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CONJUGAL TRANSFER OF HYDROGEN-OXIDIZING ABILITY OF ALCALIGENES HYDROGENOPHILUS TO PSEUDOMONAS OXALATICUS

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Conjugal transfer of hydrogen-oxidizing ability (Hox) of the hydrogen bacterium Alcaligenes hydrogenophilus was examined. Intraspecific cross of plasmid pHG21-a that encodes hydrogenases that mediate hydrogen oxidation was most frequent at 25 C; the optimal temperature for growth was 30 C. The plasmid could be transferred from A. hydrogenophilus to Pseudomonas oxalaticus OX1 and OX4, and the resulting strains gained the capacity for autotrophic growth with H_2 and CO_2. Plasmid pHG21-a was maintained in P. oxalaticus OX1 and OX4 as stably as in A. hydrogenophilus.

Alcaligenes hydrogenophilus is a gram-negative, facultative chemolithoautotrophic bacterium, which grows with H_2 as an energy source and CO_2 as the sole carbon source. This organism was isolated from soil (1). It has two hydrogenases that mediate hydrogen oxidation. One is soluble NAD^+-reducing hydrogenase and the other is membrane-bound methylene blue-reducing hydrogenase (2). Cells of A. hydrogenophilus grown with H_2 and CO_2 have ribulose bisphosphate carboxylase (RuBPCase, EC 4.1.1.39)(2), a key enzyme of autotrophic carbon dioxide fixation. In A. hydrogenophilus, soluble and membrane-bound hydrogenases are encoded on plasmid pHG21-a, and this Hox plasmid is self-transmissible (2). Hox plasmid can be exchanged between hydrogen bacteria (2). In this study, we transferred Hox plasmid of A.

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hydrogenophilus to CO_2-fixing bacteria as well as to hydrogen bacteria and cultivated these bacteria with H_2 and CO_2.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1. An auxotrophic mutant strain was isolated as a spontaneous mutant by the penicillin-cycloserine method (3). Antibiotic-resistant mutant strains were isolated as spontaneous mutants. A plasmid pHG21-a cured Hox^- strain of A. hydrogenophilus was isolated after treatment with acridine orange.

Media and culture conditions. The modified L-broth used as the nutrient broth consisted of 1 liter of water, 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), 5 g of NaCl, and 1 g of fructose (pH 7.2). Autotrophic growth was in minimal medium under a gas mixture of H_2, O_2, and CO_2 (7:2:1, vol/vol) (1). When necessary, amino acid was supplemented at a final concentration of 50 µg/ml. Solid media contained 1.5% (wt/vol) agar. All cultures were incubated at 30 C.

Conjugation. Conjugation was performed by mating on membrane filters in a modification of Murooka et al. (4). Equal volumes of exponentially growing donors and recipients were mated overnight on a membrane filter in a nutrient agar plate. Bacteria were then suspended in 2 ml of 0.9% saline, and 0.1 ml samples of suitable dilutions were spread on the selective medium. Bacterial growth for the mating period was estimated from the OD_{660} in 2 ml of bacterial suspension after conjugation. For estimation of conjugal transfer of Hox plasmid pHG21-a, donors and Hox^+ transconjugants were incubated autotrophically under H_2, O_2, and CO_2 for 7 to 10 days. When strains resistant to streptomycin (Sm) were used as recipients, Sm was added to the final concentration of 500 or 1,000 µg/ml. The transfer frequency of the plasmid

Table 1. Bacterial strains

Strain		Relevant phenotype	Source
Alcaligenes	hydrogenophilus		
	1978	Hox^+	Wild-type (1)
	CH30SR	Hox^-, Sm^r	Mutant of 1978, this study
	MT105	Hox^+, Trp^-	Mutant of 1978, this study
Pseudomonas	oxalaticus		
	OX1	Hox^-	Wild-type, NCIB 8642
	OX1-SR	Hox^-, Sm^r	Mutant of OX1, this study
	OX1-H1	Hox^+, Sm^r	Conjugant, A. hydrogenophilus MT105 x P. oxalaticus OX1-SR, this study
	OX4	Hox^-	Wild-type, NCIB 8543
	OX4-H1	Hox^+	Conjugant, A. hydrogenophilus MT105 x P. oxalaticus OX4, this study
	OX4-H2	Hox^+	Conjugant, A. hydrogenophilus MT105 x P. oxalaticus OX4, this study
	OX6	Hox^-	Wild-type, NCIB 8544
	OX23	Hox^-	Wild-type, ATCC 11884
	OX23-SR	Hox^-, Sm^r	Mutant of OX23, this study

Abbreviations: Hox, ability to oxidize hydrogen; Sm^r, resistance to streptomycin

transfer was expressed as the number of transconjugants per donor cell in mating mixture at the end of conjugation.

Isolation of plasmid. Bacterial cultures for plasmid isolation were harvested at the end of the logarithmic growing phase in nutrient broth. Crude lysates of plasmid DNA were prepared as described by Yano et al. (5), who slightly modified the method of Hansen and Olsen.

RESULTS AND DISCUSSION

Influence of temperature on conjugation. The effect of temperature on the conjugal transfer of hydrogen-oxidizing ability was investigated with an intraspecific cross of A. hydrogenophilus. A. hydrogenophilus 1978 wild type was used as the donor and plasmid pHG21-a cured Hox^- strain CH30SR (Sm^r) was used as the recipient. Conjugation was performed at 20, 25, 30, and 37 C overnight. Bacterial growth and formation of Hox^+ transconjugants for the mating period were examined at each temperature. Bacterial growth was best at 30 C, which was the optimum temperature for growth on hydrogen, but the number of Hox^+ transconjugants was largest at 25 C (Table 2). Hox plasmids of hydrogen bacteria are transferred at 30 C (6,7). The optimum temperature for the conjugation of Hox plasmid pHG21-a of A. hydrogenophilus was lower than for other hydrogen bacteria.

Table 2. Influence of temperature on conjugal transfer of hydrogen-oxidiziing ability

Temperature (C)	Growth (OD_{660})	Number of Hox^+ transconjugants (c.f.u. per filter)
20	2.4	2.1×10^6
25	7.7	1.4×10^8
30	11.8	7.8×10^7
37	8.7	1.3×10^4

A. hydrogenophilus 1978 was used as the donor, and A. hydrogenophilus CH30SR was used as the recipient. Three filter matings were performed at each temperature and mean values were estimated. Hox^+ transconjugants were selected under H_2, O_2, and CO_2 on minimal medium plates containing Sm (1 mg/ml).

Conjugal transfer of hydrogen-oxidizing ability from A. hydrogenophilus to P. oxalaticus. We examined the conjugal transfer of hydrogen-oxidizing ability from A. hydrogenophilus to CO_2-fixing bacteria as well as to hydrogen bacteria. P. oxalaticus OX1 is an oxalate-utilizer (8). P. oxalaticus OX1 metabolizes formate via the CO_2 fixation by Calvin cycle (9). A. hydrogenophilus MT105 (Trp^-) was used as the donor and the Sm^r strain of P. oxalaticus OX1 was used as the recipient. Plasmid pHG21-a was transferred at a frequency of 10^{-2} per donor in the intraspecific cross of A. hydrogenophilus, and transferred to P. oxalaticus OX1 at a frequency of 10^{-7} per donor (Table 3). Cells of P. oxalaticus OX1 harbouring Hox plasmid pHG21-a, grown with H_2 and CO_2, had soluble and membrane-bound hydrogenases (data not shown).

Microorganisms able to grow autotrophically on formate can often grow on oxalate (9). So, we examined the conjugal transfer to other oxalate-utilizers such as P. oxalaticus OX4, OX6, and OX23 (8). Plasmid pHG21-a was not transferred to P. oxalaticus OX6 and OX23 except at less than 10^{-10} per donor, but it was

Table 3. Conjugal transfer of hydrogen-oxidizing ability from A. hydrogenophilus to P. oxalaticus

Recipient	Transfer frequency (Hox^+ transconjugants per donor)
A. hydrogenophilus CH30SR	1.7×10^{-2}
P. oxalaticus OX1-SR	3.8×10^{-7}
P. oxalaticus OX4	3.3×10^{-7}
P. oxalaticus OX6	$< 10^{-10}$
P. oxalaticus OX23-SR	$< 10^{-10}$

A. hydrogenophilus MT105 was used as the donor. Conjugation was performed at 25 C. Donor and Hox^+ transconjugants were selected for minimal medium plates under H_2, O_2, and CO_2. For donor growth, tryptophane (50 µg/ml) was supplemented. When Sm-resistant recipients were used, Sm was added at 500 µg/ml.

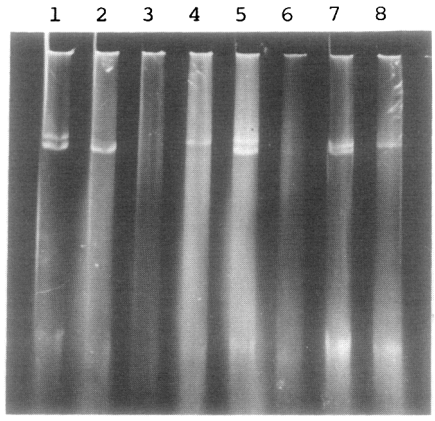

Figure 1. Agarose gel electrophoresis of plasmid DNAs from Hox$^+$ and Hox$^-$ strains of A. hydrogenophilus and P. oxalaticus. Agarose gel electrophoresis was carried out on 0.7% (wt/vol) agarose in Tris-borate buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.5) at 100 V for 8 h. lane 1. A. hydrogenophilus 1978; lane 2. A. hydrogenophilus CH30SR; lane 3. P. oxalaticus OX1; lane 4. P. oxalaticus OX1-H1; lane 5. A. hydrogenophilus 1978; lane 6. P. oxalaticus OX4; lane 7. P. oxalaticus OX4-H1; lane 8. P. oxalaticus OX4-H2.

transferred to P. oxalaticus OX4 at a frequency of 10^{-7} per donor (Table 3).

Plasmid analysis. Agarose gel electrophoresis of plasmid DNAs from Hox$^+$ and Hox$^-$ strains of A. hydrogenophilus and P. oxalaticus is shown in Fig. 1. A. hydrogenophilus 1978 harbours two large plasmids (lane 1 and 5), pHG21-a (270 Md) and pHG21-b (230 Md) as reported by Friedrich et al. (2). A. hydrogenophilus CH30SR was a plasmid pHG21-a cured Hox$^-$ strain and harboured a cryptic plasmid pHG21-b (lane 2). P. oxalaticus OX1 was plasmid-free (lane 3), and the Hox$^+$ transconjugant of P. oxalaticus OX1 had only plasmid pHG21-a (lane 4). P. oxalaticus OX4 harboured an indigenous plasmid with a intermediate size between that of plasmid pHG21-a and pHG21-b (lane 6). Hox$^+$ transconjugants of P. oxalaticus OX4 were of two types. Nine out of 11 Hox$^+$ transconjugants contained both plasmid pHG21-a and indigenous plasmid (lane 7) and the other two transconjugants had only plasmid pHG21-a (lane 8). Plasmid analysis showed that all Hox$^+$

transconjugants of P. oxalaticus OX1 and OX4 contained plasmid pHG21-a.

Stability of plasmid pHG21-a in A. hydrogenophilus and in P. oxalaticus OX1 and OX4. We examined the stability of plasmid pHG21-a in each host. Autotrophically grown cultures of A. hydrogenophilus and Hox^+ transconjugants of P. oxalaticus OX1 and OX4 were cultivated in nutrient broth overnight and were plated on nutrient plates. Then, 100 colonies of each strain on nutrient agar plates were examined for growth with H_2 and CO_2. Plasmid pHG21-a was maintained in 96% of the colonies of A. hydrogenophilus and in 97% of those of P. oxalaticus OX1. Plasmid pHG21-a was maintained in 100% of the colonies of P. oxalaticus OX4 with or without indigenous plasmid. Hox plasmid pHG21-a was maintained in P. oxalaticus OX1 and OX4 as stably as in A. hydrogenophilus.

CONCLUSIONS

Hox plasmid pHG21-a was transferred from A. hydrogenophilus to P. oxalaticus OX1 and OX4. The hydrogenase genes were expressed and coupled with originally existing enzyme systems in P. oxalaticus OX1 and OX4.

REFERENCES

1. Ohi, K., Takada, N., Komemushi, S., Okazaki, M., and Miura, Y. (1979) J. Gen. Microbiol. 25, 53-58
2. Friedrich, B., Friedrich, C. G., Meyer, M., and Schlegel, H. G. (1984) J. Bacteriol. 158, 331-333
3. Ornston, L. N., Ornston, M. K., and Chou, G. (1969) Biochem. Biophys. Res. Commun. 36, 179-184
4. Murooka, Y., Takazawa, N., and Harada, T.(1981) J. Bacteriol. 145, 358-368
5. Yano, K., and Nishi, T. (1980) J. Bacteriol. 43, 552-560
6. Reh, M., and Schlegel, H. G. (1981) J. Gen. Microbiol. 126, 327-336
7. Behki, R. M., Selvaraj, G., and Lyer, V. N. (1983) Can. J. Microbiol. 29, 767-774
8. Khambata, S. R., and Bhat, J. V. (1953) J. Bacteriol. 66, 505-507
9. Friedrich, C. G., Bowien, B., and Friedrich, B. (1979) J. Gen. Microbiol. 115, 185-192