

COMMENTS ON ARTICLE "NITRITE TEST AS AN AID IN THE IDENTIFICATION OF *BACILLUS LARVAE* GROWN IN YEAST-STARCH AGAR"

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A paper with the above title appeared in this Journal recently (Bitner, 1971). It described research in which the addition of nitrate to a culture medium for *B. larvae* followed by a colorimetric test for nitrite was proposed to distinguish this organism from one of two other bacilli found in honey bee larval tissue. Since the author makes no reference to previously published information on nitrate reduction by *B. larvae* as a diagnostic aid (or indeed for any purpose) it must be assumed that he is not aware of previous work in this area. In his work on this organism, Lochhead (1928) noted that when *B. larvae* is cultured in yeast-carrot broth or agar media containing carrot extract but no further added nitrate (p. 86) "nitrites can be readily detected . . . under conditions where all nitrate-reducing bacteria so far

tested have failed to give a nitrite reaction. There is evidence that this peculiar property may become of diagnostic value in the detection of *B. larvae*. Nitrite formation by this organism is being made the subject of special study."

Sturtevant (1932) described the results of five years work, mostly carried out at Laramie, on the possibility of spreading American foulbrood by commercial honey. He determined minimum spore concentrations needed to infect colonies and individual bee larvae, and the relationships of these numbers to the spore content found in commercial honey. In the course of this work, he found nitrite production (indicated by the α -naphthylamine-sulfanilic acid test) to be a useful diagnostic aid: (p. 270) ". . . in cases of slight or doubtful growth the vegetative growth was checked both by microscopic examination of a stained smear and by testing for nitrite pro-

duction in the culture medium . . . After a large number of such observations had been made, it was found that vegetative germination of spores of *Bacillus larvae*, almost too slight to be seen, would give a definite pink color on the addition of the reagents." Sturtevant extended Lochhead's work on nitrite production on carrot medium by other organisms by examining ten miscellaneous organisms in standard nitrate broth and in carrot-extract broth. In addition to *B. larvae*, five produced nitrite in up to 6 hour incubation, but *B. larvae* was the only one in which nitrite was detectable at 24 hours incubation.

These papers by Lochhead and by Sturtevant amply demonstrate that the diagnostic value of nitrite production by *B. larvae* was recognized forty years ago; it is recorded in Bergey's Manual (Breed et al., 1957, p. 631).

REFERENCES

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