# *STREPTOMYCES LAURENTII*, A NEW SPECIES PRODUCING THIOSTREPTON

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The taxonomic description of *Streptomyces laurentii*, a new species related to but distinguishable from the *S. fradiae* group, is presented. This new species produces thiostrepton but bears no taxonomic relationship to the known producers of the antibiotic: *S. azureus*, *S. hawaiiensis*, and *Streptomyces* X-14b.

A new species of *Streptomyces* isolated from soil collected in Lawrence Township, New Jersey was shown to produce thiostrepton. The following report presents a description of the organism and examines the taxonomic relationship of this species to members of the *S. fradiae* group *sensu latu* and to the known species producing thiostrepton.

## Materials and Methods

Types strain: *Streptomyces laurentii*, n. sp. has been deposited in the American Type Culture Collection under the accession number ATCC 31,255.

Taxonomic studies were conducted in accordance with the methods used in the International Streptomyces Project.[1] Color designations[2] are taken from the Color Harmony Manual[3] and the ISCC-NBS dictionary of color names.[4]

Chromatographic analysis of cell wall hydrolysates for the presence of the L- or meso- isomer of diaminopimelic acid was done by the method of BECKER *et al.*[5]

DNA homology studies were based on the method of DELEY *et al.*[7] adapted to streptomycetes (TREJO, W. H., manuscript in preparation).

Thiostrepton was produced by *S. laurentii* ATCC 31,255 by fermentation under submerged aerobic conditions in a medium containing: Toasted Nutrisoy Flour, 15.0 g; soluble starch, 15.0 g; glucose, 50.0 g; $CoCl_2 \cdot 6H_2O$, 0.005 g; $CaCO_3$, 10.0 g; distilled water to 1 liter. The fermentation was conducted for five days at 25°C on a rotary shaker (280 rpm; 2 in. throw) in 250-ml Erlenmeyer flasks containing 50 ml of medium. The antibiotic was isolated as previously described[8,9] and analyzed according to commonly accepted procedures.

## Results

### Description of *Streptomyces laurentii* nov. sp. ATCC 31,255

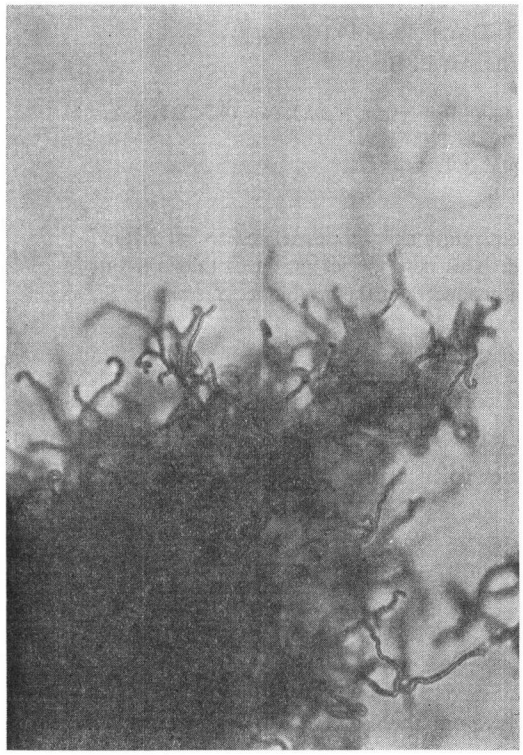
*L. laurentii* gen. n. derived from latinization of Lawrence after Lawrence Township, New Jersey, the origin of the soil isolate.

Cultural Characteristics: yeast extract malt agar (ISP medium 2): sporulation is scant as a faint pinkish blush on white aerial mycelium; reverse: burnt orange; soluble pigment: light rose.

Oatmeal agar (ISP medium 3): sporulation is good and occurs rapidly within 4 days. Aerial mycelium is grayish yellow pink. CHM No. 5 ec; dusty pink. There is no distinctive reverse color or soluble pigment.

Fig. 1. Aerial mycelium of *S. laurentii* on starch-casein agar, 7 days.

Hooks and primitive spirals more prevalent than on oatmeal agar. Magn. 350× (×0.58)



Inorganic salts-starch agar (ISP medium 4): sporulation is good, CHM No. 4 ec. light rose beige; reverse: reddish-orange; no soluble pigment.

No melanin is produced on sodium caseinate-tyrosine agar, (sodium caseinate 25.0 g/liter, $NaNO_3$ 10.0 g, *l*-tyrosine 1.0 g, tap water 1 liter).

Microscopic Characteristics: On oatmeal agar the aerial mycelium is predominantly straight (Rectus) with rare primitive spirals of a single turn; however, on starch casein agar hooks and primitive spirals predominate (Fig. 1). The spores are smooth as seen by electron microscopy (Fig. 2).

In shaken culture (18 hours at 25°C in tryptone-yeast extract broth (ISP medium 1)) a dusty pink soluble pigment is produced and the whole mycelium fragments into arthrospores and rods of varying length (Fig. 3).

Fig. 2. Electron micrograph of spores of *S. laurentii*. Magn. 9,000× (×0.60)

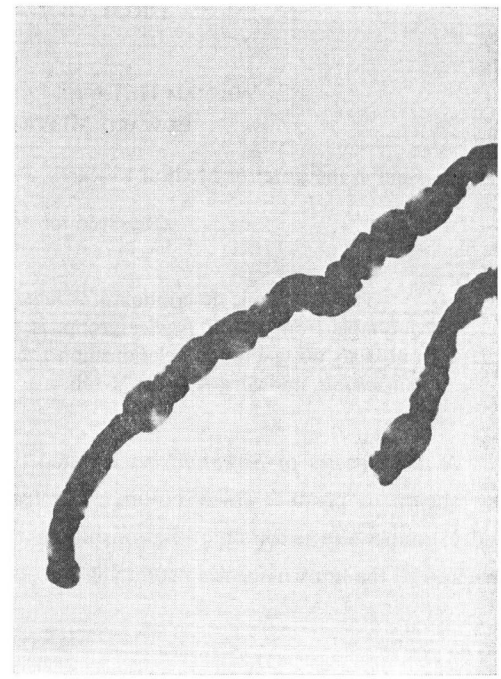
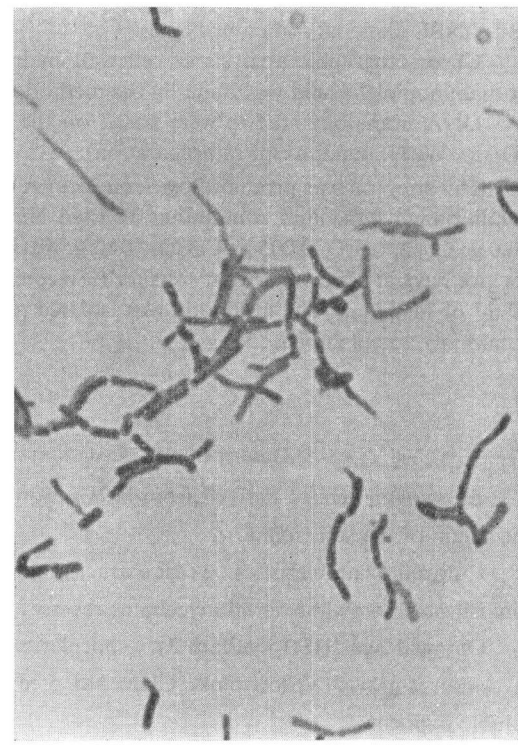


Fig. 3. Fragmentation of mycelium in 18-hour shaken culture. Magn. 2,400× (×0.58)



Physiological and Biochemical Characteristics: carbohydrate utilization was determined on the basal medium of PRIDHAM and GOTTLIEB[6] supplemented with individual carbon sources at 1% w/v. The following carbohydrates, as the sole carbon source, supported growth: glucose, xylose, galactose, melibiose, sucrose and lactose. There was no growth on mannitol, inositol, sorbitol, arabinose, rhamnose, fructose, raffinose and trehalose.

Table 1. DNA homology

| Strain pairs | Degree of binding, % |
|---|---|
| *S. laurentii, S. fragilis* | 35 |
| *S. laurentii, S. fradiae* | 35 |
| *S. fradiae, S. fragilis* | 24 |

Table 2. Comparison of the product of *S. laurentii* with the thiostrepton standard

| | Product of *S. laurentii* ATCC 31,255 | Thiostrepton* |
|---|---|---|
| 1. Melting point | 250~255°C | 240~250°C |
| 2. Specific rotation | −60° | −55.8° |
| 3. Ultraviolet absorption max (nm) | 225, 250, 280 | 225, 250, 280 |
| 4. Rf (silica gel, 10% MeOH/$CHCl_3$) | 0.38 | 0.38 |
| | (detected by UV and bioautography on *Staph. aureus* 209P) | |
| 5. NMR (100 MHz; DMSO-$d_6$) | The spectra of both samples are identical and superimposible. | |
| 6. IR (KBr) | Both samples have identical spectra. Amide I and II bands characteristic for a peptide are present. | |
| 7. Amino acid analysis: | | |
| threonine | 1.0 | 0.96 |
| alanine | 2.19 | 2.09 |
| isoleucine | 1.0 | 0.95 |
| cystine | traces | traces |

* Squibb standard

Cell wall hydrolysates contain L-diaminopimelic acid.

DNA homology as used here refers to the degree of binding of base pairs in nucleotide fragments by free reassociation as defined by DELEY *et al.*[7] DNA homologies for the three strains are shown in Table 1.

### Antibiotic Identification

A comparison of the analytical data obtained with the isolated antibiotic and that of an authentic sample (Table 2) identifies it as thiostrepton.

## Discussion

Comparison of taxonomic characters for the known thiostrepton-producing strains is shown in Table 3. These data show that *S. laurentii* ATCC 31,255 bears no relationship to *S. azureus, S. hawaiiensis* or *Streptomyces* X-14b. Thus, *S. laurentii* represents another species producing thiostrepton.

*S. laurentii* shares several key morphological characteristics with *S. fradiae* and *S. fragilis* which are representative of those species in the Red spore color series that are melanin negative and have smooth spores. A comparison of the key characteristics of *S. laurentii* and the latter two species are summarized in Table 4. While resembling *S. fragilis* in morphology, *S. laurentii* differs from both *S. fragilis* and *S. fradiae* in its carbohydrate utilization pattern with respect to arabinose, trehalose, melibiose, sucrose and lactose. Moreover, all three strains differ from each other with respect to antibiotic production. The total fragmentation of the mycelium of *S. laurentii* and the lack of this property in *S. fradiae* and *S. fragilis* also constitutes a fundamental morphological difference. Additionally, the DNA homology data strongly support the contention that these species are different

Table 3. Comparison of thiostrepton-producing strains

| | *S. laurentii* | *S. azureus* | *S. hawaiiensis* | *S.* sp.* |
|---|---|---|---|---|
| Strain No. | ATCC 31,255 | ISP 5106 | ISP-5042 | X-14b |
| Spore color series | Red | Blue | White to yellow | White |
| Morphology group | Rectus | Spira | Spira | Spira |
| Spore wall | Smooth | Smooth | Spiny | No report |
| Melanin | − | + | + | − |
| Carbohydrate utilization: | | | | |
| Glucose | + | + | + | + |
| Mannitol | − | + | + | + |
| Inositol | − | + | + | + |
| Sorbitol | − | − | + | − |
| Xylose | + | + | − | + |
| Arabinose | − | + | + | − |
| Rhamnose | − | + | + | − |
| Fructose | − | + | + | + |
| Raffinose | − | + | + | + |
| Galactose | + | + | + | + |
| Sucrose | + | + | + | + |
| Lactose | + | + | + | + |

* Due to unavailability of the culture, description is based on the published data[10].

Table 4. Comparison of cultures resembling *S. laurentii*

| | *S. laurentii* | *S. fragilis* | *S. fradiae* |
|---|---|---|---|
| Strain No. | ATCC 31,255 | ATCC 23,908 | IMRU 3535 |
| Spore color series* | Red | Red | Red |
| Morphology group* | RF⟶RA | RF⟶RA | RA |
| Spore wall | Smooth | Smooth | Smooth |
| Melanin | − | − | − |
| Reverse | YB**-orange | YB**-orange | No distinctive pigment |
| Carbohydrate utilization: | | | |
| Glucose | + | + | + |
| Mannitol | − | − | − |
| Inositol | − | − | − |
| Sorbitol | − | − | − |
| Xylose | + | + | var. |
| Arabinose | − | + | + |
| Rhamnose | − | − | − |
| Fructose | − | − | var. |
| Raffinose | − | − | − |
| Galactose | + | + | + |
| Trehalose | − | + | + |
| Melibiose | + | − | − |
| Sucrose | + | − | − |
| Lactose | + | − | − |
| Antibiotic | Thiostrepton | Azaserine[12] | Neomycin |

* Spore color series and morphology groups after PRIDHAM *et al.*[11]

** Abbreviation for yellowish-brown.

from each other. In this system similar species have a high degree of binding, 90% or greater, while homologies below 50% are suggestive of disparate species (TREJO, W. H., manuscript in preparation). While the data showing correlation between conventional taxonomic groups and DNA homology are

fragmentary, there is a high likelihood of unrelatedness if single stranded DNA from the two organisms, when paired under appropriate conditions, cannot reassociate to form duplexes.

Based on these differences, *i.e.*, carbohydrate utilization pattern, mycelial fragmentation and DNA homology, *S. laurentii* is considered to be a new species.

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