JOURNAL OF BACTERIOLOGY, Nov., 1966
Vol. 92, No. 5

Deamination of Amino Acids by *Clostridium botulinum*

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Received for publication 18 June 1966

The types of *Clostridium botulinum* were found to differ in their abilities to deaminate single amino acids. Cells were prepared from 24- to 36-hr cultures grown at 37 C in screw-cap tubes in a medium containing (grams per liter); Trypticase (BBL), 15; Phytone (BBL), 5; yeast extract, 5; K_2HPO_4, 8.5; KH_2PO_4, 1.5; NaCl, 1; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4 \cdot 7H_2O$, 0.0125; and sodium thioglycolate, 1. The cells were washed twice in distilled water, resuspended in 0.05 M phosphate buffer (*p*H 7.4) to the density of a McFarland no. 4 nephelometer tube (J. S. Simmons and C. J. Gentzkow, *Medical and Public Health Methods*, Lea & Febiger, Philadelphia, p. 649), and used within 1.5 hr. For the tests, 0.5-ml quantities of cell suspension and substrate were pipetted into Kolmer tubes and incubated for 18 to 24 hr in an anaerobic incubator in a hydrogen atmosphere (36 hr for glutamic acid). The substrates were dilutions of amino acids in the phosphate buffer supplemented with 1 mg/100 ml amounts of biotin and pyridoxal phosphate, which have been shown to be stimulatory for the deamination of certain amino acids (H. Lichstein and Chrisman, J. Biol. Chem. **175**:649, 1948; R. Kallio and A. D. Larson, Johns Hopkins Univ. McCollum Pratt Inst. Contrib. 105, p. 616, 1955), and readjusted to *p*H 7.4. The concentration (milligrams per milliliter) of amino acids varied according to solubility and buffering capacity: methionine, 0.1, arginine, citrulline, cysteine, glutamic acid; histidine, leucine, ornithine, phenylalanine, proline, 0.07; asparagine, aspartic acid, lysine, serine, threonine, valine, 0.05; cystine, tryptophan, tyrosine, 0.04; alanine, glycine, 0.03. L-Amino acids were used except for methionine and tryptophan which were DL-mixtures.

Deamination was determined by the production of ammonia, as measured by P. A. Hansen's (J. Bacteriol. **19**:223, 1930) test and nesslerization of protein-free filtrates of the reaction mixtures prepared with 12.5% trichloracetic acid, and by the determination of keto acids by the method of T. E. Friedman and G. Haugen (J. Biol. Chem. **147**:415, 1943). The protein-free filtrates were prepared for nesslerization in the same manner as for keto acid determination, except that the lower concentration of trichloracetic acid was used. Results with tests for ammonia and for keto acids were identical except with arginine, citrulline, and ornithine, when no keto acids were detected.

The designation of types of strains is that of the strains as received in our laboratory (Table 1). Differential reactions, with the limited number of strains tested, include the failure of type E strains to deaminate aspartic acid, glutamic acid, and leucine; the ability of type C and type F strains to deaminate phenylalanine and tyrosine, and inability to deaminate methionine; and the ability of type D strains to deaminate the last three amino acids. Except for one strain the,

TABLE 1. *Deamination reactions of Clostridium botulinum*[a]

Organism	No. of strains tested	Amino acids				
		Asparagine, threonine	Serine arginine, citrulline, ornithine	Aspartic acid, glutamic acid, leucine	Methionine	Phenylalanine, tyrosine
C. botulinum						
Type A..	6	+	+	+	±[b]	−
Type B..	4	+	+	+	+	−
Type C..	3	+	+	+	−	+
Type D..	2	+	+	+	+	+
Type E..	7	+	±[c]	−	−	−
Type F..	1	+	+	+	−	+
C. parabotulinum	5	+	+	+	−	−

[a] No strains deaminated alanine, cysteine, cystine, glycine, histidine, lysine, proline, tryptophan, or valine.

[b] One strain positive.

[c] Four strains positive for arginine; five, citrulline and ornithine; six, serine.

type A and type B strains could be differentiated on the basis of deamination of methionine. The one type A strain that gave positive results is also aberrant in fermenting sucrose vigorously. The strains designated as *C. parabotulinum* appear to be type A.

This investigation was supported by Public Health Service Research Grant AI 04958 from the National Institute of Allergy and Infectious Diseases.

We thank L. S. McClung of Indiana University and H. H. Hall of the Taft Sanitary Engineering Center, Cincinnati, Ohio, for various cultures used in this study.