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SHORT COMMUNICATION

METABOLIC PATHWAY OF L-TRYPTOPHAN IN *STREPTOMYCES* SPECIES

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Abstract—The tryptophan metabolism of many microorganisms is well investigated (*Agrobacterium tumefaciens*,[1] *Bacillus subtilis*,[2] *Chromobacterium violaceum*,[3] *Claviceps purpurea*,[4] *Escherichia coli*,[5,6] *Flavobacterium* species,[7] *Neurospora crassa*,[8] *Pseudomonas* species[9,10]), but nothing is known about *Streptomyces* species. In the present communication we wish to report some experiments with excess L-tryptophan and the possible pathway of tryptophan utilization in various *Streptomyces* strains.

MATERIALS AND METHODS

FOLLOWING strains of *Streptomycetes* were used in our experiments: *S. massasporeus* 5035, *S. exfoliatus* 5060, *S. flaveolus* 5061, *S. flavovirens* 5062, *S. limosus* 5131, *S. cremeus* 5147, *S. cyaneofuscatus* 5148, *S. bobiliae* 701, *S. coelicolor* 702, *S. griseus* 703, and strains 11 and 12, isolated from alder (*Alnus glutinosa*) root nodules. For experimental purposes cultures were grown on a rotary shaker for 48 hr at +30° in 500 ml flasks containing 120 ml of glucose-yeast-ammonium medium.[11] For our experiments the mycelium was freed from the nutrient medium, washed under sterile conditions and incubated in 500 ml flasks with phosphate buffer pH 6·98 containing L-tryptophan (5×10^{-4}-3×10^{-3} M), anthranilic acid (10^{-3} M) or indole (10^{-3} M) for 24 hr.

Residual tryptophan was determined by the method of Spiess and Chambers,[12] anthranilic acid by the method of Gröger *et al.*,[13] and formyl anthranilic acid as anthranilic acid after hydrolysis with equal volumes of 5% tartaric acid for 10 min in a boiling water bath. Indole was estimated by the method of Scott.[14]

RESULTS AND DISCUSSION

From the acidified replacement medium (pH 3·0) after feeding tryptophan, we isolated 2 main substances by extraction with peroxide-free ether. These substances fluoresced violet

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in the u.v. light, gave a yellow colour on thin-layer chromatograms after spraying with Ehrlich's reagent with R_f values with the solvent mixture chloroform:acetic acid 95:5[15] on silica gel D (VEB Chemiewerk Greiz/Dölau) of 0·48 and 0·31.

The faster running metabolite yielded, after extraction with 80% ethanol, an u.v. absorption spectrum which was identical with that of anthranilic acid. Paper and thin-layer chromatographical comparison of this substance with anthranilic acid in various solvent mixtures also showed identity.

The second substance reacted more slowly with Ehrlich's reagent than did anthranilic acid. Paper electrophoresis at pH 3·0 indicated that this product was more acidic than anthranilic acid. The metabolic product could not be separated from anthranilic acid electrophoretically in

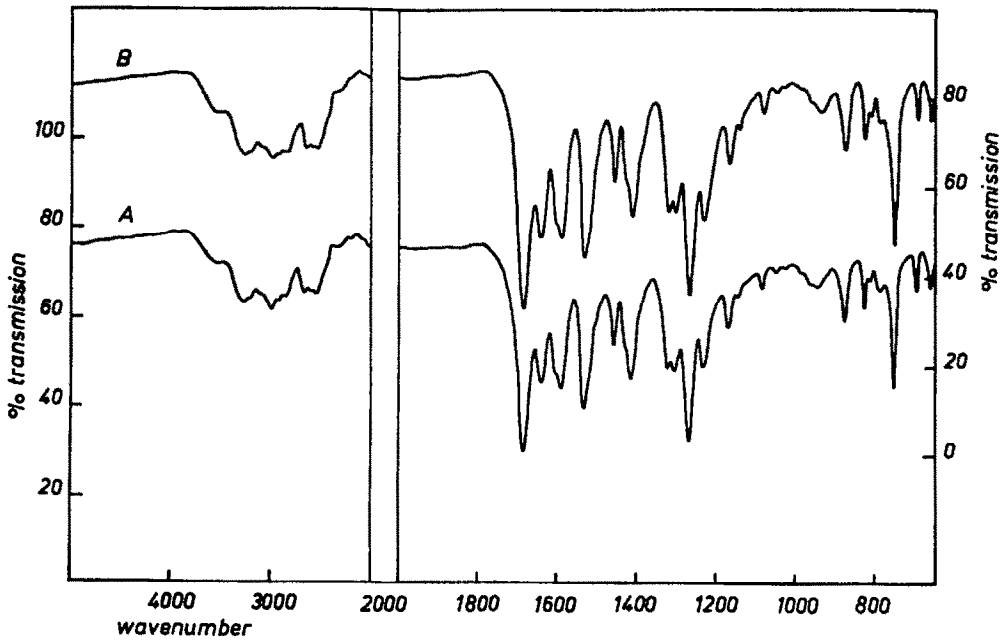

FIG. 1. COMPARISON OF I.R. ABSORPTION SPECTRA FROM EXTRACTED (A) AND SYNTHETIC (B) FORMYL ANTHRANILIC ACID, RECORDED IN KBr.

alkaline buffer (pH 9·18). The u.v. absorption spectrum of the metabolite gave, in contrast to the spectra of other tryptophan metabolites with a free aromatic amino group, no absorption maximum above than 300 nm. This led to the conclusion that the amino group must be acylated. Our conception is supported by the slow reaction with Ehrlich's reagent and by the i.r. absorption spectra (Fig. 1) which in contrast to that of anthranilic acid showed no bands at 3560 cm^{-1} and 3450 cm^{-1} (indicative of NH_2-groups). After treatment of the metabolite with dilute hydrochloric acid, however, anthranilic acid could be detected. All these facts, together with the relatively low melting point (164°) indicated that the metabolite may be either *N*-formyl or *N*-acetyl anthranilic acid. *N*-acetylanthranilic acid, synthetized according to Kaufman,[16] showed different chromatographical behaviour. *N*-Formyl

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anthranilic acid[17] (m.p. 168°), on the other hand was in all respects identical with the metabolic product. The discrepancy in the melting point of the metabolite is probably caused by a trace of free anthranilic acid which could not be avoided because of the ease of hydrolysis. Formic acid was detected after hydrolysis, by reduction to formaldehyde and application of the chromotropic acid reaction.[18]

Besides anthranilic acid and its *N*-formyl derivative, traces of indole were identified (for *Streptomyces bobiliae* less than 10 μ moles/g dry weight per 24 hr, for all other strains less than half this amount). The fact that indole when fed is only slowly metabolized shows that it could not be a main intermediate in tryptophan utilization under our experimental conditions.

Determination of the amounts of anthranilic acid and formyl anthranilic acid accumulated, and of the amounts of tryptophan degraded showed that anthranilic acid is in most cases

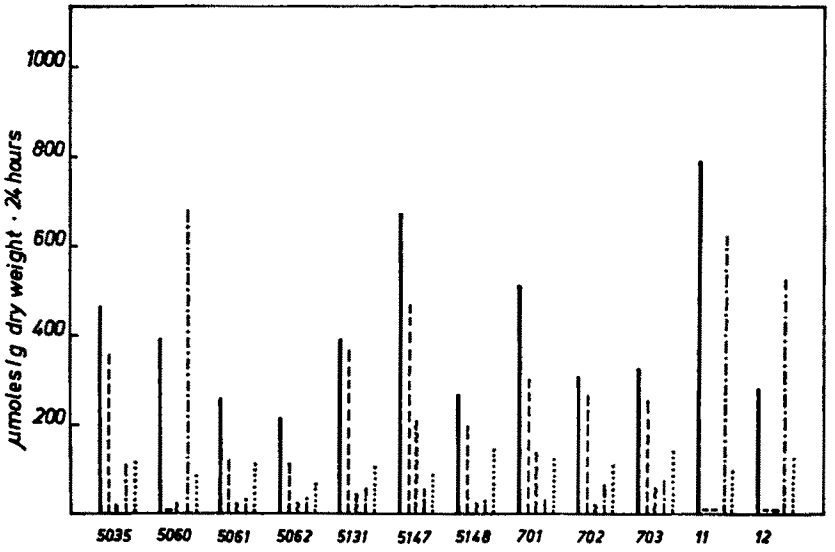

FIG. 2. METABOLISM OF TRYPTOPHAN AT VARIOUS *Streptomyces* STRAINS.
Tryptophan metabolized (———), anthranilic acid accumulated (– – – –), formyl anthranilic acid accumulated (~ ~ ~), indole metabolized (·····), and anthranilic acid metabolized (—·—·—).

the main degradation product (Fig. 2). Anthranilic acid is only slowly further metabolized by the bulk of the strains. No anthranilic acid was detectable after feeding trytophan to *Streptomyces exfoliatus* 5060 and to strains 11 and 12. This fact could be explained by the great rate of metabolism of anthranilic acid in these strains.

The amount of tryptophan that disappeared from the medium equals the sum of anthranilic acid and formyl anthranilic acid accumulated. This fact rules out the occurrence of considerable amounts of other tryptophan metabolites. Exceptions were only found in strains with a faster rate of metabolism of anthranilic acid (strains 5060, 11 and 12) and by *S. flavovirens* 5062 and *S. flaveolus* 5061. There was no evidence in our experiments for the occurrence of indolyl-3-acetic acid, 5-hydroxyindole-3-acetic acid, 3-hydroxyanthranilic acid, nicotinic acid or 2,3-dihydroxybenzoic acid.

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[18] E. EEGRIWE, *Z. Anal. Chem.* **110**, 22 (1937).

The results reported above show that the bulk of the *Streptomyces* strains degrade tryptophan quantitatively to anthranilic acid and formyl anthranilic acid. These metabolites were accumulated in the resting state and may serve as precursors for the resynthesis of tryptophan under conditions allowing growth. Only some strains are capable of degrading tryptophan completely and of utilizing also anthranilic acid as a source of energy.

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