

COMPLETE REDUCTION OF TELLURITE TO PURE TELLURIUM METAL BY MICROORGANISMS

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ABSTRACT

TUCKER, FAYNE L. (University of Southern California, Los Angeles), JOHN F. WALPER, MILO DON APPELMAN, AND JERRY DONOHUE. Complete reduction of tellurite to pure tellurium metal by microorganisms. *J. Bacteriol.* **83**:1313-1314. 1962—The black precipitate produced in the presence of potassium tellurite by growing cells of *Streptococcus faecalis* N83 and *Corynebacterium diphtheriae* was shown, by X-ray diffraction analysis, to consist of metallic tellurium. The metal was not complexed, to any significant degree, with any organic material.

Many authors have reported that the black precipitate, produced by microorganisms growing in the presence of potassium tellurite, was metallic tellurium (Klett, 1900; Scheurlen, 1900; Conradi and Troch, 1912; King and Davis, 1914; Morton and Anderson, 1941; Wachstein, 1949; Mudd, Takeya, and Henderson, 1956; Terai, Kamahora, and Yamamura, 1958). The conclusion that the precipitate in question was metallic tellurium, rather than, for example, an oxide, a chloride, or a tellurium-organic complex, has been based upon evidence which is not unequivocal. Morton and Anderson (1941) found needlelike crystals inside and outside of the cells of *Corynebacterium diphtheriae* previously grown on Chocolate Tellurite Agar. These crystals were shown in electron micrographs of whole cells incubated for 144 hr, and were said to be of high density. They reasoned that, if these crystals were actually tellurium metal, then they should dissolve in an oxidizing agent such as bromine. They found that a drop of bromine water did indeed dissolve the black material present in the colonies, and they concluded "... it appears highly probable the black color is due to tellurium metal which exists, at least in part, in the needle-like forms..." While these results are suggestive, they are not conclusively diag-

nostic for tellurium metal. The experiments reported in this paper were carried out in order to determine the true identity and nature of the black precipitate.

MATERIALS AND METHODS

Streptococcus faecalis N83 was grown in Difco folic acid assay medium (FAAM). A 24-hr culture (10 ml) was added to 90 ml of FAAM containing potassium tellurite (final concentration of tellurite being 10^{-3} M). The mixture was then incubated for 24 hr at 37 C. *C. diphtheriae* was handled in a similar fashion, except that Difco Trypticase soy broth was used with a final incubation period of 48 hr. After the indicated times of incubation in the presence of potassium tellurite, the *S. faecalis* N83 cells and the precipitates were washed three times in 40 ml of distilled water, each time centrifuging at $15,000 \times g$ for 10 min in an SS-1 Servall centrifuge. *C. diphtheriae* cells were not washed, but instead were centrifuged once at $15,000 \times g$. This force was chosen for the purpose of attaining a more complete separation of the cells from the precipitate. This method of centrifugation resulted in the cells being packed on the bottom of the centrifuge tubes while the precipitate was thrown against the periphery of the tubes. The precipitates were collected from the sides of the tubes, dried overnight in a small mortar, and then ground to a fine powder. This powder was packed into Lindemann glass capillaries, and subjected to X-ray diffraction analysis in a Norelco powder camera of nominal radius 57.3 mm. Copper K radiation was used, with exposure times of about 20 hr. The powder patterns were viewed and measured in the usual way.

RESULTS

The precipitate from *S. faecalis* N83 gave a powder pattern which was identical, with regard to both spacings and relative intensities, to that of metallic tellurium as reported by Swanson

and Tatge (1953). The lines were rather diffuse (a condition indicating very small crystallites) but quantitative determination of particle size from the line breadths was not made, since this question was not germane to the present study; nevertheless, observations of discrete lines showed that the precipitate was not amorphous, but crystalline. In addition to the tellurium lines, one very faint, but distinct, line ($d = 3.60 \text{ \AA}$) was observed. Although the origin of this line was not established, its weak intensity indicated that whatever substance was its cause could have been present in the washed precipitate to the extent of only about 5% or less. The precipitate from *C. diphtheriae* was treated with somewhat more circumspection, and its diffraction pattern showed, in addition to the expected tellurium lines, other lines which, however, could be accounted for by the presence of about 20% sodium chloride. It is interesting that the tellurium lines were much sharper than those shown by the precipitate from *S. faecalis* and that the "extra" line ($d = 3.60 \text{ \AA}$) was not detected.

DISCUSSION

Previous methods of analysis of the black precipitate resulting from the growth, in the presence of potassium tellurite, of *S. faecalis* N83, *C. diphtheriae*, and numerous other microorganisms, did not lead to its identification with complete certainty. In particular, the observations of Morton and Anderson (1941) that the black precipitate was soluble in bromine water and that the precipitate was of high density can scarcely be said to prove the presence of tellurium metal. It is conceivable, also, that the electron beam could have further reduced the black material present in the colonies, had the precipitate been, for example, tellurium oxide. The results from X-ray studies, on the other hand, showed without doubt that metallic tellurium was present, and, moreover, that no appreciable quantity of other crystalline material was present in the washed precipitate of the experiments

involving *S. faecalis* N83. The amount of sodium chloride which was present in the precipitates from *C. diphtheriae* can possibly be accounted for from the fact that these precipitates were not washed.

It is, perhaps, not out of place to point out here that evidence of constitution based on nonspecific reactions or observations should not be accepted as *proof* of a particular conjecture. In the present study, the X-ray method of analysis leads to an unexceptionable result.

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