Immunological relationships among transaldolases in the genus *Bifidobacterium*

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Antisera were prepared against electrophoretically homogeneous transaldolase (dihydroxyacetone transferase, E.C. 2.2.1.2.) of *Bifidobacterium thermophilum* (*B. ruminale*) RU326 (ATCC 25866), *B. cuniculi* RA93 (ATCC 27916) and *B. 'minimum'* (homology group) F392 (ATCC 27538). Crude extracts of eighty six strains previously assigned to twenty one species of the genus *Bifidobacterium* on the basis of deoxyribonucleic acid (DNA) homology (DNA-DNA hybridization), were compared by double diffusion tests on Ouchterlony plates. Eight groups of identical antigenic specificity were recognized. By analysis of the spur formation, the groups of identical specificity were arranged in preliminary sequences of decreasing similarity to each of the three homologous transaldolases used as reference points. The relationships between immunological data and the genetic similarity among the species of the genus measured by means of DNA-DNA hybridization were discussed together with some relevant points of bifidal ecology.

**INTRODUCTION**

In the last edition (VIII) of Bergey’s Manual of Determinative Bacteriology eleven species of the genus *Bifidobacterium* are listed (Rogosa, 1974). Six additional species, namely *B. angulatum* Scardovi and Crociani (1974), *B. animalis* (Mitsuoka) Scardovi and Trovatelli (1974), *B. catenulatum* Scardovi and Crociani (1974), *B. dentium* Scardovi and Crociani (1974), *B. magnus* Scardovi and Zani (1974) and *B. pullorum* Trovatelli et al. (1974), have been accepted by the Committee of the Judicial Commission of the JCBS (1976). Four new species, isolated from a variety of habitats, have been quite recently described by Scardovi et al. (1979); two small groups of bifids have been described as distinct DNA homology groups by Scardovi and Trovatelli (1974) but up to now they have not been proposed as new species; these two groups, namely ‘minimum’ and ‘subtile’ are here referred to as specific taxa.
The speciation of the genus *Bifidobacterium* has thus reached a rather high level of complexity. In line with the modern trend of bacterial taxonomy, the separation of bifidobacteria into species has been based primarily upon the deoxyribonucleic acid (DNA) relationships. Different levels of genetic similarity (DNA homology) were shown to exist among the recognized species of the genus *Bifidobacterium*: e.g. 70–80% between *B. thermophilum* and *B. boum*, or *B. infantis* and *B. longum*; 40–60% between *B. indicum* and *B. asteroides*, or *B. animalis* and *B. globosum*; and levels generally lower than 20% in most other cases.

Several distinctive characteristics, such as carbohydrate degradation flowing through the 'fructose-6-phosphate shunt', general cellular morphology, relation to oxygen, composition of cell wall peptidoglycan, habitat etc., which are shared by this bacterial group, might be taken as evidence for it being a single evolutionary group among bacteria.

This assumption remains, however, to be proved, since a similar situation could well be evoked through the possible action of convergent evolution from different ancestors. The differences in the catalytic properties of the key-enzyme of the bifidobacterial glucose metabolism, namely fructose-6-phosphate phosphoketolase (FPPK), purified from species of different habitats (‘human’ and ‘animal’, see also Scardovi, Sgorbati and Zani, 1971), provided a first, albeit tenuous, indication that this possibility may exist (Sgorbati et al., 1976). Immunological properties of isofunctional enzymes are a tool for exploring evolutionary relationships (see Stanier, 1971; Gasser and Gasser, 1971; London and Kline, 1973). The enzyme transaldolase (dihydroxyacetone transferase, E.C. 2.2.1.2) is an ‘all-through’ enzyme in the carbohydrate metabolism of bifidobacteria (Scardovi and Trovatelli, 1965; De Vries, Gerbrandy and Stouthamer, 1967); it is logical to suppose that, on account of its metabolic role, this enzyme underwent limited changes during evolution of the bifidobacterial species (see Gillespie and Kojima, 1968; Johnson, 1974). This enzyme was therefore selected for study.

Prior to this study, an extensive survey was made of the electrophoretic forms of transaldolase and 6-phosphogluconate dehydrogenase among more than 1200 strains assigned to the known species of the genus *Bifidobacterium* by means of DNA-DNA hybridization: 14 different transaldolase isozymes were recognized and their distribution among species was reported (Scardovi et al., 1979).

For this study three specific antisera were prepared against the purified transaldolases of the type strains of *B. euniculi*, *B. thermophilum* and *B. 'minimum'*. These species were chosen because i) their transaldolases were electrophoretically quite distinct from each other, and ii) they were little or not related at DNA-DNA hybridization. The bacterial extracts used in the double diffusion experiments were obtained from strains selected on the basis of their transaldolase electrophoretic forms in order to include all possible variants of this enzyme (Scardovi et al., 1979).