METABOLIC STUDIES ON BRUCELLA NEOTOMAE (STOENNER AND LACKMAN)

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Stoenner and Lackman (1957) isolated an organism from the wood rat Neotoma lepida and, on the basis of its behavior on differential dye media, CO₂ requirements, and H₂S production, regarded it as a new species of Brucella, for which they proposed the name Brucella neotomae. Although recognition of a new species may be justified on the basis of these conventional techniques, it was considered advisable to investigate the oxidative metabolic pattern of the proposed new species on substrates of amino acids, carbohydrates, and intermediates in the Kreb's cycle. Meyer and Cameron (1958) have reported that each of the three recognized species display metabolic patterns that would aid in defining the species. Cultures of the proposed new species were obtained from Dr. Stoenner and subjected to manometric studies to determine if the oxidative pattern differed significantly from those exhibited by the three recognized species.

MATERIALS AND METHODS

Seven strains of B. neotomae were examined concurrently with the following typical species: B. abortus strain 2308, a standard virulent strain; B. suis strain 148A and B. melitensis strain S90, both of which were isolated from human cases of brucellosis by the California State Department of Public Health Laboratory. Cultures were smooth, as determined by the methods of Braun and Boneestell (1947) and White and Wilson (1951).

Tryptose agar (Difco) dispensed in Roux flasks, slants, and plates was used as the growth medium throughout the investigation. Conventional procedures for differentiating species were as recommended by Huddleson (1957). To obtain resting cells for manometry, Roux flasks were inoculated with 2.5 ml of a saline suspension of the desired culture, incubated 24 hr at 37.5°C, and the resultant growth harvested, washed, and suspended in Sorenson's 0.066 M phosphate buffer at pH 7.0. Cell concentrations were adjusted on a spectrophotometer and cellular nitrogen determined as described earlier by Cameron and Meyer (1953, 1955).

The substrates were dissolved in Sorenson's 0.066 M phosphate buffer and, where necessary, the pH of the solution adjusted to 7.0 by the addition of sodium hydroxide. Conventional manometric technique was employed to determine oxygen uptake (Umbreit et al. 1945). Each flask contained 1.0 ml cells, 0.5 ml solution containing 5 mg of the desired substrate, 1.4 ml buffer, and 0.1 ml alkali. Endogenous respiration rates were determined for each experiment and all experiments were repeated on several harvestings of cells from various lots of media. The figures given in the results are Q₀(N) values with the endogenous rates subtracted.

RESULTS

The H₂S production and the effect of basic fuchsin and thionin on the growth of seven strains of the proposed new species as compared to a representative strain of each of the recognized species is shown on table 1. No growth of the atypical species was observed on either dye; the H₂S production resembled that associated with the growth of B. suis.

Table 2 shows the oxidative metabolism of typical strains of the three species on amino acid and carbohydrate substrates as compared to the unclassified organism. Included in the table are only those substrates which contributed to a pattern differing significantly from that observed with recognized species. The following substrates were also used: D- and L-alanine, L-lysine, and L-proline, fructose, glucose, D-ribose, and D-xylose. All exhibited activity usually associated with the genus but in a pattern that would not serve to differentiate a species. The oxidative pattern on substrates of intermediates in the Kreb's cycle was similar to that observed in the three species.

As a result of inoculating dye plates heavily,
colonies were obtained that would grow when transferred to the respective dye medium. Such colonies, although growing on either basic fuchsin or thionin, exhibited no difference in their metabolic pattern from the original strain that failed to grow on either dye media.

**DISCUSSION**

Stoener and Lackman (1957) have shown that actively proliferating cells of *B. neotomae* display growth and biochemical characteristics which distinguish these organisms from the three recognized species of *Brucella* and all subclassifications (types) within the species (Huddleson 1957). The bacteriostatic effect of the dyes upon *B. neotomae* is identical to that of *B. abortus*, type II. However, all three types of *B. abortus* require increased carbon dioxide tension for initial isolation. According to Huddleson (1957), this characteristic, rather than the dyes, apparently is sufficient to identify the species since growth on dyes is different in each type. Because it does not depend on CO₂ for initial isolation, the new organism would not appear to belong in the species *B. abortus*. *B. neotomae* does not share common characteristics with *B. melitensis*.
The former is vigorous in its production of hydrogen sulfide, and since it behaves the opposite of \textit{B. melitensis} on basic fuchsin and thionin, it is decidedly not in this species. On the basis of the above characteristics, classification into the \textit{B. suis} species could be arguable. The outstanding characteristic it shares with \textit{B. suis} is the excellence of its hydrogen sulfide production. However, none of three types of \textit{B. suis} share its inability to grow in the presence of thionin.

The oxidative metabolic pattern of \textit{B. neotomae} also distinguishes this organism from the other species. Although the biochemical features of proliferating cells, such as dye tolerance and H$_2$S production, are more closely similar to \textit{B. suis}, the metabolic pattern on amino acid substrates clearly separates it from this species. The pattern of \textit{B. suis} is dissimilar to both \textit{B. abortus} and \textit{B. melitensis} and distinctively defines this species. \textit{B. suis} will oxidize L-arginine, dl-citruline, dl-ornithine, and L-lysine. It will not oxidize D-asparagine, and shows consistently low oxidative rates on L-asparagine, L-aspartic and L-glutamic acids, and L-proline. These data are in agreement with those previously reported by Meyer and Cameron (1958). \textit{B. neotomae}, on the basis of amino acid metabolism, is apparently not a strain of \textit{B. suis} that displays atypical sensitivity to thionin. In its amino acid metabolism it resembles \textit{B. melitensis} only in utilizing the D- and L-isomers of asparagine at comparable rates. It differs, however, from that species in the oxidation of L-aspartic acid.

In carbohydrate metabolism significant differences also were observed. These were apparent on adonitol, where the rates were considerably higher in \textit{B. neotomae} than in any of the other species; on L-arabinose where \textit{B. suis} and \textit{B. neotomae} rates were similar; on D-galactose where again there was a strong similarity between those same organisms. In carbohydrate metabolism the proposed species resembles \textit{B. suis}. On the other hand, in amino acid metabolism it is distinctly different from that species. Numerous reports are available on “atypical” strains of \textit{Brucella}, based chiefly on H$_2$S production, CO$_2$ dependency, and dye tolerance. Variations, through temporary modifications or mutation and selection may occur in the latter characteristic. From the results reported herein, a change in dye tolerance does not affect the oxidative metabolic pattern, which apparently is a relatively stable characteristic.

Whether or not a new species is warranted in the case of the organism isolated from the wood rat may be debatable. It can be said, however, that it differs significantly in oxidative metabolism from the three typical species of \textit{Brucella} and there would seem to be considerable justification for naming it a separate species.

**SUMMARY**

The species \textit{Brucella neotomae} was isolated from the wood rat and identified as a new species by Stoenner and Lackman on the basis of conventional methods for speciating the genus \textit{Brucella}. These observations were substantiated by manometric techniques in that the oxidative metabolic pattern of the proposed new species is not identical to the patterns of any of the three recognized species.

**REFERENCES**


