# Short Communications

## Indole-3-glycerol formation by cell suspensions of *Lactobacillus plantarum*

It was reported by RHULAND AND BARD[1] that either anthranilic acid or indole could replace tryptophan as a growth factor for *Lactobacillus plantarum* (*Lactobacillus arabinosus*). Further, cell suspensions of this organism could metabolise anthranilic acid or indole when incubated in phosphate buffer with glucose to an unknown compound or compounds and tryptophan was not formed. The spectrum of the supernatant, after cells had been incubated with anthranilic acid, suggested the possibility that indole-3-glycerol, a more recently described compound, might have been formed.

Indole-3-glycerol has been shown to be excreted by auxotrophs of *Escherichia coli*[2,3], *Salmonella typhi-murium*[3], *Aerobacter aerogenes*[5], *Bacillus subtilis*[6] and *Neurospora crassa*[7], which require tryptophan for growth. Indole-3-glycerol appears to arise from the dephosphorylation of indole-3-glycerol phosphate, which is formed by cell extracts of *E. coli*[2,3,8].

It was of interest to repeat the experiments of RHULAND AND BARD to see whether cell suspensions of *Lactobacillus plantarum*, in fact, converted indole and anthranilic acid to indole-3-glycerol.

The organism used in these experiments was *L. plantarum* 3061, kindly supplied by Dr. L. E. RHULAND. The media and conditions of cultivation were those described by RHULAND AND BARD[1] although more dilute cell suspensions were used to minimise the amount of substances interfering with ultraviolet spectroscopy.

Experiments were carried out by incubating cell suspensions (0.5 mg dry wt./ml) with the following substrates: D-glucose, $4 \cdot 10^{-2}\,M$; phosphate buffer (pH 7.8), $10^{-1}\,M$ and indole or anthranilic acid, $10^{-4}\,M$. The tests were incubated at 37° for 3 h. Estimation of indole by Ehrlich's reagent[9] and anthranilic acid by absorbancy at 310 mμ (ref. 10) showed that about 75% of each compound was removed. The cells were removed by centrifugation and in the supernatants was found a compound which appeared to be identical with indole-3-glycerol. For reference the compound

TABLE I

COMPARISON BETWEEN *L. plantarum* COMPOUND AND INDOLE-3-GLYCEROL

| *Test* | *L. plantarum 3061 supernatant from indole experiment* | *L. plantarum 3061 supernatant from anthranilic acid experiment* | *E. coli 7-8 supernatant* | *$10^{-4}\,M$ + indole* | *$10^{-4}\,M$ − anthranilic acid* |
|---|---|---|---|---|---|
| SALKOWSKI[12,13] reaction | + | + | + | — | — |
| BRATTON AND MARSHALL[10,14] test | bluish-mauve | pale greyish pink | pale greenish blue | bright pink | bright magenta |
| Oxidation to indole-3-aldehyde[13] | + | + | + | — | — |

* Experiments with suspension of *E. coli* 7–8 were carried out as described by GIBSON, JONES AND TELTSCHER[9].

was compared with that appearing in supernatants of experiments with cell suspensions of *E. coli* 7–8, a mutant known to produce indole-3-glycerol[3,11].

The tests set out in Table I indicate some of the similarities between the compound formed by *L. plantarum* and indole-3-glycerol formed by *E. coli* 7–8.

Supernatants from experiments with *L. plantarum* and *E. coli* were chromatographed in methanol–butanol–benzene–water (2:1:1:1) and the papers sprayed with Ehrlich's reagent. In both cases purple spots, $R_F$ 0.33, were obtained after heating.

The spectrum of the compound formed by *L. plantarum* had the characteristic form of that given by indole-3-glycerol, with peaks at 278 and 287 mμ (refs. 3, 11).

Further evidence was obtained which indicated that indole-3-glycerol phosphate did not accumulate in the cell suspensions of *L. plantarum*. Thus incubation of experimental samples with crude extracts of *E. coli* possessing high tryptophan synthetase activity and with serine and pyridoxal phosphate showed that the compound previously identified as indole-3-glycerol was not removed. Indole-3-glycerol phosphate would have been converted to tryptophan[15].

It seems therefore that the compound formed by cell suspensions of *L. plantarum* from indole or anthranilic acid is probably indole-3-glycerol.

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## The stimulation of the phosphogluconate oxidation pathway by pyruvate under anaerobic conditions in diaphragm muscle and in rat brown adipose tissue

It is generally accepted that glucose metabolism in diaphragm muscle proceeds solely by way of the glycolytic pathway; experiments carried out with specifically labelled [$^{14}$C]glucose have given no evidence for the occurrence of the phosphogluconate