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NOTES

Indole-3-Lactic Acid as a Tryptophan Metabolite Produced by *Bifidobacterium* spp.

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Fifty-one strains of the genus *Bifidobacterium* have been found to accumulate indole-3-lactic acid in culture broth. The isolated metabolite was identified through mass and nuclear magnetic resonance spectroscopy. All the microorganisms tested, as resting cells, have been shown to be able to convert L-tryptophan into L-indole-3-lactic acid.

Recently, there has been increasing interest in bacteria belonging to the genus *Bifidobacterium* (9-17, 19). Although it has been widely demonstrated that aerobic microorganisms are able to release amino acids, relatively little information is available concerning this ability by anaerobic microorganisms. Recently it was demonstrated (4, 10) that microbial strains belonging to the genus *Bifidobacterium* release significant amounts of amino acids into culture broth; however, tryptophan has not been accumulated by most of the examined strains. This could be correlated with possible tryptophan transformation by strains of the genus *Bifidobacterium*. In this connection, this report is concerned with the isolation and identification of indole-3-lactic acid as a tryptophan metabolite produced by 51 strains of the genus *Bifidobacterium*.

The following strains of *Bifidobacterium* spp., obtained from the culture collection of V. Scardovi (Institute of Agrarian and Technical Microbiology, University of Bologna, Bologna, Italy), were tested: *B. adolescentis* (ATCC 15705, F2, F20, F147, F200, F227, RU424), *B. angulatum* (ATCC 27535, ATCC 27669, ATCC 27670, ATCC 27671), *B. animalis* (ATCC 27536, ATCC 27672, ATCC 27673, ATCC 27674), *B. bifidum* (E319f), *B. breve* (B627, B628, B648, B828, B860, B943), *B. catenulatum* (ATCC 27539, ATCC 27675, ATCC 27676, ATCC 27677, B1331), *B. dentium* (ATCC 27534, ATCC 27678, ATCC 27679, ATCC 27680), *B. longum* (ATCC 15708, B612, B654, F13, F33, F66, F130, F149), *B. magnum* (ATCC 27540, ATCC 27682), *B. pullorum* (ATCC 27685), *B. suis* (ATCC 27533); also, *Bifidobacterium* sp. "*minimum*" group (ATCC 27538) (15), *Bifidobacterium* sp. "*subtile*" group (ATCC 27683, ATCC 27684) (15), *Bifidobacterium* sp. unassigned homology group I (RA161) (19), and *Bifidobacterium* sp. (ATCC 27686, ATCC 27917, ATCC 27918, ATCC 27919).

The strains were maintained on Trypticase-Phytone (BBL, Microbiology Systems)-glucose medium (16) and incubated at 37°C in a vacuum incubator, model TS 50 (Mazzali, Monza-Milan, Italy), under N_2-CO_2 (9:1).

Production of the metabolite was studied in Trypticase-Phytone-glucose broth cultures incubated under anaerobic conditions. After incubation the cultures were acidified to pH 2 with 5 N HCl, extracted with methylene chloride-ethyl acetate (1:1), and examined by thin-layer chromatography (silica gel plates [DC, Merck], 0.25 mm; solvent system: i, benzene-ethyl acetate-acetic acid-water [46:40:10:4]; ii, chloroform-acetic acid [95:5]; iii, acetone-isopropanol-7% NH_4OH [5:40:10]). After drying, the plates were sprayed with H_2SO_4-water (1:1) or Ehrlich reagent (18).

All of the extracts prepared from 3-day-old cultures of bifidobacterial strains showed a spot on thin-layer chromatography (solvent i, R_f 0.35; solvent ii, R_f 0.15; solvent iii, R_f 0.26), visible with ultraviolet light or by Ehrlich reagent. This unknown metabolite could be detected in the cell-free spent culture medium, and it was not observed in extracts of cells collected by centrifugation.

To isolate a sufficient amount of this unidentified product for identification, 2 liters of *B. breve* B828 culture broth was adjusted to pH 2 with 5 N HCl and extracted three times with ethyl acetate (800-ml total volume). The combined extracts were dried over Na_2SO_4 and evaporated to dryness at 40°C under vacuum. The oily residue (95 mg) was applied to a column (15 by 1 cm) of silica gel (Kieselgel 60, Merck) and eluted (1 ml/min, 2 ml/fraction) with solvent i

used in thin-layer chromatography. The fractions (7 to 14) containing the Ehrlich-positive metabolite were collected (44 mg).

The metabolite isolated by the above-described method could not be crystallized, and its identification was carried out by preparation of derivatives.

The isolated substance was dissolved in chloroform and methylated with diazomethane. The methyl ester was purified by column chromatography on silica gel (solvent system: hexane-ethyl acetate [6:4]). This methyl ester was acetylated by treatment with acetic anhydride in pyridine (1:10), and then the purification of methyl ester acetate was carried out by silica gel column chromatography (solvent system: hexane-ethyl acetate [8:2]).

The mass spectrum of methyl ester (LKB 9000 spectrometer, 70 eV; *m/e* [relative intensity]: 219 [M^+, 18%], 160 [3%], 130 [100%], 103 [5%], 91 [6%], 77 [16%], 59 [4%]) and the nuclear magnetic resonance spectrum of methyl ester acetate (Perkin-Elmer R 10 spectrometer; solvent, $CDCl_3$; internal standard, trimethylsilane; δ 2.05 [s, 3H], 3.32 [d, 2H], 3.68 [s, 3H], 5.30 [t, 1H], 7.0 to 7.4 [m, 5H], 8.24 [broad s, 1H]) suggested that the isolated metabolite was indole-3-lactic acid. Identical spectra were obtained when the methyl ester and methyl ester acetate of an authentic sample of indole-3-lactic acid (BDH Chemicals Ltd., Poole, England) were examined.

By optical activity determination ($[\alpha]_D^{25} = -30°$, CH_3OH, c 1; model 141 Perkin-Elmer polarimeter) the isolated acid resulted in the L-(−)-isomer.

The transformation of tryptophan into indole-3-lactic acid, which was sometimes found together with indole-3-pyruvic, indole-3-propionic, and indole-3-acetic acids, was previously reported for *Agrobacterium tumefaciens* (8), *Clostridium sporogenes* (7; J. A. Boezi and R. D. DeMoss, Bacteriol. Proc. **124**:79, 1959), *Endomycopsis vernalis* (5), and *Rhizobium leguminosarum* (6). Thus, the metabolic derivation of indole-3-lactic acid from tryptophan was also studied for *Bifidobacterium* spp.: tryptophan was incubated under anaerobic conditions in the presence of resting cell cultures. The cells, harvested from cultures after 48 h of incubation in Trypticase-Phytone-glucose, were washed twice by centrifugation and resuspended in 0.02 M phosphate buffer (pH 7), to give a total N content of 0.3 mg/ml in the presence of 0.05% L-tryptophan (BDH Chemicals Ltd.).

After 4 days of incubation, thin-layer chromatography gave evidence of indole-3-lactic acid as a transformation product.

The ability to convert tryptophan into indole-3-lactic acid, even if at a different rate, was found in all 51 *Bifidobacterium* strains.

Among the alternative routes of tryptophan deamination by microorganisms, the most probable is the one involving indole-3-pyruvic acid. We examined the ability of *B. breve* B828 to transform indole-3-pyruvic acid. As this compound was converted to indole-3-lactic acid by growing or resting cells, it has been assumed to be a reasonable pathway: tryptophan → indole-3-pyruvic acid → indole-3-lactic acid. Research is in progress on the mechanism of this transformation.

Furthermore, since the bifidobacteria represent one of the most important bacterial groups of the intestinal flora of mammals, this activity could be considered significant in the complicated pattern of amino acid transformation by intestinal microorganisms, particularly regarding the tryptophan metabolic products involved in various pathogeneses (1–3).

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