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Cyanocobalamin in mycelium of Neurospora sitophila

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Abstract

Cyonocobolamine in *N. sitophila*

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Cyanocobalamine has not yet been described as on essential component of Neurospora. Therefore we studied this compound in mycelio by microbiological assay with Poteriochromonas stipitata. N. sitophila 299 (FGSC#348), a pyridoxine requirer, was used for our experiments. The fungus was cultured at 30°C in 300 ml Erlenmeyer flasks for different timer on the medium of Ryan et al. (1943 Am. J. Botany 30: 784), containing pyridoxine at a concentration of 150 µg/l. Ammonium tartrate was used as the sole nitrogen source (5 g/l) and glucose as the sole carbon source (20 g/l). Cobalt was not oddcd to the medium additionally. Nevertheless, using the Zeiss ultra-spectrophotometer Q24 we found about 30 µg Co/l medium. This quantity war brought into the culture medium with the tmce elements. We followed the growth of the fungus by measuring the weights of myceliol pods after drying at 80°C. For cyanocobalamine determinations, the mycelial pods were washed, dried, homogenized by grinding in a mortar and then extracted with distilled water for 20 min. at 100°C in the presence of 0.05% NaCN. The vitamin B_{12} concentrations in these extracts were determined by the method described by Muecke and Dummler (1960 Pharmazie 15: 305) using P. stipitata as test organism.

Table 1. Growth and cyanocobalamine content of N. sitophila 299.

Age of culture (days)	Mycelial dry wt. (mg/flask)	Cyanocobalamine of mycelium (pg/mg dry wt.)	(pg/flask)
1	2	6	12
2	30	16	480
3	61	31	1891
4	111	35	3885
5	136	16	2176
6	159	12	1908
9	175	7	1225

Vegetative cultures of N. sitophila produce demonstrable amounts of cyanocobalamine, as shown in Table 1. As growth pmceeds, the concentration of cyanocobalamine increases. Maximal content was found at the 4th day. At this time only somewhat more than 10% of the exogenous cobalt is incorporated into the cyanocobalamine molecules, calculated from the B_{12} content. 'n this case, therefore, the cobalt concentration of the medium is sufficient for cyanocobalamine biosynthesis. At the end of the ,ctive growth phase, cyanocobalamine concentration showed a marked decline. ▪ ▪ ▪ Institute of Physiological Chemistry, Karl Marx University, Leipzig, Germany.