Production of Hydrogen Sulfide by *Streptomyces odorifer*

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**Abstract**

Collins, R. P. (University of Connecticut, Storrs), and H. D. Gaines. Production of hydrogen sulfide by *Streptomyces odorifer*. Appl. Microbiol. 12:335-336. 1964.—By use of various trapping systems, as well as lead acetate papers, *Streptomyces odorifer* was shown to produce hydrogen sulfide. Other sulfur-containing compounds may be produced by *S. odorifer*, but the amounts obtained were too small for detailed analysis. It was suggested that hydrogen sulfide might be a part of the earthy-odor complex produced by *S. odorifer*.

A number of microorganisms are known to produce hydrogen sulfide as a metabolic by-product. The compound appears to originate from sulfur-containing amino acids. Tarr (1933) showed that, with washed cells of *Proteus vulgaris*, L-cystine undergoes anaerobic decomposition to form two molecules each of hydrogen sulfide, ammonia, acetic acid, and formic acid. Desnuelle (1939a, b) and Desnuelle and Fromageot (1939) showed that *Escherichia coli* produces hydrogen sulfide from cysteine. Binkley (1943) showed that brewer's and baker's yeasts contain a cysteine desulfurase which produces hydrogen sulfide from cysteine.

In the present study, the actinomycete *Streptomyces odorifer* (Rullmann emend. Lachner-Sandoval) was investigated for the production of hydrogen sulfide and other sulfur-containing compounds. Previous experiments (Gaines and Collins, 1963) had shown that *S. odorifer* produced a number of compounds which might partially make up the earthy odor characteristic of this organism; however, sulfur-containing compounds were not investigated.

**Materials and Methods**

The culture of *S. odorifer* was obtained from the American Type Culture Collection and maintained on nutrient agar slants at 20 C. The inoculum for 250-ml Erlenmeyer shake flasks was obtained by transferring a small piece of agar containing spores and mycelium to each shake flask and incubating the flasks at 25 C for approximately 60 hr. The contents of these flasks then served as the inoculum for six large (2,500 ml) pecticoat flasks containing 800 ml of nutrient broth. These flasks were incubated on a reciprocal shaker for approximately 60 hr at 25 C.

The factors in the initial experiments which suggested that hydrogen sulfide might be present were the odor of the culture medium and the blackening of lead acetate papers held at the tip of the condenser during the distillation process.

In later experiments, the contents of six large shake flasks were emptied into a 10-liter distilling flask equipped with a reflux condenser and a gas inlet for nitrogen. The contents of the flask were heated to 80 C, and the evolved vapor was passed through a series of traps containing reagents specific for various classes of sulfur compounds. The trapping system used was similar to those used by Walker (1959) and Kiribuchi and Yamaniski (1963), and contained calcium chloride (trap 1), crystalline lead acetate (trap 2), a saturated solution of mercuric cyanide (trap 3), and a saturated solution of mercuric chloride (trap 4). In some experiments, traps 1 and 2 were replaced with one containing an acid solution of cadmium sulfate [5% (w/v) in 0.2 N HCl].

The calcium chloride in trap 1 served to remove water vapor, and the lead acetate in trap 2 served to remove hydrogen sulfide. Dry mercaptans do not react with lead acetate, but they do react with mercuric cyanide (trap 3). The mercuric chloride in trap 4 served to remove organic sulfides and disulfides. The cadmium sulfate trap served to selectively remove hydrogen sulfide.

**Results and Discussion**

A stream of nitrogen was passed through the heated culture medium for 5 hr. The trap containing lead acetate showed considerable darkening, indicating the formation of lead sulfide. There was a small amount of precipitate in the trap containing mercuric chloride, suggesting the presence of organic sulfides and disulfides; however, this result was inconclusive, as any hydrogen sulfide not trapped in lead acetate would be trapped in mercuric chloride. A portion of the mercuric chloride precipitate was warmed with dilute sodium hydroxide, but no odor was detected, which indicated the absence of volatile organic sulfides. A portion of the mercuric chloride precipitate was also acidified and refluxed, and the evolved vapors were trapped as above. Only hydrogen sulfide was detected.

In another series of experiments, the calcium chloride and lead acetate traps were replaced with a trap containing cadmium sulfate. A yellow precipitate was formed in the cadmium sulfate traps, indicating the presence of cadmium sulfide. The other traps in the series were devoid of a precipitate.
It was concluded that the major sulfur-containing compound produced by \textit{S. odorifer} was hydrogen sulfide. It is interesting that, in a recent publication describing the genera and species of the actinomycetes, \textit{S. odorifer} is said not to produce hydrogen sulfide (Waksman, 1961). Tresner and Danga (1958) also reported that \textit{S. odorifer} does not produce hydrogen sulfide. These authors used a Peptone Iron Agar medium for determining the presence of hydrogen sulfide. Recently, however, Kuster and Williams (1964) showed that blackening of Peptone Iron Agar does not clearly indicate the presence of hydrogen sulfide.

Organisms such as \textit{S. odorifer} may contribute to the foul or pigpen-like odors found in certain water reservoirs and often said to be due to decaying vegetation. That hydrogen sulfide can play an important role as a flavor constituent in cheese was shown by Barnicoat (1950), Kristofferson and Nelson (1955), and Walker (1959). Walker (1959) stated that the flavor of cheddar cheese was due primarily to carbonyl and sulfur compounds. Hydrogen sulfide has also been shown to be a flavor constituent in beer by Brenner, Owades, and Golyziak (1954).

The nature of the odor produced by \textit{S. odorifer} is conditioned, at least in part, by the culture medium. The sulfuriferous odor is not found if the organism is grown on a glycerol-starch-glutamate medium. This finding is not too surprising, in that the amino acid precursors would not be as readily available as in the nutrient broth medium.

The fine precipitate found in the mercuric chloride traps indicates that other sulfur-containing compounds might be produced by \textit{S. odorifer}; however, the amount of precipitate formed was too small to permit detailed analysis.

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**Literature Cited**


