INDUCED MUTANTS OF THIOBACILLUS THIOOXIDANS REQUIRING ORGANIC GROWTH FACTORS

SYDNEY C. RITTENBERG AND ROBERT P. GRADY

Department of Bacteriology, University of Southern California, Los Angeles 7, California

Received for publication June 20, 1950

Thiobacillus thiooxidans, one of the few obligate autotrophic bacteria, grows in a strictly mineral medium with sulfur or thiosulfate as its energy source and requires a very acid pH. Vogler and Umbreit and their group (Umbreit: Bact. Revs., **11**, 157, 1947) have shown that despite the unique requirements of this organism, it is basically similar to the heterotrophic bacteria in many facets of its metabolism. O'Kane (J. Bact., **43**, 7, 1942), for example, isolated the following growth factors from organisms grown on a completely inorganic medium: nicotinic acid, pantothenic acid, biotin, riboflavin, thiamine, and pyridoxine. It is reasonable to assume that these compounds are serving the same function within the cells of *T. thiooxidans* as they do for heterotrophic bacteria and that the compounds are essential for its metabolism. If this is the case, then it should be possible to produce a "deficiency" for one or more of these compounds by some mutagenic procedure. This note reports the results of such an attempt using ultraviolet irradiation for inducing mutations.

The culture of *T. thiooxidans*[1] was grown in a mineral salts medium with sulfur as the sole energy source (pH 3.5). In this medium the drop in pH is closely correlated with increase in population, and the cultures were suitable for irradiation 24 hours after the pH fell to 2.0 (about 5 days after inoculation). Five ml of the culture were irradiated in a quartz tube at a distance of 6 inches with a Westinghouse "sterilamp" for 15 minutes. Three-tenths ml of the irradiated suspension was spread over the surface of a solid medium containing thiosulfate as the energy source and 0.1 per cent yeast extract as a source of growth factors for any deficiency mutants that might be present. The parent strain is not inhibited by yeast extract and grew normally on this medium, giving visible colonies in 5 days at 30C. Transfers from all colonies that developed were made to the same medium and simultaneously to the thiosulfate medium without yeast extract (minimal medium). All colonies that grew on the complete medium and failed to grow on the minimal medium were considered possible mutants.

A total of about 300 colonies were tested in this manner. Of these, all but 4 grew initially on both media. Two isolates failed to grow on the minimal medium on initial testing but did so after the first transfer through complete medium. A third isolate persisted in its need for yeast extract through three serial subcultures and then reverted to a nonrequiring type. The fourth isolate persisted in its need for the yeast extract through 20 serial subcultures over a period of $4\frac{1}{2}$ months. Isolates 3 and 4 were mutant strains of *T. thiooxidans* and not heterotrophic contaminants capable of growing on yeast extract at low pH, as

[1] Kindly furnished by Dr. R. L. Starkey, Rutgers University.

was shown by the following: the organisms were morphologically identical with the parent strain; they would not grow in a yeast extract medium without thiosulfate or sulfur; and they would grow in a liquid medium containing yeast extract, mineral salts, and sulfur at pH 3.5, oxidizing the sulfur and causing a marked drop in pH.

An attempt was made to determine the exact nature of the requirement of isolate 4 by testing its ability to grow in the mineral salts sulfur medium to which various growth factors were added both singly and in combination. The strain mutated back to a nonrequiring type before the studies were completed, but the available data indicated that the requirement was not for an amino acid and that one or more of the B vitamins was probably involved.

The fact that mutants requiring exogenous organic compounds for growth were obtained from this "obligate" autotrophic organism illustrates further the close relationship between autotrophic and heterotrophic organisms and further strengthens the concept of the basic unity of the metabolic processes in diverse forms of life.