THE IDENTIFICATION OF FERMENTATIVE PHENOTYPES IN SACCHAROMYCES[1]

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In a recent article in this JOURNAL, Douglas and Condie have analyzed the effects of 2 pairs of genes on the ability of the cell to ferment galactose. The four genotypes, the 3 tetrads and their phenotypes are listed in table 1. They speak of G_1 G_2 as a "fermenter" and G_1 g_2 as a "nonfermenter". The "name" of a genotype appears in this case to have more importance than ordinarily since they have concluded that two "nonfermenters" (G_1 g_2 and g_1 G_2) on hybridization may produce "fermenter" (G_1 G_2) offspring. This terminology leads to some confusion since G_1 g_2 clearly ferments galactose after 7 days. Their careful study showed that the appearance of gas after 7 days by G_1 g_2 was not due to mutation and selection as is the case with *Saccharomyces chevalieri* and other "slow" or "delayed" fermenters (Mundkur, Genetics, **37**, 484, 1952). (The "slow" fermentation of g_1 G_2, however, which occurs irregularly in some tubes after 8 days, was found to be due to mutation presumably from g_1 to G_1 and selection of the mutant.) The data established to their satisfaction that the complete enzymatic machinery for the fermentation of galactose is present in G_1 g_2 after 48 hours' contact with galactose, and that it could be elicited sooner at higher galactose concentrations and demonstrated in cell-free preparations.

TABLE 1

Genotype	Phenotype*		Genotype	Phenotype		Genotype	Phenotype	
	At 2 days	After 7 days		At 2 days	After 7 days		At 2 days	After 7 days
G_1G_2	+	+	G_1g_2	−	+	G_1G_2	+	+
G_1G_2	+	+	G_1g_2	−	+	G_1g_2	−	+
g_1g_2	−	−	g_1G_2	−	−	g_1G_2	−	−
g_1g_2	−	−	g_1G_2	−	−	g_1g_2	−	−

* (+) means gas production in galactose broth; (−) means no gas in galactose broth.

If one may designate an organism capable of producing the entire enzyme system controlling galactose fermentation as a nonfermenter by adjusting the testing conditions, disastrous confusion in the analysis of genotypes is inevitable. Nonfermenters of galactose, so designated by us, are completely incapable of utilizing or fermenting galactose under any conditions which we have been able to devise.

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EXPERIMENTS WITH NITRAMIDE AS A POSSIBLE INTERMEDIATE IN BIOLOGICAL NITROGEN FIXATION[1]

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Nitramide (NO_2NH_2) has been implicated as an intermediate in bacterial denitrification by Allen and van Niel (J. Bacteriol., **64**, 397, 1952). If the first reaction in nitrogen fixation is an oxidation, then a compound at the oxidation level of hyponitrite or nitramide should be produced.

To test the possible function of nitramide in nitrogen fixation, nitramide of high purity was

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synthesized (Booth, Inorg. Syntheses, **1**, 68, 1939). Crystalline nitramide, though labile, was stored successfully for several weeks in an evacuated tube immersed in an ethanol-dry ice mixture. In Warburg respirometers, the nitramide when decomposed evolved 95–100 per cent of the theoretical N_2O.

A 22 hour culture of *Azotobacter vinelandii* was diluted with fresh nitrogen-free 2 per cent sucrose medium and placed in respirometer vessels carrying KOH in their center wells. Dry nitramide or ammonium acetate solution to give 20 ppm N final concentration was placed in the side arms. The flasks were evacuated and filled with 20 per cent O_2-80 per cent H_2. After 5 minutes in the 30° C bath, the side arm contents were mixed, and oxygen uptake was followed to measure growth of the organisms. *A. vinelandii* did not grow appreciably on nitramide as a nitrogen source, as the curve for growth on nitramide was like that for growth on the nitrogen-free medium and of much lower slope than the curve on ammonia. Other experiments with 100 ml cultures in shaken flasks, in which total cellular nitrogen was determined after 24 hours' incubation with nitramide, confirmed this conclusion.

The rapid decomposition of nitramide observed in control flasks made it apparent that nitramide survived but briefly in the medium. Nitramide added to Burk's medium buffered at pH 4.5, 5.0, 5.5, 6.0, or 6.7, decomposed almost completely within 5 minutes as evidenced by N_2O evolution (measured manometrically and identified mass spectrometrically). This commonly recognized instability precludes a critical test of nitramide as an intermediate in denitrification under the experimental conditions employed by Allen and van Niel (our nitramide decomposed rapidly in their yeast autolysate medium). Kluyver and Verhoeven (Antonie van Leeuwenhoek J. Microbiol. Serol., **20**, 241, 1954) also have reached this conclusion from their tests of nitramide as a possible intermediate in denitrification.

To employ a more sensitive test of the ability of *A. vinelandii* to utilize nitramide, N^{15}-labeled nitramide was synthesized (over-all yield of 10 per cent from NH_3) from N^{15}-labeled urethan by the method indicated earlier (urethan was synthesized from $N^{15}H_3$ and ethyl chloroformate). When labeled nitramide (3.4 atom per cent N^{15} excess) was supplied to growing cultures of *A. vinelandii* at 20 ppm N, no N^{15} could be detected in the cells. To avoid exposure of the bacteria to toxic concentrations of nitramide and to minimize the effect of its rapid decomposition, it was added in some trials in 10 portions during 100 minutes; each portion contributed 2 ppm N (3.4 atom per cent N^{15} excess). No enrichment of the cells with N^{15} was detected.

Our trials with N^{15}, though based upon a highly sensitive test, have supplied no evidence for the utilization of nitramide as a source of nitrogen for *A. vinelandii*.