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Oxygen-dependent Respiration and Proton Extrusion in *Wolinella succinogenes*

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When H_2 was provided as the electron donor, optimum O_2 levels for growth of *Wolinella succinogenes* ATCC 29543 were 2% and 8% on brucella agar and in brucella broth, respectively. No growth occurred under 21% O_2, and scant or no growth occurred under anaerobic condition. O_2 uptake was inhibited by cyanide and 2-heptyl-4-hydroxyquinoline *N*-oxide. Protons were translocated out of the cell when oxygen was used as the terminal electron acceptor. The H^+/O ratios with H_2 and formate as an electron donor were 1.97 and 1.49, respectively. Proton translocation was inhibited by the protonophore carbonylcyanide *m*-chlorophenylhydrazone.

KEY WORDS □ microaerophilic, *Wolinella succinogenes*, respiration, electron transport, proton translocation

Wolin *et al.* (32) reported the isolation of cytochrome-producing, motile vibrio *Vibrio succinogenes* (now classified as *Wolinella succinogenes*) from bovine rumen fluid. It is gram-negative and asaccharolytic, and requires formate or H_2 as an energy source (28, 29).

Although *W. succinogenes* has been described as an anaerobe (29), there have been many reports suggesting the idea that it may be better to account the organism as a microaerophile. It is oxidase-positive, a characteristic usually associated with organisms that can respire with O_2. Moreover, the organism was reported to use O_2 as an electron acceptor under microaerobic condition (8, 32) and to possess cytochrome *b* and cytochrome *c* (8, 9, 15-19, 32). *W. succinogenes* belongs to the rRNA group which also contains *Helicobacter pylori* (formerly called *Campylobacter pylori* (5)), *C. cinaedi*, and *C. fennelliae*, which are microaerophiles (21, 26, 30).

Although *W. succinogenes* is known to exhibit oxygen-dependent growth, there is little or no information about the optimum O_2 level for growth, inhibition of oxygen uptake by cyanide or other respiratory inhibitors, respiration-driven proton translocation with O_2 as the terminal electron acceptor, and the inhibition by the protonophore, carbonylcyanide *m*-chlorophenylhydrazone (CCCP). The present report provides quantitative measurements of these fundamental characters of the organism.

MATERIALS AND METHODS

Bacterial strain

W. succinogenes ATCC 29543 (type strain) was used in this study. Stock cultures were grown under an atmosphere containing 6% O_2, 3% CO_2, 23% N_2, and 68% H_2 at 37°C in semisolid brucella medium (brucella broth (Difco) with 0.15% agar) and transferred weekly.

Inocula and general cultivation conditions

The top 1-cm was removed from a 2-day-old culture grown in semisolid brucella medium and mixed to yield a homogeneous suspension. One drop (0.05 m*l*) of this suspension was inoculated into each 5-m*l* portion of test broth. For inoculation of agar media, one loopful of the suspension was streaked over the entire surface of the test slant or plate.

For testing growth response in liquid media, the organism was cultured in 5-m*l* volumes of media contained in 50-m*l* cotton-stoppered serum bottles. For testing growth response on solid media, the organism was grown on 10-m*l* agar slants or in petri plates containing 20-m*l* of medium.

Cultures were incubated statically at 37°C in sealed vessels (Oxoid anaerobic jar system) equipped with a vent to allow filling with various gas mixtures. Gas atmospheres were obtained manometrically by evacuating the Oxoid jar and refilling with various combinations of CO_2, H_2, N_2, and O_2. In order to make anaerobic conditions to use H_2 as the electron donor, O_2 was omitted and an activated palladium catalyst was used to remove residual O_2. When anaerobic conditions were required to use formate as the electron donor, O_2 in the broth or slants was replaced by flushing the media with oxygen-free N_2 gas which

had been passed through heated copper coil.

For mass cultures of oxygen uptake rate and respiration-driven proton translocation experiments, cells were grown in a biphasic culture system (50 m*l* brucella broth overlaid onto 200 m*l* of brucella agar containing 2.5% agar) at 37°C for 24 h under an atmosphere of 6% O_2, 3% CO_2, 23% N_2, and 68% H_2.

Measurement of growth

Growth responses in liquid media were estimated turbidometrically at 660 nm with a Bausch and Lomb Spectronic 2000 spectrophotometer using an 1-cm cuvette. Growth responses on solid media were estimated by washing the cells from the agar surface with 5 m*l* of physiological saline (0.85% NaCl) and measuring the turbidity of the suspension.

Oxygen uptake rate

Cells were harvested by centrifugation and suspended in 40 mM Tris-HCl buffer (pH 7.0). Oxygen consumption was measured with an oxygen electrode (YSI model 53 Oxygen Monitor, Yellow Springs Instruments Co.) inserted into a water-jacketed Clark cell-type chamber (Gilson Medical Electronics, volume = 1.7 m*l*) maintained at 37°C with a circulating water bath. The system was calibrated for dissolved O_2 concentrations by the method of Robinson and Cooper (25). O_2 uptake rates were linear with time and were expressed in nanomoles of O_2 consumed per min per mg of protein. Protein concentrations were determined by the method of Bradford (1).

For measuring the effects of cyanide, rotenone, and 2-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) on O_2 uptake, the inhibitors were added initially to the cell suspension in the absence of electron donor. After steady-state oxygen consumption was maintained for 2 minutes, formate (1 mM final concentration) was added to the cell suspension. Formate was also added to control cell suspensions lacking the inhibitor.

Respiration-driven proton translocation

Cells harvested by centrifugation were washed with 3 mM glycylgylcine/140 mM KCl buffer (pH 7.2) containing 100 mM potassium thiocyanate and resuspended in the same buffer. For maintaining the temperature of cell suspension, a water-jacked chamber (volume = 5.0 m*l*) was used into which an oxygen electrode was inserted from one side and a pH electrode was inserted through an adjustable top. The reaction mixture contained 3 mM glycylglycine, 140 mM KCl, 100 mM potassium thiocyanate, and 100 μg of carbonic anhydrase (Sigma). The cell suspension (protein concentration = 0.8 to 1.0 mg/m*l*) was maintained for 30 to 60 min in the chamber to use up endogenous substrates, followed by being bubbled with oxygen-free N_2 gas to make an anaerobic condition. Residual oxygen was removed by the addition of 2 mM (final concentration) of potassium formate, which was also utilized as the electron donor. To measure H^+/O ratio with H_2 as the electron donor, H_2 gas was vigorously bubbled for 5 min. The pH meter (Fisher Accumet, Model 925) was used to monitor changes in pH. Adjustments in pH were made by addition of either 0.01 N HCl or 0.01 N KOH to achieve a starting pH of 7.2. The pH changes were recorded with a Kipp and Zonen recorder at 1 mV scale. An appropriate volume of 140 mM KCl solution saturated with air was injected to provide O_2 as the electron acceptor. The concentrations of O_2 in the air-saturated KCl solution were measured by the method of Robinson and Cooper (25); changes in temperature and barometric pressure were taken into account in all experiments. The acidification in the system was calibrated by addition of 10 μl of anaerobic 0.01 N HCl (equivalent to 100 nmoles H^+). Calculation of H^+/O ratio was determined from the ΔpH as described by Mithchell and Moyle (23, 24) and corrected for collapse of the pH gradient by the method of Scholes and Mitchell (27). *Paracoccus denitrificans* was used as a control. To measure the effect of the protonophore CCCP on the proton translocation, an ethanolic solution of CCCP was injected to give final concentrations of 5, 10, or 20 μM.

RESULTS

Oxygen dependent growth

W. succinogenes showed the typical characteristic of microaerophilic growth in brucella broth and on brucella agar slants when H_2 or formate was supplied as the electron donor (Table 1). They exhibited only very slight growth under anaerobic conditions and no growth under 21% O_2. Optimum levels of O_2 for growth on brucella agar slants and in brucella broth were 2% and 8%, respectively, indicating that optimum O_2 level for growth of *W. succinogenes* on agar slants is lower than that for growth in brucella broth. In this experiment, 10% H_2 which was provided as an electron source was sufficient to support growth of *W. succinogenes* (Table 2).

Oxygen uptake

Cell suspension of *W. succinogenes* exhibited O_2 uptake when either H_2 (0.11 mM), formate (1 mM), or succinate (10 mM) was provided as the electron donor (Table 3). Rates of O_2 uptake were highest with formate.

O_2 uptake was inhibited by KCN (Table 4). One millimolar KCN inhibited 52% of the rate of oxygen uptake measured in the absence of the inhibitor. Ten and twenty millimolar KCN caused 95 and 100% inhibition, respectively. O_2 uptake was inhibited 86% by 1 mM HOQNO. Rotenone

Table 1. *Effect of O_2 concentrations on the growth of W. succinogenes during growth with H_2 or formate as an electron donor*[a]

O_2 concn (%)	Turbidity[b]			
	In broth[c]		On slants[c]	
	H_2	Formate[d]	H_2	Formate[d]
0	0.04± 0.00	0.01± 0.02	0.03± 0.00	0.03± 0.00
2	0.32± 0.03	0.20± 0.02	**0.78± 0.06**	**0.49± 0.05**
4	0.33± 0.03	0.19± 0.02	0.11± 0.03	0.46± 0.06
6	0.37± 0.04	0.20± 0.03	0.07± 0.03	0.08± 0.02
8	**0.42± 0.04**[e]	**0.23± 0.04**	0.04± 0.02	0.04± 0.01
10	0.25± 0.01	0.18± 0.01	0.03± 0.02	0.04± 0.03
12	0.01± 0.00	0.00± 0.00	0.02± 0.01	0.04± 0.01
14	0.01± 0.00	0.00± 0.00	0.01± 0.00	0.01± 0.00
16	0.01± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
18	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
21	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00

[a] Cultures were grown in 5-m*l* brucella broth or 10-m*l* agar slants for 48 h at 37°C under different levels of O_2 as described in methods.
[b] Turbidities were measured at 660 nm as described in methods. The values are the averages± standard deviations for five to seven replicate cultures.
[c] Gas atmospheres for cultivation of the bacterium were obtained manometrically by evacuating the Oxoid jar and refilling with various combinations of CO_2, H_2, and N_2 to give the desired residual levels of oxygen manometrically as described in methods. To make anaerobic conditions, a palladium catalyst was used to remove the residual O_2 for the experiments using H_2 as the electron donor and oxygen-free N_2 was used to flush the broth or the slants for the experiments using formate as the electron donor.
[d] The final concentration of sodium formate as the electron donor was 30 mM.
[e] Values in bold face type represent maximum growth responses.

caused only slight inhibition of O_2 uptake.

Respiration-driven proton translocation

When the air-saturated KCl solution was injected as the source of O_2, respiration-driven proton translocation, i.e., acidification out of the cell, occurred (Fig. 1). The more air-saturated KCl solution was injected, the more acidification occurred. When the protonophore CCCP was applied into the system, however, the acidification was collapsed. When 10 μM of CCCP was added into the system using formate as the electron donor, the proton translocation was completely inhibited. When H_2 was used as the electron donor, the proton translocation was not inhibited completely at the final concentration of 20 μM CCCP. The H^+/O ratios with H_2 and formate as an electron donor were 1.97± 0.14 and 1.49± 0.15, respectively (Table 5).

Table 2. *Effect of H_2 concentrations on the growth of W. succinogenes*[a]

H_2 concn (%)[b]	Turbidity[c]
0	0.00± 0.00
2.5	0.03± 0.01
5	0.26± 0.06
10	0.31± 0.07
15	0.31± 0.09
20	0.30± 0.09
30	0.29± 0.08
40	0.32± 0.06
50	0.30± 0.04

[a] Cells were cultivated in 5-m*l* brucella broth under a 4% O_2 in the presence of various concentrations of H_2 as described in methods.
[b] Initially, jars containing the bottles were partially evacuated to give 4% residual oxygen. Carbon dioxide (final concentration, 3%) and various concentrations of H_2 were added, and the jars were restored to 1 atm (101.29 kPa) with N_2.
[c] See Table 1, footnote b.

Table 3. *Rates of O_2 uptake in W. succinogenes in the presence of various electron donors*[a]

Electron donor	Concn (mM)	O_2 uptake rate[b]
Formate	1	874± 24
H_2	0.11	275± 31
Lactate	1	0± 0
Malate	1	0± 0
Succinate	1	0± 0
	10	8± 1
NADH	1	0± 0
	20	166± 11

[a] Cells were grown on brucella medium in a biphasic system at 37°C for 24 h under an atmosphere of 6% O_2, 3% CO_2, 23% N_2, and 68% H_2 as described in methods.
[b] Values are expressed as nmoles O_2 taken up per min per mg protein. The values are the averages± standard deviations for three replicates.

DISCUSSION

According to the definition of Krieg and Hoffman (13), microaerophilic organisms exhibit O_2-dependent growth under microaerobic conditions; they do not grow under anaerobic conditions unless suitable alternative electron acceptors are provided, fail to grow or grow only poorly at the level of O_2 present in air (21% O_2), and are capable of utilizing O_2 as a terminal electron acceptor. *W. succinogenes* studied in this experiment was found to fit well to these criteria.

Table 4. *Effect of electron transport inhibitors on the O_2 uptake of W. succinogenes*[a]

Inhibitor[b]	Final concn (mM)	% inhibition[c]
KCN	1	52
	2	60
	5	83
	10	95
	20	100
Rotenone	20	9
HOQNO	1	86

[a] Cells were grown as described in Table 3, footnote a. To determine the level of inhibition of oxygen uptake, the inhibitors were added to the cell suspension, and then sodium formate (1 mM final concentration) was injected.
[b] Stock solutions of KCN, rotenone, and HOQNO were prepared by dissolving the inhibitors in 40 mM Tris-HCl buffer (pH 7.0), N,N-dimethylformamide (DMFA), and 0.01 N KOH, respectively.
[c] The percentage of inhibition by KCN or HOQNO was determined as follows: [1-(O_2 uptake rate in the presence of inhibitor/O_2 uptake rate in the absence of inhibitor)]×100. The percentage of inhibition by rotenone was determined as follows: [1-(O_2 uptake rate in the presence of rotenone plus DMFA)/(O_2 uptake rate in the presence of DMFA alone)]×100.

Table 5. *H^+/O ratio of W. succinogenes*[a]

Electron donor	H^+/O ratio[b]
H_2	1.97±0.14
Formate	1.49±0.15

[a] Cells were grown as described in Table 3, footnote a. The reaction mixture contained 3 mM glycylglycine, 140 mM KCl, 100 mM potassium thiocyanate, and 100 μg carbonic anhydrase in the 5.0 ml volume of chamber. The reaction temperature was maintained at 37°C. Details are described in methods.
[b] The values are the averages± standard deviations for at least five determinations.

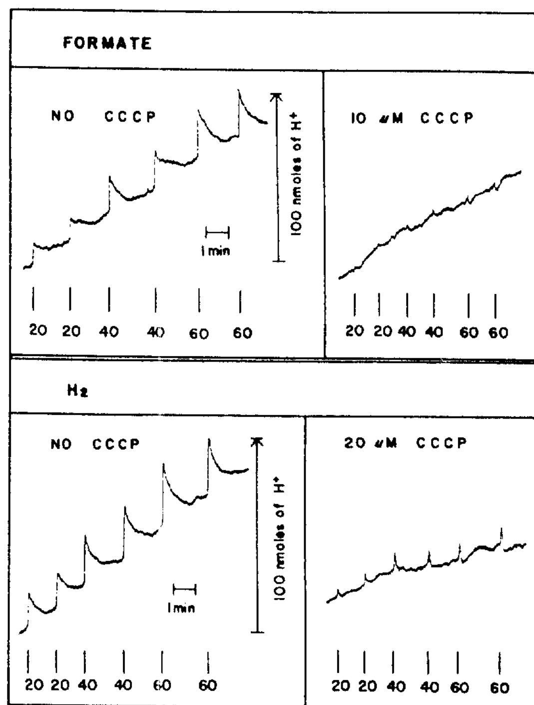

Fig. 1. *Effect of CCCP on the respiration-driven proton translocation of W. succinogenes. Proton translocation in the presence of H_2 or formate as an electron donor was measured as described in methods. The vertical arrow indicates an acidification of the medium equivalent to 100 nmoles of H^+. The numbers indicate the amounts (μl) of air-saturated 140 mM KCl buffer injected. 20 μl buffer injected contains 9.37 nmoles of atomic oxygen.*

It grew in the presence of low level of O_2, exhibited no or only slight growth on brucella media under anaerobic conditions in the absence of any electron acceptors, and failed to grow under 21% O_2. Its ability to use O_2 as a terminal electron acceptor was indicated by inhibition of O_2 uptake by KCN and HOQNO, and to a slight extent, by rotenone.

Under anaerobic conditions, it showed slight growth in the absence of any electron acceptor such as fumarate which can be utilized as a terminal electron acceptor. The growth might be due to aspartate and asparagine which are undoubtedly present at low level in complex media such as brucella media and the presence of asparaginase and aspartase activities in *W. succinogenes* (2, 12).

As proposed by Jacobs and Wolin (9), *W. succinogenes* takes up O_2 during growth on formate via a formate oxidase system or an unknown electron transport system through either cytochrome *b* or cytochrome *c*. I found in this experiment that O_2 uptake for the oxidation of formate is completely inhibited by 20 mM KCN, an inhibitor of cytochrome oxidase in electron transport system (3, 11). HOQNO, an inhibitor of electron transport system between cytochrome *b* and cytochrome *c* (3, 6, 20), at 1 mM showed 86% inhibition. The rate of O_2 uptake was high with 20 mM NADH, but it was affected only slightly by 20 mM rotenone. These together with the report of Kröger *et al.* (18) suggest that formate is oxidized by formate oxidase and electrons

might flow directly to cytochrome *b* in *W. succinogenes*. The present results strongly support that electrons from formate do not flow via flavoproteins between NAD and CoQ/or cytochrome *b*, which is susceptible by rotenone. The bacterium also showed O_2 uptake with H_2 and succinate as an electron donor.

As shown in Fig. 1, when oxygen was injected, the acidifications out of the cell in the reaction mixture occurred and protonophore, CCCP, inhibited the proton translocation. This strongly supports that oxygen is used as the terminal electron acceptor, not as the substrate for another oxidase system. This proton gradient may be utilized further for oxidative phosphorylation, motility, reverse electron transport, and any transport system, if any, for amino acid or TCA cycle intermediates, etc. The H^+/O ratios with H_2 and formate as an electron donor were 1.97 and 1.49, respectively. These values are smaller than those of other microaerophiles or facultative anaerobe *Escherichia coli*; H^+/O ratios of *C. jejuni* with H_2 and formate were 3.72 and 2.50, respectively (7). H^+/O ratio of *C. sputorum* biovar bubulus with formate was 3.2 (31) and that of *E. coli* with H_2 was 3.62 (10). The ratio of *W. succinogenes* with H_2, howver, is larger than that of *C. mucosalis* (H^+/O ratio=0.7, (4)). The H^+/O ratio with endogenous substrates of *P. denitrificans*, an aerobic bacterium, is larger than that of *W. succinogenes* (H^+/O ratio=7~8, (22, 27)). In *W. succinogenes*, the H^+/O ratio with formate is similar to or a little smaller than H^+/fumarate with formate as the electron donor (H^+/O ratio=1.6, (14)).

In conclusion, although many authors have described *W. succinogenes* as an anaerobe (28, 29), the present results show that the organism is not an anaerobe but a microaerophile, as already mentioned by Wolin *et al.* (32) and Jacobs and Wolin (8, 9). From these and the report of Tanner and Socransky (29), the description of *W. succinogenes* can be changed as follows; *W. succinogenes* is a H_2- or formate-requiring microaerophile. It grows under anaerobic conditions in the presence of any alternative electron acceptors such as fumarate, malate, nitrate, and a couple of amino acids such as aspartate and asparagine.

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REFERENCES

1. **Bradford, M.M.,** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248-254.
2. **Bronder, M., H. Mell, E. Stupperich and A. Kröger,** 1982. Biosynthetic pathways of *Vibrio succinogenes* growing with fumarate as terminal electron acceptor and sole carbon source. *Arch. Microbiol.*, **131**, 216-223.
3. **Carlone, G.M. and J. Lascelles,** 1982. Aerobic and anaerobic respiratory systems in *Campylobacter fetus* subsp. *jejuni* grown in atmospheres containing hydrogen. *J. Bacteriol.*, **152**, 306-314.
4. **Goodhew, C.F., A.B. Elkurdi and G.W. Pettigrew,** 1988. The microaerophilic respiration of *Campylobacter mucosalis*. *Biochim. Biophys. Acta*, **933**, 114-123.
5. **Goodwin, C.S., J.A. Armstrong, T. Chilvers, M. Peters, M.D. Collins, L. Sly, W. McConnell and W.E.S. Harper,** 1989. Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae*. comb. nov., respectively. *Int. J. Syst. Bacteriol.*, **39**, 397-405.
6. **Harvey, S. and J. Lascelles,** 1980. Respiratory systems and cytochromes in *Campylobacter fetus* subsp. *intestinalis*. *J. Bacteriol.*, **144**, 917-922.
7. **Hoffman, P.S. and T.G. Goodman,** 1982. Respiratory physiology and energy conservation efficiency of *Campylobacter jejuni*. *J. Bacteriol.*, **150**, 319-326.
8. **Jacobs, N.J. and M.J. Wolin,** 1963a. Electron-transport system of *Vibrio succinogenes*. I. Enzymes and cytochromes of the electron-transport system. *Biochim. Biophys. Acta*, **69**, 18-28.
9. **Jacobs, N.J. and M.J. Wolin,** 1963b. Electron-transport system of *Vibrio succinogenes*. II. Inhibition of electron transport by 2-heptyl-4-hydroxyquinoline *N*-oxide. *Biochim. Biophys. Acta*, **69**, 29-39.
10. **Jones, R.W.,** 1980. Proton translocation by the membrane-bound formate dehydrogenase of *Escherichia coli*. *FEMS Microbiol. Lett.*, **8**, 167-171.
11. **Jurtshuk, P.Jr., T.J. Mueller and W.C. Acord,** 1975. Bacterial terminal oxidases. *Crit. Rev. Microbiol.* **3**, 399-468.
12. **Kafkewitz, D. and D. Goodman,** 1974. L-asparaginase production by the rumen anaerobe *Vibrio* succinogenes. *Appl. Microbiol.*, **27**, 206-209.
13. **Krieg, N.R. and P.S. Hoffman,** 1986. Microaerophily and oxygen toxicity. *Ann. Rev. Microbiol.*, **40**, 107-130.
14. **Kröger, A.,** 1975. *In* Electron transfer chains and oxidative phosphorylation (E. Quagliariello *et al.*, eds.), pp. 265-270, North-Holland, Amsterdam.
15. **Kröger, A.,** 1978. Fumarate as terminal acceptor of phosphorylative electron transport. *Biochim. Biophys. Acta*, **505**, 129-145.
16. **Kröger, A. and A. Innerhofer,** 1976a. The function

of menaquinone, covalently bound FAD and iron-sulfur protein in the electron transport from formate to fumarate of *Vibrio succinogenes*. *Eur. J. Biochem.*, **69**, 487-495.

17. **Kröger, A. and A. Innerhofer,** 1976b. The function of the b cytochromes in the electron transport from formate to fumarate of *Vibrio succinogenes*. *Eur. J. Biochem.*, **69**, 497-506.
18. **Kröger, A., E. Dorrer and E. Winkler,** 1980. The orientation of the substrate sites of formate dehydrogenase and fumarate reductase in the membrane of *Vibrio succinogenes*. *Biochim. Biophys. Acta*, **589**, 118-136.
19. **Kröger, A., E. Winkler, A. Innerhofer, H. Hackenberg and H. Schäger,** 1979. The formate dehydrogenase involved in electron transport from formate to fumarate in *Vibrio succinogenes*. *Eur. J. Biochem.*, **94**, 465-475.
20. **Lascelles, J. and K.M. Calder,** 1985. Participation of cytochromes in some oxidation-reduction system in *Campylobacter fetus*. *J. Bacteriol.*, **164**, 401-409.
21. **Lau, P.P., B. DeBrunner-Vossbrinck, B. Dunn, K. Miotto, M.T. MacDonell, D.M. Rollins, C.J. Pillidge, R.B. Hespell, R.R. Colwell, M.L. Sogin and G.E. Fox,** 1987. Phylogenetic diversity and position of the genus *Campylobacter*. *Syst. Appl. Microbiol.*, **9**, 231-238.
22. **Meijer, E.M, H.W. van Verseveld, E.G. van Der Beek and A.H. Stouthamer,** 1977. Energy conservation during aerobic growth in *Paracoccus denitrificans*. *Arch. Microbiol.*, **112**, 25-34.
23. **Mitchell, P. and J. Moyle,** 1967. Acid-base titration across the membrane system of rat-liver mitochondria. *Biochem. J.*, **104**, 588-600.
24. **Mitchell, P. and J. Moyle,** 1968. Proton translocation coupled to ATP hydrolysis in rat liver mitochondria. *Eur. J. Biochem.*, **4**, 530-539.
25. **Robinson, J. and J.M. Cooper,** 1970. Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. *Anal. Biochem.*, **33**, 390-399.
26. **Romaniuk, P.J., B. Zoltowska, T.J. Trust, D.J. Lane, G.J. Olsen, N.R. Pace and D.A. Stahl,** 1987. *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. *J. Bacteriol.*, **169**, 2137-2141.
27. **Scholes, P. and P. Mitchell,** 1970. Respiration-driven proton translocation in *Micrococcus denitrificans*. *J. Bioenerg.*, **1**, 309-323.
28. **Tanner, A.C.R., S. Badger, C.H. Lai, M.A. Listgarten, R.A. Visconti and S.S. Socransky,** 1981. *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin *et al.*) comb. nov., and description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. *Int. J. Syst. Bacteriol.*, **31**, 432-445.
29. **Tanner, A.C.R. and S.S. Socransky,** 1984. Genus *Wolinella*, pp. 646-650. *In* N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore, M.D.
30. **Thompson, L.M. III, R.M. Smibert, J.L. Johnson and N.R. Krieg,** 1988. A phylogenetic study of the genus *Campylobacter*. *Int. J. Syst. Bacteriol.*, **38**, 190-200.
31. **de Vries, W., H.G.D. Niekus, M. Boellaard and A.H. Stouthamer,** 1980. Growth yields and energy generation by *Campylobacter sputorum* subspecies *bubulus* during growth in continuous culture with different hydrogen acceptors. *Arch. Microbiol.*, **124**, 221-227.
32. **Wolin, M.J., E.A. Wolin and N.J. Jacobs,** 1961. Cytochrome-producing anaerobic vibrio, *Vibrio succinogenes*, sp. n. *J. Bacteriol.*, **81**, 911-917.

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초 록: *Wolinella succinogenes*의 산소 의존성 호흡과 양자 방출

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수소를 전자공여체로 사용하였을 때 *Wolinella succinogenes*의 생장을 위한 최적 산소 농도는 brucella 한천배지와 액체배지에서 각각 2%와 8%이었다. 21% 신소를 갖는 일반 대기조건에서 이 세균은 전혀 생장하지 않았으며, 무산소 조건에서서는 미미한 정도 자라거나 거의 자라지 않았다. 전자전달저해제인 cyanide나 2-heptyl-4-hydroxyquinoline *N*-oxide에 의해 세균의 산소 흡수가 저해 되었다. 무산소 조건에서 산소를 최종 전자수용체로 주입하였을 때, 양자가 세포 안으로부터 밖으로 방출됨을 볼 수 있었다. 수소와 개미산을 전자공여체로 사용하였을 때, 무산소 조건에서 산소를 최종 전자수용체로 주었을 때 H^+/O의 비율은 각각 1.97과 1.49를 나타내었다. 양자의 세포내로부터 밖으로의 방출은 uncoupler인 carbonylcyanide *m*-chlorophenylhydrazone에 의해 저해됨을 볼 수 있었다.