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NOTES

Expression of Hydrogenase in *Alcaligenes* spp. Is Altered by Interspecific Plasmid Exchange

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Alcaligenes hydrogenophilus was found to contain a soluble and a particulate hydrogenase whose control and structure differed in part from that in *Alcaligenes eutrophus*. One of at least two plasmids indigenous to *A. hydrogenophilus* determines hydrogenase genes (Hox). The interspecific exchange of Hox-encoding plasmids generated transconjugants which expressed the structural and regulatory Hox phenotype of the donor.

To gain further insight into the genetic regulation of hydrogen-oxidizing enzyme systems, we have comparatively studied *Alcaligenes hydrogenophilus* (Table 1). This gram-negative facultatively autotrophic bacterium has been isolated recently from soil (10). The guanine-plus-cytosine content of its DNA (70.2 mol%) was reported to be higher than that of *Alcaligenes eutrophus* (66.5 mol%). Both species belong to the fast-growing lithoautotrophs, with doubling times of 175 and 230 min, respectively.

The majority of hydrogen-oxidizing bacteria are characterized by the possession of a single, membrane-bound hydrogenase. The presence of a soluble NAD^+-reducing hydrogenase (hydrogen:NAD^+ oxidoreductase, EC 1.12.1.2) had been restricted to *A. eutrophus*, *Alcaligenes ruhlandii*, and *Nocardia opaca* (2). In this communication, we provide evidence that cells of *A. hydrogenophilus* cultivated with hydrogen and carbon dioxide also contain NAD^+-reducing hydrogenase activity (see Table 2). Moreover, extracts derived from those cells exhibit activity of a particulate methylene blue-dependent hydrogenase and of ribulose bisphosphate carboxylase (RuBPC, EC 4.1.1.39), a key enzyme of autotrophic carbon dioxide fixation. Cross-reactivity studies with antisera raised against homogenous soluble hydrogenase and particulate hydrogenase from *A. eutrophus* H16 (Table 1) revealed a close immunochemical relationship between the corresponding enzymes of the two species. The precipitin bands of the NAD^+-dependent hydrogenases were completely identical. However, the cross-reaction of the particulate hydrogenase from *A. hydrogenophilus* was one of only partial identity (data not shown), indicating some structural variation in the protein. Unlike *A. eutrophus* (5), which is only able to grow heterotrophically at 37°C, *A. hydrogenophilus* grew well at this temperature with hydrogen and carbon dioxide, and the cells exhibited a high level of hydrogenase and RuBPC activity (Table 2). Formate, another one-carbon compound, was utilized for growth. The high activity of RuBPC in formate-grown cells suggests that the substrate is metabolized via the Calvin cycle, as in *A. eutrophus* (4). On selected organic substrates supporting either fast growth, such as succinate, or allowing only slow growth, such as isoleucine, almost no hydrogenase activity could be detected (Table 2). The weak expression of hydrogenase activity under heterotrophic conditions indicates that the formation of these enzymes is subject to induction in the presence of hydrogen.

We have shown earlier that hydrogen-oxidizing ability (Hox) is a plasmid-encoded property in several wild-type strains of *A. eutrophus*. Most of them harbor a single self-transmissible plasmid of large size, approximately 300 megadaltons (8). Electrophoretic analysis of lysates from *A. hydrogenophilus* resolved the presence of at least two different plasmid molecules (Fig. 1, lane 2). The slowly moving plasmid band (pHG21-a) was only slightly smaller (270 megadaltons) than plasmid pHG1 from *A. eutrophus* (Fig. 1, lane 1). The fast-moving DNA band (pHG21-b) corresponded to an approximate molecular size of 230 megadaltons, compared with plasmids from *Rhizobium meliloti* (data not shown), whose contour lengths are known (13). The

TABLE 1. Bacterial strains

Strain	Relevant phenotype[a]	Reference or source
A. eutrophus		
H16(pHG1)	Hox Ts, Hox d	Wild type: ATCC 17699, DSM 428
HF151(pHG1)[-b]	Hox[-] Sm[r] Na[r]	9
HF41(pHG1)	Hox[+] Cfx[-] Met[-]	8
HF157(pHG21-a)	Hox Tr, Hox i	Transconjugant of HF151, this study
A. hydrogenophilus		
M50(pHG21-a, pHG21-b)	Hox Tr, Hox i	Wild type (10)
M55(pHG21-a[-],pHG21-b)	Hox[-]	Mutant of M50, this study
M111(pHG1, pHG21-b)	Hox Ts, Hox d	Transconjugant of M55, this study

[a] Phenotypes are designated as follows: Hox, ability to oxidize hydrogen; Hox Ts and Hox Tr, inability and ability, respectively, to grow with hydrogen at 37°C; Hox d, energy-derepressible hydrogenase control; Hox i, hydrogen-inducible hydrogenase control; Cfx, ability to fix carbon dioxide autotrophically; Sm[r], resistance to 500 μg of streptomycin per ml; Na[r], resistance to 100 μg of nalidixic acid per ml.
[b] [-] indicates loss of the plasmid.

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TABLE 2. Enzymes of lithoautotrophic metabolism in *A. hydrogenophilus*

Substrate[b]	Doubling time (h)	Enzyme activity (U/mg of protein)[a]		
		SH	PH	RuBPC
$H_2 + CO_2$				
30°C	2.8	0.186	0.818	0.128
37°C	2.9	0.147	0.758	0.114
Formate	2.6	0	0	0.117
Fructose	2.3	0.005	0	0.017
Succinate	1.4	0	0	0.001
Isoleucine	5.8	0	0	0.030

[a] SH, Soluble hydrogenase was assayed by hydrogen-dependent NAD^+ reduction with whole cells as described (7, 12); PH, particulate hydrogenase was assayed from the particulate fraction by spectrophotometric determination of hydrogen-dependent methylene blue reduction (11); RuBPC was determined from cell-free extracts by the method of Bowien et al. (1).

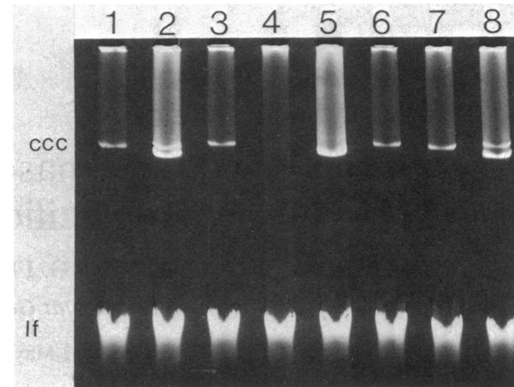

FIG. 1. Plasmid DNA in wild-type strains, Hox mutants, and Hox^+ transconjugants. Lysates of the following strains were prepared and subjected to electrophoresis in an agarose gel as described (8): *A. eutrophus* H16 ($pHG1^+$; lanes 1, 3 and 6); *A. hydrogenophilus* M50 (pHG21-a^+, pHG21-b^+, lane 2); *A. eutrophus* HF151 ($pHG1^-$; lane 4); *A. hydrogenophilus* M55 (pHG21-a^-, pHG21-b^+; lane 5), *A. eutrophus* HF157 (pHG21-a^+, $pHG1^-$; lane 7) and *A. hydrogenophilus* M111 ($pHG1^+$, pHG21-a^-, pHG21-b^+, lane 8), lf, Linear fragments; ccc, covalently closed circular DNA.

intense fluorescence of plasmid pHG21-b DNA may be due to the presence of a doublet band in this region.

We have reported in an accompanying paper on the identification of plasmid-cured, hydrogenase-deficient mutants (Hox^-) among spontaneous antibiotic-resistant mutants derived from *A. eutrophus* (9). Accordingly, we screened drug-resistant colonies from *A. hydrogenophilus* for the loss of hydrogenase function. In fact, Hox mutants were detected among nalidixic acid-resistant and tetracycline-resistant derivatives. They turned out to be incapable of reverting to the Hox^+ phenotype. Plasmid analysis revealed that all of these isolates had lost the large plasmid pHG21-a. An example is shown in Fig. 1, lane 5.

Interspecific mating experiments were conducted with plasmid-harboring Hox^+ donors of *A. eutrophus* and plasmid pHG21-a cured Hox^- recipients derived from *A. hydrogenophilus*. The donor strain HF41 (Table 1) carried as counterselectable markers a deficiency for methionine and for carbon dioxide-fixing ability. Transconjugants which were able to grow autotrophically with hydrogen occurred at a frequency of approximately 10^{-3} per donor. They had acquired plasmid pHG1 from *A. eutrophus* (Fig. 1, lane 8). Reciprocal crosses were performed between wild-type *A. hydrogenophilus* and the double antibiotic-resistant Hox^- recipient HF151 (Table 1; Fig. 1, lane 4). Hox^+ transconjugants were isolated on minimal agar containing nalidixic acid and streptomycin (9). As Fig. 1, lane 7, shows, the transconjugants harbored a plasmid whose size was identical with that of pHG21-a from *A. hydrogenophilus*. Plasmid pHG21-a could be easily differentiated in the gel from the *A. eutrophus* resident plasmid pHG1 (Fig. 1, lane 6).

We further examined the extent to which the exchange of plasmid DNA affected the structure and regulation of the hydrogenases in Hox^+ transconjugants. As is evident from Table 3, the transfer of the *A. hydrogenophilus* plasmid pHG21-a to *A. eutrophus* HF151 resulted in transconjugants like HF157, which expressed the Hox regulatory phenotype of the donor. They were temperature resistant (Hox Tr) and incapable of derepressing hydrogenase activity under heterotrophic conditions. Likewise, the acquisition of plasmid pHG1 rendered Hox^+ transconjugants of *A. hydrogenophilus* such as M111 temperature sensitive. Moreover, the

TABLE 3. Regulation of hydrogenase formation in Hox^+ transconjugants

Strain	Plasmid	Phenotype	Growth conditions[a]	Hydrogenase activity (U/mg of protein)[b]	
				SH	PH
A. eutrophus H16	pHG1	Hox Ts, Hox d	$CO_2 + H_2$	0.250	0.860
			FGN	2.700	1.030
A. hydrogenophilus M50	pHG21-a, pHG21-b	Hox Tr, Hox i	$CO_2 + H_2$	0.370	1.800
			FGN	0	0
A. eutrophus HF151	$pHG1^-$	Hox^-	FGN	0	0
A. hydrogenophilus M55	pHG21-a^-, pHG21-b	Hox^-	FGN	0	0
A. eutrophus HF157	pHG21-a	Hox Tr, Hox i	$CO_2 + H_2$	0.261	3.400
			FGN	0	0.008
A. hydrogenophilus M111	pHG1, pHG21-b	Hox Ts, Hox d	$CO_2 + H_2$	0.941	5.100
			FGN	2.390	2.980

[a] Cells were cultivated either autotrophically with CO_2 plus H_2 or heterotrophically in fructose-glycerol-ammonium-containing minimal medium (FGN) as described (7).

[b] Abbreviations and assay conditions are described in Table 2, footnote *a*.

transconjugants formed hydrogenases under heterotrophic conditions in the presence of energy limitation, as described for the natural host *A. eutrophus* H16 (3, 6). Immunological studies of the particulate hydrogenase from transconjugant HF157 (Table 1) revealed a cross-reactivity of only partial identity compared with that of the parent strain H16. Conversely, transconjugant M111 had acquired a particulate hydrogenase of complete immunochemical homology (data not shown).

The results support the proposal that the species *A. eutrophus* and *A. hydrogenophilus* are very similar, if not identical, except for their plasmid content. Furthermore, the observations are in agreement with our conclusion that Hox-encoding pHG plasmids carry structural and regulatory hydrogenase genes (9).

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