

tivity is not inhibited by cholera antimucinase sera.

At this time it is not known whether the depolymerization of ovomucin by a supernatant

of clostridial culture is merely another manifestation of a previously described enzyme or is due to a hitherto unknown enzyme. Additional studies will be required to elucidate this point.

OBLIGATE ANAEROBES WHICH FORM SKATOLE

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Although the presence of skatole in decomposing protein materials and in feces has frequently been reported, very rarely have microorganisms been shown to form skatole in pure culture. Two species of the genus *Clostridium* have been described as producing skatole: *Clostridium skatol* Fellers and Clough (J. Bacteriol., **10**, 130, 1925), which had been characterized earlier by Fellers (Abstr. Bacteriol., **7**, 351, 1923), and *Clostridium nauseum* Spray (J. Bacteriol., **55**, 839, 1948). The two organisms resemble each other in several aspects although a distinctive property of *C. skatol*, the formation of propionic, valeric, and caproic acids, has not been described in *C. nauseum*.

Five clostridial cultures isolated from ensiled grass and closely resembling each other were found to form skatole, one in trace amounts only. The presence of skatole could be demonstrated with the Ehrlich and dimethylaniline reagents in cultures on peptone-yeast extract-glucose media and in ether extracts or steam distillates of the cultures. On paper chromatograms of ether extracts (Dalglish, Biochem. J., **52**, 3, 1952) a single violet colored spot having the same R_f as a pure sample of skatole appeared a few minutes after spraying with the Ehrlich reagent. No immediate red color appeared on spraying, indicating the absence of indole. Skatole formation was inhibited by the addition to the medium of 0.1 per cent (w/v) cysteine, partially inhibited by 0.05 per cent (w/v) sodium thioglycolate or 0.03 per cent (w/v) sodium sulfide, and was unaffected by 0.1 per cent (w/v) ascorbic acid.

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Acetic, propionic, butyric, valeric, and caproic acids were formed in cultures on peptone-yeast extract-glucose media, as shown by paper partition chromatography; washed cell suspensions produced acetic, propionic, butyric, and valeric acids from glucose.

The formation of skatole and of all the volatile fatty acids from acetic to caproic is probably a distinctive combination of characters. Since in their other properties the isolates from silage appear to conform to the description given by Fellers, they have been identified as *C. skatol*. This species has not received general acceptance (Breed, Murray, and Smith, *Bergey's Manual of Determinative Bacteriology*, 7th ed., 1957), possibly because of certain omissions from the original characterization. The following properties, found in the recent isolates, may be added to those given by Fellers:

Iron-litmus-milk unchanged. Iron-glucose-gelatin not liquefied; slightly blackened. Gas in corn-liver mash; gel not liquefied. Nitrite not formed from nitrate. Acetylmethylcarbinol not produced. No hemolysis on ox blood agar. Acid and small amounts of gas from glucose, fructose, mannose, xylose, arabinose, rhamnose, and glycerol. No action on lactose, maltose, galactose, inulin, dextrin, starch, mannitol, or sorbitol. Lactic, citric, tartaric, malic, succinic, and malonic acids with or without acetate not fermented. Skatole formed, but its production suppressed in some media. Growth occurs at 14 and 40 C but not at 10 or 45 C.

The differentiation of *C. skatol* and *C. nauseum* remains to be clarified. Milk, maltose, and glycerol appear to afford distinctions, but information is lacking on the formation of volatile fatty acids by *C. nauseum*.