MICROBIOLOGY

Tocopherols in Micro-organisms

ALTHOUGH the tocopherols, and in particular α-tocopherol, are widely distributed in Nature, they have not so far been identified in micro-organisms. The only micro-organism that has been adequately studied is bakers' yeast, which has been found to contain no tocopherol, although it contains another substance with vitamin E-like activity[1]. In view of recent work connecting α-tocopherol with some fundamental cellular processes, such as the reduced diphosphopyridine nucleotide-cytochrome *c* reductase system[2], the regulation of succinate oxidation in rat liver mitochondria[3] and the synthesis of adenosine triphosphate[4], it was of interest to investigate the occurrence of tocopherols in a range of micro-organisms. The following organisms were grown in deep culture, using suitable media, the general composition of which is shown in Table 1: three strains of *Esch. coli.*, 8196 and two vitamin B_{12}-requiring mutants, N.I.R.D. and *M*200 (*C*181 strain); *Streptococcus fœcalis R.*; *Bacillus cereus* (strain 5); *Staphylococcus aureus* 4163; the nitrogen-fixing bacterium, *Rhizobium leguminosarum* 317; three sulphur-requiring, chlorophyll-containing bacteria, *Chromatium* 8379, *Chlorobium thiosulphatophilum* and *Thiopedia*; two chlorophyll-containing protozoa, *Ochromonas malhamensis* and *Euglena gracilis*; and one non-chlorophyll containing protozoan, *Tetrahymena pyriformis*.

After suitable incubation periods, when heavy growth was obtained, the micro-organisms were centrifuged off, washed and weighed. Quantities varying between 2 and 20 gm. were taken for analysis. Each batch of micro-organisms was extracted exhaustively with boiling ethanol, and the lipid fraction obtained by ether extraction after dilution with water. This was analysed for tocopherols by methods including two-dimensional paper chromatography previously described[5,6].

The results (Table 1) show (*a*) that α-tocopherol is the only tocopherol observed in these organisms, (*b*) that its presence is not restricted to any one type of organism, (*c*) that it is found only in organisms containing chlorophyll, although it was not found in all of them. The results support a previous suggestion[6] that tocopherol in plants might be formed from the same phytol precursor as chlorophyll. That there is a close relationship between tocopherol and chlorophyll synthesis is further borne out by the difference in the figures for α-tocopherol in *Ochromonas*, depending on whether this organism is grown in the light or the dark. Under dark conditions, *Ochromonas* develops very little green pigmentation and the corresponding tocopherol analysis is only about a sixth of that obtained from the same organism grown in daylight. Dam *et al.*[7,8] have considered synthesis of vitamin K to be linked with chlorophyll synthesis. It is perhaps of interest that *Chromatium*, which did not contain detectable amounts of tocopherol, was found to contain large amounts of vitamin K_2 and ubiquinone 50. The latter substances were identified by paper chromatography and ultra-violet absorption curves on the eluted fractions.

Table 1. TOCOPHEROLS IN MICRO-ORGANISMS

Organism	Medium	Tocopherols (μgm./gm. wet wt.) α	non-α
Esch. coli (3 strains)	Lactose-peptone broth.	ND	ND
Strept. fœcalis R	Buffered peptone yeast ext. glucose.	ND	ND
B. cereus (strain 5)	Peptone-Lemco.	ND	ND
Staph. aureus (4163)	" "	ND	ND
Rhizobium leguminosarum 317	Salts + mannitol + yeast ext.	ND	ND
Chromatium 8379	Synthetic + Na_2S	ND	ND
Chlorobium thiosulphatophilum.	Synthetic + Na_2S	1·7	ND
Thiopedia	Synthetic + Na_2S	ND	ND
Ochromonas malhamensis (grown in light)	'Vitamin B_{12} inoculum'	88·0	ND
Ochromonas malhamensis (grown in dark)	'Vitamin B_{12} inoculum'	14·2	ND
Euglena gracilis	Acetate-pepsin-yeast ext.	155	ND
Tetrahymena pyriformis	Proteose-peptone-yeast ext. glucose.	ND	ND

ND, not detected.

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Production of Colicines in Simmons's Citrate Agar

ANTIBIOTIC substances produced by strains of the family Enterobacteriaceæ are known as colicines[1,2]. A recent review by P. Fredericq[3] indicates that these substances are of particular biological interest: colicinogenic strains resemble in some aspects lysogenic strains, and colicines have certain characteristics similar to those of bacteriophages. Colicinogenic strains behave as pathogenic agents, which can be transduced from colicinogenic to non-colicinogenic strains by culture in common[3-5].

In some experiments on the transduction of colicinogeny from cultures producing colicine I to non-colicinogenic cultures, I observed that colicinogeny was transduced when cultures where plated together on nutrient agar (for details of technique see refs. 3–5), but not when plated on Simmons's citrate agar with glucose 1 °/₀₀ w/v.

It was therefore thought that colicine I is not produced in Simmons's citrate agar, and a number of strains producing colicine I, as well as some other colicinogenic strains, were tested in this synthetic medium for production of colicine.

The colicinogenic strains were stabbed on plates of Difco Simmons's citrate agar with glucose (1 °/₀₀ w/v), eight per plate, according to Fredericq's technique[3], and incubated at 37° for 48 hr. After sterilization with chloroform vapour, a sensitive indicator strain was seeded over the surface of the plates[3]. After 24 hr. incubation at 37°, around the cultures which produce colicines in the synthetic medium, clear zones of inhibition of the sensitive strain are produced.

Table 1 shows results for 73 strains; 61 of these strains were typed in our Laboratory and 9 are type cultures of the collection of P. Fredericq and produce