of the lysozyme-sensitive organisms *Micrococcus lysodeikticus* and *Bacillus megaterium* previously shown to be digested by crystalline egg-white lysozyme (Salton, 1952*b*; 1953*a*) are dissolved by the lytic enzymes produced by various actinomycetes. In addition to the lytic enzymes degrading bacterial cell walls, some of the actinomycetes and myxobacteria lyse the cell walls of the yeast *Candida pulcherrima*.

The method for the isolation of cell-wall decomposing micro-organisms described in this paper has not provided organisms possessing lytic enzymes for Gram-negative bacteria. Attempts to isolate such organisms for *Escherichia coli* from cell-wall enrichment cultures have so far failed. The high lipid contents and the nature of the protein components of the cell walls of Gram-negative bacteria (Salton, 1953*b*) may confer a greater degree of resistance to enzymic digestion than that exhibited by the cell walls of certain Gram-positive bacteria.

This work was commenced during the tenure of a Merck International Fellowship at Prof. R. Y. Stanier's laboratory, University of California, Berkeley; I should like to thank Prof. Stanier for his hospitality and interest and Merck and Co. for the award of a Fellowship. I wish to thank Dr H. L. Jensen for cultures of actinomycetes, Dr McCarty for the strain of *Streptomyces albus*, Dr B. Bachmann for strains of *Myxococcus fulvus* and Mr K. Harvey for photographing the cell-wall agar plates. These investigations were continued during the tenure of a Broodbank Fellowship.

REFERENCES

- Bergey's Manual of Determinative Bacteriology*, 6th ed. (1948). Ed. by BREED, R. S., MURRAY, E. G. D. & HITCHENS, A. P. London: Baillière, Tindall and Cox.
- BORN, G. V. R. (1952). The extracellular bacteriolytic enzymes of a species of *Streptomyces*. *J. gen. Microbiol.* 6, 344.
- CONN, H. J. (1921). The use of various culture media in characterizing actinomycetes. *Tech. Bull. N.Y. St. agric. Exp. Sta.* no. 83.

- MCCARTY, M. (1952*a*). The lysis of group A hemolytic streptococci by extracellular enzymes of *Streptomyces albus*. I. Production and fractionation of the lytic enzymes. *J. exp. Med.* **96**, 555.
- MCCARTY, M. (1952*b*). The lysis of group A hemolytic streptococci by extracellular enzymes of *Streptomyces albus*. II. Nature of the cellular substrate attacked by the lytic enzymes. *J. exp. Med.* **96**, 569.
- MUGGLETON, P. W. & WEBB, M. (1952). The exocellular bacteriolytic system of soil actinomycetes. II. A further investigation of the lytic system. *Biochim. Biophys. Acta*, **8**, 431.
- SALTON, M. R. J. (1952*a*). The nature of the cell walls of some Gram-positive and Gram-negative bacteria. *Biochim. Biophys. Acta*, **9**, 334.
- SALTON, M. R. J. (1952*b*). Cell wall of *Micrococcus lysodeikticus* as the substrate of lysozyme. *Nature, Lond.* **170**, 746.
- SALTON, M. R. J. (1953*a*). Cell structure and the enzymic lysis of bacteria. *J. gen. Microbiol.* **9**, 512.
- SALTON, M. R. J. (1953*b*). Studies of the bacterial cell wall. IV. The composition of the cell walls of some Gram-positive and Gram-negative bacteria. *Biochim. Biophys. Acta*, **10**, 512.
- SALTON, M. R. J. & HORNE, R. W. (1951). Studies of the bacterial cell wall. II. Methods of preparation and some properties of cell walls. *Biochim. Biophys. Acta*, **7**, 177.
- STANIER, R. Y. (1947). Studies on nonfruiting myxobacteria. I. *Cytophaga johnsonae*, n.sp., a chitin-decomposing myxobacterium. *J. Bact.* **53**, 297.
- TAI, T. Y. & VAN HEYNINGEN, W. E. (1951). Bacteriolysis by a species of *Streptomyces*. *J. gen. Microbiol.* **5**, 110.
- WELSCH, M. (1947). *Phénomènes d'antibiose chez les actinomycètes*. Gembloux: Imprimerie J. Duculet.

EXPLANATION OF PLATE

- Fig. 1. Lytic activities of the seven strains of *Streptomyces* spp. and *S. albus* on *Streptococcus faecalis* cell-wall agar; 5 days incubation at 30°.
- Fig. 2. Lytic activities of *Micromonospora* spp., *Nocardia* spp. and *Streptomyces albus* on *Streptococcus faecalis* cell-wall agar; 5 days incubation at 30°.
- Fig. 3. Lytic activities of the seven strains of *Streptomyces* and *S. albus* on *Bacillus megaterium* cell-wall agar; 5 days incubation at 30°.
- Fig. 4. Lytic activities of *Micromonospora* spp., *Nocardia* spp. and *Streptomyces albus* on *Bacillus megaterium* cell-wall agar; 5 days incubation at 30°.
- Fig. 5. *Escherichia coli* cell-wall agar inoculated with *Micromonospora* spp., *Nocardia* spp. and *Streptomyces albus*; 7 days incubation at 30°.
- Fig. 6. Lysis of heat-killed *Escherichia coli* by *Micromonospora* spp., *Nocardia gardneri* and *Streptomyces albus* after 3 days incubation at 30°.

Key to test organisms shown in Figs. 1-6:

1	<i>Streptomyces</i> sp.	1A	9	<i>Micromonospora</i> sp.	G1
2	<i>Streptomyces</i> sp.	1B	10	<i>Micromonospora chalceae</i>	SG5
3	<i>Streptomyces</i> sp.	2A	11	<i>Micromonospora chalceae</i>	G7
4	<i>Streptomyces</i> sp.	3·1	12	<i>Nocardia corallina</i>	K6
5	<i>Streptomyces</i> sp.	4A	13	<i>Nocardia corallina</i>	227
6	<i>Streptomyces</i> sp.	5A	14	<i>Nocardia corallina</i>	H2
7	<i>Streptomyces</i> sp.	7·2	15	<i>Nocardia gardneri</i>	NCTC 6531
8	<i>Streptomyces albus</i>				

(Received 24 May 1954)