N o t e

NAD and NADP utilization by hetero-lactic acid bacteria

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The enzyme activities of hetero-lactic acid bacteria, including seven strains of *Leuconostoc* and two strains of *Lactobacillus* were measured with cell-free extracts. 6-Phosphogluconate dehydrogenases in two strains were NAD-specific, on the other hand, glucose-6-phosphate dehydrogenase in all strains used both NAD and NADP as the coenzyme. All the strains had D-lactate dehydrogenase and two had L-lactate dehydrogenase, and these enzymes used only NAD. All the strains had alcohol dehydrogenase that used both NAD and NADP.

Key words : hetero-lactic acid bacteria, cell-free extract, enzyme activity, NAD, NADP

We isolated the strains SHO-54 and SHO-47, which contained large amounts of NAD-specific 6-phosphogluconate dehydrogenase (6-PGDH; EC 1.1.1.44).¹⁾ From the DNA-DNA hybridization and the G+C content of DNA, it was concluded that the strain SHO-54 belongs to *Leuconostoc lactis* and the growth condition of the strain was determined.²⁾ In this report to reveal the NAD and NADP utilization by hetero-lactic acid bacteria including these strains, five enzyme activities of nine hetero-lactic acid bacteria were measured with cell-free extracts.

The methods of isolation of the strains, preparation of the cell-free extracts, the protein determination in the cell-free extract, and the analytical method of 6-PGDH were reported previously.¹⁾ The reaction mixture to assay D-lactate dehydrogenase (D-LDH; EC 1.1.1.28) or L-lactate dehydrogenase (L-LDH; EC 1.1.1.27) contained: Tris-HCl buffer (pH 8.0), 0.5 M; D or L-lactic acid (Sigma Chemical Co.; St. Louis, MO, U.S.A.), 56 mM; NAD or NADP, 2.6 mM. The reaction mixtures of alcohol dehydrogenase (ADH; EC 1.1.1.1) and glucose-6-phosphate dehydrogenase (G-6-PDH; EC 1.1.1.49) were prepared according to Bergmeyer *et al.*^{3,4)} Though the assays were performed with either NAD or NADP as the coenzyme in these methods, both NAD and NADP were used in this

work. The NAD (grade I), NADP (NAD free), NADH (grade I), and NADPH were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μ mol of NADH or NADPH per min at 30 °C and then the specific activities of these enzymes were calculated in units per mg of protein. DNA isolation, DNA-DNA hybridization and the determination of G+C contents were carried out as described previously.²⁾

The strain SHO-47 produces acid from lactose, while SHO-54 does not. It would be interesting to learn if the strains SHO-47 and SHO-54 are the same genus. As such, the DNA homology between the strains and G+C contents were determined. The DNA homology between strain SHO-47 and NRIC 1540 ranged from 92.9% to 96.9%. The G+C contents of the DNA from SHO-47 and *Leu. lactis* NRIC 1540 were 42.4 mol% and 42.7 mol%, respectively. Thus it was concluded that the strain SHO-47 belongs to *Leu. lactis*, as does SHO-54. Moreover, the DNA homology between strain SHO-47 and SHO-54 ranged from 74.5% to 81.6%.

The cells grew well, and 4.8-8.0 mg protein was yielded from one milliliter of cell-free extract. As shown in Table 1, G-6-PDH and ADH in all the strains used both NAD and NADP. All the strains had D-LDH, while SHO-82 and SHO-94 had L-LDH. These enzymes could not use NADP. The strain SHO-47 and SHO-54 had NAD-specific 6-PGDH, though other

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Table 1. The specific activity of G-6-PDH, 6-PGDH, D-LDH, L-LDH, and ADH in hetero-lactic acid bacteria

Strain	Protein (mg/ml)	Specific activity (units/mg protein)									
		G-6-PDH		6-PGDH		D-LDH		L-LDH		ADH	
		NAD	NADP	NAD	NADP	NAD	NADP	NAD	NADP	NAD	NADP
<i>Leu. lactis</i> SHO-47	4.8	5.29	2.67	4.42	0.00	8.06	0.00	0.00	0.00	1.00	0.67
<i>Leu. lactis</i> SHO-54	5.6	3.20	2.34	4.04	0.00	3.13	0.00	0.00	0.00	0.79	0.46
<i>Lac. brevis</i> SHO-82	7.7	2.30	0.87	0.14	1.53	0.39	0.00	0.49	0.00	0.10	0.19
<i>Leu. paramesenteroides</i> SHO-89	5.3	5.60	3.74	1.89	0.08	2.00	0.00	0.00	0.00	0.26	0.40
<i>Leu. mesenteroides</i> subsp. <i>cremoris</i> SHO-90	6.3	0.48	0.27	0.10	0.03	1.62	0.00	0.00	0.00	0.49	0.02
<i>Leu. mesenteroides</i> subsp. <i>mesenteroides</i> SHO-93	6.2	0.34	0.84	1.32	0.02	1.40	0.00	0.00	0.00	0.23	0.02
<i>Lac. viridescens</i> SHO-94	8.0	2.21	3.39	1.46	0.16	1.43	0.00	0.06	0.00	0.08	0.01
<i>Leu. mesenteroides</i> subsp. <i>dextranicum</i> NRIC 1539	4.9	4.86	3.16	1.69	0.04	3.08	0.00	0.00	0.00	0.47	0.84
<i>Leu. lactis</i> NRIC 1540	6.7	1.88	1.39	1.60	0.01	2.64	0.00	0.00	0.00	0.09	0.04

Abbreviations: *Lac.*, *Lactobacillus*; *Leu.*, *Leuconostoc*.

strains had non-specific one. It was conventionally reported, regarding *Leu. mesenteroides*,^{5,6)} that the metabolic pathway for these hetero-lactic acid bacteria was as follows: glucose was catabolized into xylose 5-phosphate via the pentose phosphate cycle,⁷⁾ and then divided into pyruvic acid and acetyl phosphate; the former was reduced to lactic acid, and the latter to ethanol. In the case of SHO-47 and SHO-54, the NADH required to convert pyruvic acid to D-lactic acid would be produced in oxidation of 6-phospho-gluconate to ribulose 5-phosphate by NAD-specific 6-PGDH, while the NADPH required to convert acetaldehyde to ethanol would be produced in the oxidation of glucose-6-phosphate to glucono- δ -lactone 6-phosphate by G-6-PDH.

The function of 6-PGDH in cell metabolism has been described as having two different roles. One is regeneration of NADPH for reductive synthesis^{8,9)} and synthesis of ribose 5-phosphate as a precursor for nucleic acids and coenzymes. The other role of 6-PGDH is the biosynthetic reaction for lipogenesis, which specifically requires NADPH¹⁰⁾ as a reductant. Thus 6-PGDH in SHO-47 and SHO-54 may not serve as the reductant for lipogenesis, rather G-6-PDH mainly reduced NADPH.

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REFERENCES

- 1.H. Ohara, K. Uchida, M. Yahata, and H. Kondo, *Biosci. Biotechnol. Biochem.*, **60**, 692-693 (1996).
- 2.H. Ohara, M. Yahata, K. Uchida, and H. Kondo, *World J. Microbiol. Biotechnol.*, **14**, 143-144 (1998).
- 3.H.U. Bergmeyer, M. Graßl, and H.E. Walter, in "Methods of enzymatic analysis," 3rd ed., Vol. 2, ed by Bergmeyer, Verlag Chemie, Weinheim, 1983 pp 141-142.
- 4.H.U. Bergmeyer, M. Graßl, and H.E. Walter, in "Methods of enzymatic analysis," 3rd ed., Vol. 2, ed by Bergmeyer, Verlag Chemie, Weinheim, 1983 pp 204-205.
- 5.R.D. DeMoss, R.C. Bard, and I.C. Gunsalus, *J. Bacteriol.*, **62**, 499-511 (1951).
- 6.J. Hurwitz, *Biochim. Biophys. Acta*, **28**, 599-602 (1958).
- 7.I. Gunsalus and M. Gibbs, *J. Biol. Chem.*, **194**, 871-875 (1952).
- 8.J.P. Flatt and E.G. Ball, *J. Biol. Chem.*, **239**, 675-685 (1964).
- 9.J. Katz and R. Rognstad, *Biochemistry*, **6**, 2227-2247 (1967).
- 10.I.B. Fritz, *Physiol. Rev.*, **41**, 52-129 (1961).

ヘテロ乳酸発酵型乳酸菌によるNADおよびNADPの利用

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要 約

ヘテロ乳酸発酵型乳酸菌である7菌株の *Leuconostoc* と2菌株の *Lactobacillus* より無細胞抽出液を調製し, その酵素活性を測定した。供試菌株のうち2菌株において6-phosphogluconate dehydrogenase はNADを特異的に補酵素として利用し, 全菌株においてglucose-6-phosphate dehydrogenase はNADおよびNADPを補酵素として利用した。また, 全菌株がD-LDHに活性を示し, 2菌株はL-LDHにも活性を示した。なお, これらのLDHはいずれもNADのみを補酵素として利用した。さらに, 全菌株がalcohol dehydrogenaseに活性を示し, NAD, NADPのいずれかをも補酵素として利用した。

Key words : hetero-lactic acid bacteria, cell-free extract, enzyme activity, NAD, NADP