

## Vitamin B<sub>1</sub>, Vitamin B<sub>6</sub> and Biotin as Growth Substances for some Ascomycetes

IN continuation of earlier published investigations<sup>1</sup> on the significance of vitamin B<sub>1</sub> (aneurin, thiamin) and biotin (vitamin H) for the growth of different fungi, the need for growth substances of a number of Ascomycetes has been more closely studied. Leaving aside yeast-fungi, only relatively few of these have been more thoroughly examined in this respect<sup>2</sup>.

The genus *Ophiostoma* (*Ceratostomella*) was found to include species with the most varying need of accessory growth substances. All the nine species examined required one or several growth substances to grow on synthetic media.

TABLE 1. THE EFFECT OF DIFFERENT GROWTH SUBSTANCES ON 9 SPECIES OF OPHIOSTOMA IN SYNTHETIC MEDIUM. (Bn = BIOTIN METHYL ESTER, PYR = PYRIMIDINE, Thi = THIAZOLE.)

Species tested	Effective growth substances	
	Indispensable for growth	Growth-stimulating but not indispensable
<i>O. piceae</i>	B <sub>1</sub> (Pyr)	—
<i>O. stenoceras</i>	B <sub>1</sub> (Pyr)	Bn
<i>O. coeruleum</i>	B <sub>1</sub> (Pyr)	B <sub>6</sub> , Bn
<i>O. quercus</i>	B <sub>1</sub> (Pyr)	B <sub>6</sub> , Bn
<i>O. pini</i>	B <sub>1</sub> (Pyr), Bn	B <sub>1</sub> (Thi)
<i>O. ulmi</i>	B <sub>6</sub>	B <sub>1</sub> (Pyr ?)
<i>O. fagi</i>	B <sub>6</sub>	Bn
<i>O. piliferum</i>	B <sub>6</sub>	Bn
<i>O. multiannulatum</i>	B <sub>1</sub> (Pyr + Thi), B <sub>6</sub>	—

The significance of vitamin B<sub>6</sub> (adermin, pyridoxin) is noteworthy for several of the examined species; at least six species reacted positively on an addition of this growth substance, and a closer examination showed that four of these had no, or in any case insufficient for growth, ability to synthesize vitamin B<sub>6</sub>. One species, *O. multiannulatum*, required B<sub>1</sub> besides B<sub>6</sub>. The table shows, furthermore, that B<sub>1</sub>-heterotrophy occurred within a number of species; in one particular case, *O. pini*, combined with biotin-heterotrophy. Generally there was no need to add the entire B<sub>1</sub>-molecule; supplied with only pyrimidine (2-methyl-4-amino-5-aminomethyl-pyrimidine hydrochloride) the organism itself was able to produce for the synthesis of vitamin B<sub>1</sub> the necessary thiazole.

Successful experiments to effect 'artificial symbiosis' proved that it was a question in these cases of a more or less complete loss of synthetic ability. Two species—no matter which two—one requiring B<sub>1</sub> (but producing B<sub>6</sub>), the other requiring B<sub>6</sub> (but producing B<sub>1</sub>), could consequently grow together on a substratum without addition of growth substances.

The weakest concentration of vitamin B<sub>6</sub> which exercised an observable effect was about 10<sup>-10</sup>; it was nearly the same as earlier established, being valid for vitamin B<sub>1</sub> and biotin for those fungi reacting to these growth substances. An especially favourable object for studies of the quantitative effect of B<sub>6</sub> proved to be *O. multiannulatum*. This fungus with the addition of suboptimal quantities of B<sub>6</sub> to a nutrient solution, otherwise optimal, produced quantities of mycelium, which, within certain limitations, were directly proportional to the added B<sub>6</sub>-quantities. (The same holds good also for *O. ulmi*, which, however, showed a certain lability in regard to the

B<sub>6</sub>-heterotrophy.) The mycel./B<sub>6</sub>-quotient thus obtained was of the same magnitude—about 500,000—as the earlier ascertained mycel./B<sub>1</sub>-quotient of other fungi. Therefore with *O. multiannulatum* as test-object, it should be possible to carry out quantitative B<sub>6</sub>-determinations in roughly the same manner as is employed with the B<sub>1</sub>-determinations by means of Schopfer's *Phycomyces*-test<sup>3</sup>.

Other ascomycetes which have here been examined have generally proved to be auxo-autotrophic or B<sub>1</sub>-heterotrophic. Besides earlier known representatives of the latter type, *Discula pinicola* and *Diaporthe detrusa* may be mentioned here.

From certain points of view *Mitrula paludosa* is an interesting example of a fungus requiring biotin. Already it has been shown that biotin is a necessary factor for germination of the spores. For good growth in a synthetic medium vitamin B<sub>1</sub> also is required. The need of biotin for growth seems, however, in a peculiar way to be connected with the source of nitrogen; for example, *M. paludosa* with NH<sub>4</sub>-nitrogen is unable to grow without the addition of biotin, while the fungus in question, on the other hand, grows quite well without biotin with NO<sub>3</sub>-nitrogen, especially if the substratum is relatively acid.

A detailed account will be published in *Symbolae Botanicae Upsalienses*.

NILS FRIES.

Institute of Physiological Botany,  
University of Uppsala.  
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<sup>1</sup> Fries, N., *Symb. Bot. Upsal.*, 3, 2 (1938).

<sup>2</sup> Cf. Hawker, L., *NATURE*, 142, 1038 (1938). Robbins, W. J., and Kavanagh, F., *Amer. J. Bot.*, 25, 229 (1938). Schopfer, W. H., and Blumer, S., *Arch. Mikrob.*, 11, 205 (1940). Melin, E., and Midén, G., *Svensk Bot. Tidskr.*, 35, 333 (1941).

<sup>3</sup> Schopfer, W. H., and Jung, A., C. R. 5e Congr. Int. Techn. Chim. Indust. Agric. Schévéningue, 22 (1937).

## Breakdown of Paraffin Wax by Bacteria: A Source of Error in Corrosion Tests

IN work carried out by the British Non-Ferrous Metals Research Association on the corrosion of metals by domestic waters, glassware used for sampling water and for carrying out corrosion tests was coated with paraffin wax to prevent pick-up of alkali silicates. Tests carried out in waxed containers, or with water conveyed in them, gave inconsistent and non-reproducible results. It was observed from time to time that the wax coatings of some of the carboys were attacked and gradually disintegrated. In view of our previous experience of bacteria in altering the corrosive properties of waters, the possibility of bacterial action was considered.

Water from a carboy showing disintegration of the wax was inoculated into a sterile nutrient solution<sup>1</sup> containing no other source of carbon than flakes of the paraffin wax, and incubated at 30° C. After a few days a growth of bacteria was obtained which was attacking the wax. The species of organism responsible for the fermentation was not fully worked out, but it was found to be a coccus and possibly was a strain of the *Micrococcus paraffinæ* cited by Söhnngen<sup>2</sup>, who states that these bacteria are aerobic, of soil origin, and are commonly found in surface waters.

Bacterial activity in any water is bound to change its characteristics, and one of the variations inves-