FIXATION OF ISOTOPIC NITROGEN BY CLOSTRIDIUM[1]

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Although several previous workers (Bodily, 1938; McCoy, Higby, and Fred, 1928; Sjolander and McCoy, 1937) have reported fixation of atmospheric nitrogen by several species of the genus *Clostridium*, this result is frequently not mentioned in either texts or monographs. Bergey's sixth edition, for example, lists only *Clostridium butyricum* and its subsidiary species, *C. pasteurianum*, as nitrogen fixers and gives this as a *distinctive* characteristic of *C. pasteurianum*. Part of this apparent reluctance to accept ability to fix nitrogen as being widespread in the genus arises from the fact that the quantity reported fixed by some of the species is so small that unequivocal demonstration with the Kjeldahl technique is difficult. Difficulties depending on sensitivity and accuracy of analysis are readily resolved by using the isotope N^{15} to detect fixation (Burris, Eppling, Wahlin, and Wilson, 1943). Preliminary to a study of the mechanism of fixation by *C. pasteurianum* we have tested 15 species of the *Clostridium* genus for the ability to fix free N_2^{15}.

TABLE 1

Fixation of isotopic nitrogen by species of Clostridium

SPECIES	MANIFOLD	ANAEROBIC JAR
C. aceticum	0.102; 0.184; 0.137	0.518
C. acetobutylicum		0.015; 0.09
C. acidiurici	0.00; 0.012	0.00
C. beijerinckii		0.60
C. butyricum		0.063; 0.165; 0.612
C. butylicum		0.564
C. felsineum 195	3.22; 3.38	
C. kluyverii	0.302	
C. lactoacetophilum	1.16; 1.39	0.518
C. madisoni		0.059; 0.554
C. pasteurianum W5	2.80; 4.97	0.208; 2.38; 2.23; 1.08
C. pectinovorum 73	2.46; 1.81	
C. perfringens		0.008; 0.027; 0.00
C. tetanomorphum		0.245
C. sporogenes		0.00; 0.035

All data in atom per cent N^{15} excess; gain necessary for significance: 0.05. Each datum is from a separate experiment.

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Cultures were grown on a modified Winogradski medium (20 g glucose, 1 g tryptone, 1 g agar, 2 g $CaCO_3$ per liter); ethyl alcohol plus sodium acetate served as the source of carbon for *C. kluyverii*, uric acid as the source of both carbon and nitrogen for *C. acidiurici*. One ml of such stocks was added to 15 ml of a modified Winogradski N-free medium in which the tryptone was omitted and 1 ml of yeast water (1 mg N per ml) added per liter as a source of growth factor. The cultures were placed in an atmosphere of N_2 enriched with N^{15}, incubated for 6 days, and then analyzed for content of isotopic nitrogen. Originally the excess of N^{15} in the atmosphere was 6.2 per cent, but repeated recovery, purification, and reuse probably moderately diluted this concentration. Some trials were made on the manifold apparatus described by Burris *et al.* (1943), others in test tubes kept in a Brewer anaerobic jar. In each trial *C. pasteurianum* was included as a positive control. As can be seen from the data in table 1 only 3 of the 15 species tested failed consistently to fix N_2. Possibly the three negative ones, *C. sporogenes*, *C. perfringens*, and *C. acidiurici* were unable to grow in the simple medium used to test for fixation. The first two are known to have complex nutritional requirements and the third must be supplied purines for both carbon and nitrogen (Barker and Beck, 1942). This nutritional factor may also be responsible for the apparent poor fixation noted in some of the species, for example, *C. acetobutylicum*.

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