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NEW ASPECTS OF CO-REGULATION OF DECARBOXYLATION AND DEMETHYLATION ACTIVITIES IN *NOCARDIA* *

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Vanillic acid at 0.2% concentration in the medium of *Nocardia autotrophic* DSM 43100 leads to cyclic production of guaiacol; protocatechuic and *p*-hydroxybenzoic acids as well as catechol appear at the same time in the medium instead of isovanillic acid, which accumulates at lower vanillic acid concentration. Transformation of catechol formed into guaiacol by methylation with formaldehyde, and succesively into protocatechuic acid by carboxylation seems possible. Succesive reactions of methylation/demethylation and carboxylation/decarboxylation result in cyclic production of guaiacol.

Guaiacol appears at the initial stage of transformation of vanillic acid in *Nocardia* sp. This indicates that decarboxylation precedes metabolism of methoxyl group. Besides, conversion of guaiacol into isovanillic acid evidences reversibility of the process [1]. Introduction of [^{14}C]formaldehyde into the transformation process caused accumulation of ^{14}C in guaiacol [2]. The above reactions were accompanied by constant presence of catechol and protocatechuic acid in the medium. In view of a possible induction of *O*-methylase in *Nocardia* by methoxy compounds [3], the effects of concentration of methoxyphenolic substrates on the course of vanillic acid transformation became of interest.

MATERIALS AND METHODS

Nocardia autotropica DSM 43100 [1] was grown in the liquid, mineral culture with the addition of 0.025% of potassium succinate as a carbon source,

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for 48 h. Then the cells were transferred to the mineral medium containing 0.2% of vanillic acid in the form of potassium salt both as an inducer of specific oxygenases and as a carbon source. Determination of oxygen uptake by *Nocardia* cells started with the addition of vanillic acid and was carried out using the oxygen electrode in the presence, alternatively, of five substrates (Fig. 1). Thin-layer chromatograms visualized the pattern of metabolites in the medium.

RESULTS AND DISCUSSION

During growth of *Nocardia autotrophica* cells in the presence of vanillic acid, guaiacol appeared at rather regular intervals in the medium with the maxima after 30, 48, 66, 84 and 102 h of incubation (Fig. 1A). In addition to

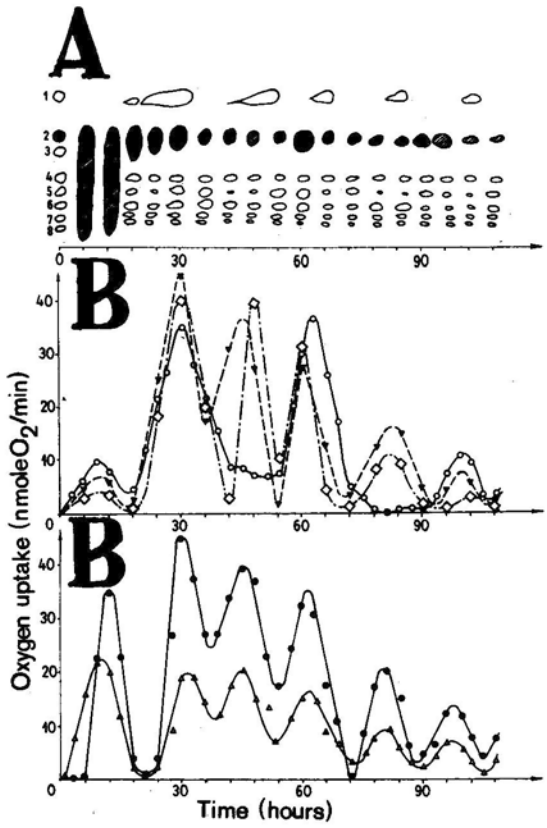
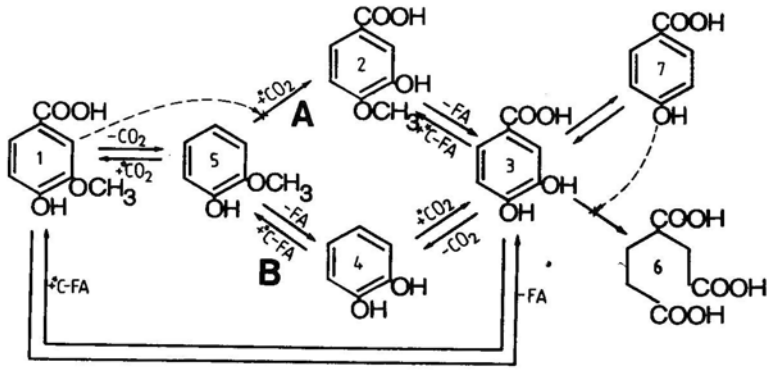


Fig. 1. Oscillating changes in oxygen uptake with different respiratory substrates (designated as symbols) by *Nocardia autotrophica* cells grown in the presence of 0.2% vanillic acid. B. In the upper part, chromatogram A of the transformation products (designated by figures): 1, guaiacol, ▼; 2, vanillic acid, ○; 3, isovanillic acid, ◇ ; 4, catechol, Δ; 5, *p*-hydroxybenzoic acid; 6, protocatechuic acid, ● ; 7, 8, unidentified compounds

vanillic acid and guaiacol, also protocatechuic and *p*-hydroxybenzoic acid were constantly detected. At concentration of vanillic acid used, isovanillic acid was not found, although it was present when the concentration of vanillic acid did not exceed 0.1%.

Out of seven transformation products detected, five were used as respiratory substrates for *Nocardia* cells, namely guaiacol, isovanillic acid, vanillic acid, protocatechuic acid and catechol. The intensity of oxygen uptake, irrespective of the respiratory substrate used, oscillated, when measured in growing cells (Fig. 1B). These oscillations with vanillic acid were by half slower than with other substrates. Four oxygenase activities maxima for vanillic acid and six maxima for other substrates were detected (Fig. 1B). This implies participation of more than one enzyme and/or differences in substrate affinities and different induction of the enzymes by vanillic acid.

The results indicate that vanillic acid transformations take different courses depending on its initial concentration (Scheme 1). At lower concentrations



Scheme 1. Transformations of guaiacol in the *Nocardia autotrophica* DSM 43100 culture in the presence of vanillic acid at concentration below 0.2% (pathway A) or above 0.2% (pathway B). 1, Vanillic acid; 2, isovanillic acid; 3, protocatechuic acid; 4, catechol; 5, guaiacol; 6, carboxy-*cis*,*cis*-muconic acid; 7, *p*-hydroxybenzoic acid. FA, formaldehyde

(below 0.2%) vanillic acid is transformed mainly into guaiacol which, in turn, is converted into isovanillic acid by carboxylation (Fig. 2). At higher vanillic acid concentration, examined in this report, *p*-hydroxybenzoic acid appears in the medium, while catechol and protocatechuic acid are permanently present (Fig. 1A) as the result of 3.0 and 4.0 demethylating activity [3]. The metabolite concentration seems to decide which pathway transformation into catechol will take, since catechol can be formed also by protocatechuic acid decarboxylation [3] (Scheme 1). This explains the presence of both these metabolites in the growth medium. In phenol transformation, *p*-hydroxybenzoic acid is commonly detected [4]. As shown earlier the excees of *p*-hydroxybenzoic acid inhibits dearomatization of the ring [4].

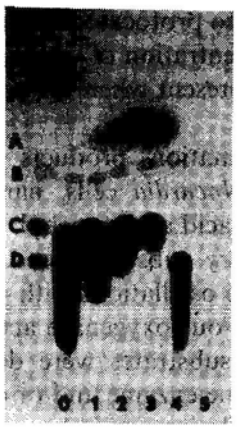


Fig. 2. Conversion of vanillic acid of 0.1% concentration in *Nocardia autotropica* culture. Spots on the chromatogram: A, guaiacol; B, methoxybenzoic acid; C, vanillic acid; D. isovanillic acid. 0 - 5, days of culture

Methylation of catechol leads again to formation of guaiacol, with formaldehyde as a C_1 donor. This last one derives from demethylation of $-OCH_3$ groups [2].

As shown earlier [2], formaldehyde contributes to remethylation of hydroxyl groups. This alternate involvement in the methylation/demethylation processes explains frequent appearance of this metabolite in the medium. A specific trigger system based on the double feedback between the two subsystems involving biosynthesis of the inter-dependent enzymes and their products is responsible for the course of these processes [5]. This also explains oscillation in the oxygenase activity reflected by changes in the rate of oxygen uptake [6].

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