

## Stimulation of $\beta$ -Carotene Synthesis in *Blakeslea trispora* by Pyruvate and Intermediates of the Tricarboxylic Acid (TCA) Cycle

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Anderson, Ciegler and co-workers showed that the synthesis of  $\beta$ -carotene in mated cultures of *B. trispora* is stimulated by  $\beta$ -ionone<sup>1,2</sup> and also by citrus oils, citrus pulp, or citrus molasses.<sup>3</sup> Pazola, Ciegler and Hall identified the main stimulatory substance in citrus molasses as citrate.<sup>4</sup> During studies on the activation of carotenogenesis in *B. trispora* we have observed occasional effects of certain other intermediates of the TCA cycle. The present study was undertaken to investigate this subject more thoroughly.

Organisms, growth media, conditions of fermentation and methods of  $\beta$ -carotene determination were similar to those described elsewhere.<sup>5</sup> Mated cultures of *Blakeslea trispora*, NRRL 2456(+) and 2457(-), kindly provided by Dr. Hesselstine of the Northern Regional Research Laboratory, Peoria Ill., USA, were used throughout. The organic acids were added as Na salts after two days of fermentation to the final concentration of 1.5, 3.0, 4.5, 6.0, 9.0, and 12 mM. The amounts of  $\beta$ -carotene produced in the presence of these substances were compared to those obtained in their absence in parallel controls, using aliquots of the same inoculum.

$\beta$ -Carotene amounted to 80–90 % of all the pigments extracted from the dried mycelium.  $\beta$ -Carotene in the extracts from mycelia without activator was 1.6–3.0  $\mu\text{g/g}$  of mycelium solids with occasional extremes of 0.4 and 4.0  $\mu\text{g/g}$ . Fig. 1 shows the effects of the organic acid anions on growth and on  $\beta$ -carotene synthesis. It plots mean values from 5–9 series, each with four parallel fermentations for every concentration of the activator. Standard

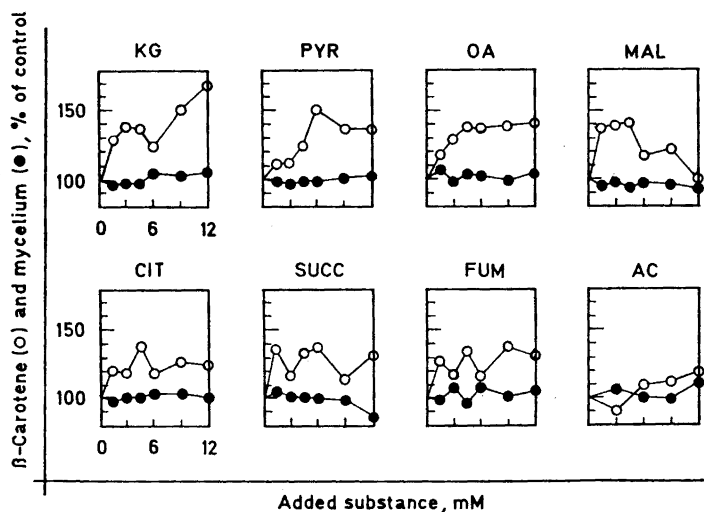


Fig. 1. Stimulation of  $\beta$ -carotene synthesis in *Blakeslea trispora* by pyruvate and intermediates of the TCA cycle added as Na salts. Rings: yield of  $\beta$ -carotene; dots: yield of mycelium, both expressed as % of control values without activator. KG:  $\alpha$ -ketoglutarate; PYR: pyruvate; OA: oxaloacetate; MAL: malate; CIT: citrate; SUCC: succinate; FUM: fumarate; AC: *cis*-aconitate.

errors were 10–20 %. Statistical analysis revealed significant effects.

Distinct stimulation of the carotene synthesis took place in 7 of 8 cases, whereas mycelium growth was unaffected. The greatest stimulation was by  $\alpha$ -ketoacids:  $\alpha$ -ketoglutarate, pyruvate, and oxaloacetate, increasing with rising concentration.

The stimulating effect of malate and, to a certain extent, also that of citrate, succinate, and fumarate was more pronounced at the lower concentrations, but fell off at the higher concentrations. *cis*-Aconitate had insignificant effect, but it is currently excluded from the TCA cycle.

One possible explanation of the pyruvate effect could be increase of the acetyl-CoA pool, the starting substance for isoprenoid synthesis. The effect of oxaloacetate could then be explained by its being decarboxylated to pyruvate, and that of  $\alpha$ -ketoglutarate and other intermediates of the TCA cycle by an increase in the pool of oxaloacetate. A more attractive hypothesis, however, would be specific action of the organic acids or their derivatives on some key enzyme(s) in carotene biosynthesis. Two enzymes which appear interesting in this context are: 1. thiolase (acetyl-CoA: acetyl-CoA C-acetyl transferase, EC 2.3.1.9) and 2. acetyl-CoA-carboxylase (EC 6.4.1.2).

Thiolase is now generally considered to catalyze the first step of isoprenoid synthesis from acetyl-CoA,<sup>6</sup> although the equilibrium constant of the formation of acetoacetyl-CoA indicates a degrading function of the enzyme rather than a biosynthetic one. The occurrence of thiolase in carotene producing microorganisms has not been investigated earlier. Recently, we have obtained a 180-fold purified preparation of thiolase from *Blakeslea trispora* and studied the effects on this enzyme of the organic acid anions, which were found to stimulate the carotenogenesis *in vivo*.<sup>7,8</sup> No significant effects have been observed so far.

According to recent findings, the malonate pathway, which normally initiates fatty acid synthesis, may also be of importance for carotenoid synthesis in

certain systems (for review, *cf.* Ref. 9). The key enzyme in this pathway, acetyl-CoA-carboxylase, is known to be activated by citrate and certain other tri- and dicarboxylic acid anions, at least when the enzyme is isolated from pig heart,<sup>10,11</sup> adipose tissue,<sup>12</sup> or liver.<sup>13</sup> Other regulatory effects of citrate have been suggested as well (for review, *cf.* Ref. 14).

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