Immunological Relationships of Glucose-6-Phosphate Dehydrogenase of *Leuconostoc mesenteroides* NCDO 768 (=ATCC 12291)

FRANCIS GASSER AND MIREILLE HONTEBEYRIE

Unité de Physiologie Cellulaire, Département de Biochimie et Génétique Microbienne, Institut Pasteur, 75015 Paris, France

An antiserum against the purified glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (G-6-P-DH) of *Leuconostoc mesenteroides* NCDO 768 has been prepared. Cross-reactions were observed with extracts of other strains of *Leuconostoc*, comprising named strains of *L. lactis*, *L. mesenteroides*, *L. dextranicum*, *L. cremoris*, and *L. paramesenteroides*. Cross-reactions were not obtained with *L. oenos*. The groups of identical specificity and their order of decreasing similarity toward the homologous enzyme were determined, and the results are consistent with those obtained previously with another G-6-P-DH antiserum from *L. lactis* NCDO 546. Thus, except for *L. oenos*, many strains of *Leuconostoc* comprising multiple (three or more) species might be considered as phenotypically diverse but genetically rather uniform in their line of descent from a common ancestor.

Previously (4), we purified to a state of electrophoretic homogeneity (3) two distinct isofunctional enzymes—D-lactate dehydrogenase (D-LDH, EC 1.1.1.28) and glucose-6-phosphate dehydrogenase (G-6-P-DH, EC 1.1.1.49)—from *Leuconostoc lactis* NCDO 546 (3). The pure enzymes from this strain were used separately as immunogens to prepare anti-D-LDH and anti-G-6-P-DH sera. Crude extracts were prepared from 50 strains of *Leuconostoc* comprising *L. cremoris* (two strains), *L. lactis* (nine strains), *L. paramesenteroides* (nine strains), *L. dextranicum* (six strains), *L. mesenteroides* (19 strains), *L. oenos* (five strains) and 13 strains of heterofermentative members of *Lactobacillus* comprising *L. brevis* (two strains), *L. buchneri* (one strain), *L. fermentum* (three strains), *L. viridescens* (one strain), unnamed heterofermentative strains (six strains), and one strain of the homofermentative species, *L. jensenii*. Any cross-reactions produced by these crude extracts in the presence of appropriate pure antisera were studied by a double agar diffusion method whose experimental conditions were described previously (2, 4, 5). There were no detectable cross-reactions between the D-LDH and G-6-P-DH antigen-antisera systems from *L. lactis* NCDO 546. None of the extracts from the *Lactobacillus* strains reacted with either antiserum. There were seven groups of antigenic specificity (A to G) for the D-LDH and six groups (a to f) for the G-6-P-DH. The important fact was that (except for *L. oenos*) the grouping pattern for the *Leuconostoc* strains was exactly the same for both enzymes.

In this paper, we describe the immunological

TABLE 1. Groups of identical antigenic specificity obtained with the serum anti-G-6-P-DH from *leuconostoc mesenteroides* NCDO 768^a

Nomenspecies	Immunological group
<i>Leuconostoc lactis</i>	
NCDO 532–533–534–535	a'
NCDO 536–546–549–956–959	
<i>Leuconostoc mesenteroides</i>	
CIP 54171–NCIB 3351	b'
<i>Leuconostoc mesenteroides</i>	
NCIB 9317–8013–9319–8710	d'
NCDO 522–523	
ATCC 10830–V16, 1533	
CIP 54168, 54170	
Le 512–13–Le 512–17–Le 513–17–Le 513–12	
<i>Leuconostoc dextranicum</i>	
NCDO 571–529–812	d'
NCIB 3739–2706–3356	
<i>Leuconostoc cremoris</i>	
NCDO 543–829	d'
<i>Leuconostoc mesenteroides</i>	
NCDO 768–882	e'
<i>Leuconostoc paramesenteroides</i>	
NCDO 803–870–871–872–873	f'
NCDO 885–886–955–CIP 54169	
<i>Leuconostoc oenos</i>	
ATCC 23278–WL D1–WL BG31	No cross-reaction
WL B6–WL LA1–SC 122	

^a These groups were determined by pairwise comparison of crude extracts located in adjacent wells on Ouchterlony plates. The production of a continuous line of precipitate without spur formation indicates the appurtenance to a same group of specificity. Group e' is the homologous group since it gathers strains, extracts of which do not produce a spur against the homologous enzyme purified from strain NCDO 768. Group c' is not represented in this table since it contains only one strain, NCIB 9317, which is included in the group d'. The symbol c', however, has been kept by analogy with the previous findings (4).

FIG. 1. Hierarchy of immunological relatedness of the different groups of identical specificity of *Leuconostoc* G-6-P-DH, based on precipitin cross-reaction with G-6-P-DH antiserum of *L. mesenteroides* NCDO 768. By definition, extracts of strains of different groups of identical specificity produce a continuous line of precipitin and a spur against the antiserum when they are placed in two adjacent wells of an Ouchterlony plate. The direction of the arrow indicates the groups, the extract of which produces the spur in the paired comparison. The equal sign indicates that no spur occurs in such a comparison.

cross-reactions obtained with another G-6-P-DH antiserum produced against the enzyme of a strain of group e, *L. mesenteroides* NCDO 768 (ATCC 12291). This strain is one of the suggested reference strains for this species cited in the 8th edition of *Bergey's Manual* (1). Its G-6-P-DH was purchased from Sigma Chemical Co. (reference G 2256) and repurified by preparative polyacrylamide gel electrophoresis as described previously (3, 5). One band of protein was obtained after analytical electrophoresis in polyacrylamide gel of 120 μ g of purified enzyme. The antiserum obtained with this material produced a single line of precipitin against a crude extract of *L. mesenteroides* NCDO 768 submitted to a 2-h immunoelectrophoretic run (4). Experimental conditions and interpretations were the same as employed previously (2, 4, 5).

All the crude extracts of the strains of *Leuconostoc* tested gave a precipitin line with the G-6-P-DH antiserum except extracts of *L. oenos* (recognized as group G with D-LDH antiserum from *L. lactis* NCDO 546 [4]). This lack of cross-reactivity already observed is related to a low enzyme activity in our extracts. Among lactobacilli, only *Lactobacillus viridescens* produced a cross-reaction.

Table 1 shows that the 50 strains of *Leuconostoc* can be arranged into six groupings (a' to f') of identical antigenic specificity, containing the same strains within each group whether the homologous G-6-P-DH system was from the group a—*L. lactis* NCDO 546—(4) or whether the homologous system was from the group e'—*L. mesenteroides* NCDO 768 (ATCC 12291), as in the present work.

Figure 1 shows the direction of the spurs obtained between the different groups of identical specificity. The spurs between the b' and d' groups and between the b' and e' groups were always weak and were not produced between all the extracts tested. From this table we deduce the following order of decreasing similarity toward the homologous enzyme: e' > d' = c' > b' > a' > f'.

This order is consistent with the result previously obtained with the G-6-P-DH antiserum of *L. lactis* NCDO 546 (group a): a > c > b > d > e > f. The relative position of group a toward group f and of group a' toward group f' suggests that a double spur might have been expected between groups d and f.

We may note that such a double spur was obtained with the D-LDH antiserum from *L. lactis* NCDO 546 (4) which displays this order of decreasing similarity: A > B > C > (D/F) > E. These results confirm the great similarity of groups (B), (C), (D) in terms of phylogeny of their D-LDH and G-6-P-DH as revealed by their respective immunological cross-reactivity. Not only do they always appear as neighbors in all orders of similarities but they sometimes fail to produce any spur as in the case of b', d', and c'.

FIG. 2. Relative evolutionary position of the different groups of identical antigenic specificity organized in a hypothetical phylogenetic tree as suggested by the immunological relationships of the D-LDH and the G-6-P-DH enzymes in the genus *Leuconostoc*. Underlined letters totalizes the different groups symbolized by the corresponding letter: for example (A) totalizes the groups A, a, and a'. Group (G) (*L. oenos*) is determined with the D-LDH antiserum only since its extracts do not produce a cross-reaction with G-6-P-DH antisera.

These convergent results for two different enzymes suggest a hypothetical phylogenetic tree (Fig. 2).

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REPRINT REQUESTS

Address reprint requests to: Dr. Francis Gasser, Unité de Physiologie Cellulaire, Département de Biochimie et Génétique Microbienne, Institut Pasteur, 28 Rue du Docteur Roux, 75015 Paris, France.

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