

Prototrophic colonies from a combination between WAc-114 and a mutant of WAc-115 which formed yellow vegetative mycelia (WAc-124) produced aerial hyphae and yellow vegetative mycelia. Other results were identical to those previously given except that WAc-124 occurred more frequently during dissociation (table 1).

These findings, which differ from previous ones (Sermonti and Spada-Sermonti, *Nature*, **176**, 121, 1955) in that (1) parental types dissociate

from the prototrophs and (2) presumptive recombinants which express the mutant phenotypes of each parent actually contain the complete genomes of both parents, are consistent with heterokaryosis. These data could result from parasexuality (Pontecorvo, G., *Proc. 9th Intern. Congr. Genetics*, **1**, 193, 1954) if the genetic markers employed were closely linked. A recombinant nucleus might go undetected in a heterokaryon.

## INFLUENCE OF CULTURE CONDITIONS ON UPTAKE AND DISTRIBUTION OF MOLYBDENUM IN *AZOTOBACTER VINELANDII*<sup>1</sup>

RICHARD F. KEELER

*Department of Biochemistry, College of Agriculture, The Ohio State University, Columbus 10, Ohio*

Received for publication October 22, 1956

The uptake and distribution of molybdenum in *Azotobacter* is of interest because of the absolute requirement of these organisms for molybdenum while fixing nitrogen. In a previous paper (Keeler, *et al.*, *J. Bacteriol.*, **72**, 394-396, 1956) the isolation from disrupted *Azotobacter vinelandii* cells of a particulate fraction (R-144-6) was reported. This fraction had a high 260/280  $\mu$  ratio, the highest level of Mo<sup>99</sup> per mg protein, and a major fraction of the total Mo<sup>99</sup> taken up by the cells.

Similar studies for cells grown on NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> as a nitrogen source have been conducted. Results indicated a marked variability in Mo<sup>99</sup> uptake per unit growth which was found to be more closely related to the degree of aeration than to nitrogen source. It was also apparent from the results that Mo<sup>99</sup> distribution was not a unique function of nitrogen source. Shake cultures revealed that either low iron concentration or a high degree of aeration approximately doubled Mo<sup>99</sup> uptake per unit growth regardless of nitrogen source. These results, in the light of the interesting proposal of Lenhoff *et al.* (*J. Biol. Chem.*, **220**, 983-995, 1956) of an alternate molybdoflavoprotein terminal oxidase pathway in pseudomonads, led to a study of Fe<sup>59</sup> uptake as influenced by aeration and molybdenum levels. For cells growing on N<sub>2</sub>, iron uptake per unit growth was constant for molybdenum levels

varying from 0.001-10.0 ppm. However, iron uptake per unit growth was strongly influenced by aeration. Four times as much Fe<sup>59</sup> was taken up by stagnant cultures as by rapid shake cultures. In all instances growth was determined by optical density readings (at 660  $\mu$ ) of the log phase cells. The uptake of radioactivity was determined by the differences in radioactivity of the cell-free media before and after growth.

Because of the considerable influence of aeration and iron concentration on Mo<sup>99</sup> uptake, it was desirable to know the effects of these factors on Mo<sup>99</sup> distribution as well as on uptake. Table 1 shows such data for cells grown on NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and N<sub>2</sub> in 6-L cultures where the aeration was controlled by the line pressure of the laboratory air supply. Although the effect of iron and aeration levels on Mo<sup>99</sup> uptake is very large, the effect on Mo<sup>99</sup> distribution is not significant except in the case of the N<sub>2</sub> grown cells. A large proportion of the total molybdenum taken up is found in the R-144-6 fraction, except where cell breakage was unusually high, indicating a high proportion of particulate rupture.

Further evidence concerning the disposition of the Mo<sup>99</sup> taken up by *A. vinelandii* was obtained by the following experiments. Cells were grown on the three nitrogen sources in 1 ppm of Mo<sup>99</sup> until most of the molybdenum was taken up. The cells were then harvested and washed at 0 C three times in molybdate-free medium. In all

<sup>1</sup> This work supported in part by a grant from the Research Corporation, New York.

TABLE 1  
*Mo<sup>99</sup> Uptake and distribution as a function of culture conditions\**

Conditions	Fraction†	260/280 $\mu$ Ratio	Total Activity in Fraction	Total Protein per Fraction	Specific Activity of Fractions	Total Mo <sup>99</sup> Uptake per Unit of Protein	Cell Breakage
			<i>counts/min.</i>	<i>mg</i>			<i>%</i>
NH <sub>4</sub> <sup>+</sup> 0.1 ppm Fe, high aera- tion	R-144-1/2	1.31	2,060	199	11	53	43
	R-144-6	1.71	13,700	116	118		
	S-144-6	1.77	7,880	135	58		
100 ppm Fe, low aera- tion	R-144-1/2	1.04	266	277	1	3	86
	R-144-6	1.79	168	11	16		
	S-144-6	1.63	462	53	9		
NO <sub>3</sub> <sup>-</sup> 0.1 ppm Fe, high aera- tion	R-144-1/2	1.29	2,310	183	13	70	43
	R-144-6	1.70	22,600	147	154		
	S-144-6	1.54	16,700	267	63		
100 ppm Fe, low aera- tion	R-144-1/2	1.23	1,320	210	6	36	43
	R-144-6	1.59	17,600	277	64		
	S-144-6	1.63	6,560	214	31		
N <sub>2</sub> 0.1 ppm Fe, high aera- tion	R-144-1/2	1.42	1,650	91	18	68	75 <sup>u</sup>
	R-144-6	1.69	7,200	48	147		
	S-144-6	1.62	8,930	124	72		
100 ppm Fe, low aera- tion	R-144-1/2	1.37	1,070	60	17	23	16
	R-144-6	1.44	1,120	62	18		
	S-144-6	1.62	3,000	102	30		

\* 1 ppm Mo<sup>99</sup> added.

† Fractionation procedure and nomenclature follows that previously used (Keeler *et al.*, J. Bacteriol., **72**, 394-396, 1956).

cases about 15 per cent of the Mo<sup>99</sup> taken up was lost from the cells. When the cells were washed at 0 C in a medium containing 100 ppm of molybdenum, about 30 per cent of the Mo<sup>99</sup> was lost. Labeled cells allowed to incubate for 4 hr at 34 C in a new medium containing 100 ppm of molybdenum also lost about 30 per cent of their Mo<sup>99</sup>. There were no differences in the loss of

Mo<sup>99</sup> as a result of these treatments that could be ascribed to the different nitrogen sources. In addition, the Mo<sup>99</sup> in the R-144-6 fractions from the cells grown on different nitrogen sources was not removed appreciably in any case by dialysis against 10<sup>-3</sup> cyanide at pH 7.2. It therefore seems probable that most of the molybdenum taken up is incorporated into molybdoprotein(s).