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Change of the Terminal Oxidase from Cytochrome a_1 in Shaking Cultures to Cytochrome *o* in Static Cultures of *Acetobacter aceti*

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***Acetobacter aceti* has an ability to grow under two different culture conditions, on shaking submerged cultures and on static pellicle-forming cultures. The respiratory chains of *A. aceti* grown on shaking and static cultures were compared, especially with respect to the terminal oxidase. Little difference was detected in several oxidase activities and in cytochrome *b* and *c* contents between the respiratory chains of both types of cells. Furthermore, the results obtained here suggested that the respiratory chains consist of primary dehydrogenases, ubiquinone, and terminal ubiquinol oxidase, regardless of the culture conditions. There was a remarkable difference, however, in the terminal oxidase, which is cytochrome a_1 in cells in shaking culture but cytochrome *o* in cells grown statically. Change of the culture condition from shaking to static caused a change in the terminal oxidase from cytochrome a_1 to cytochrome *o*, which is concomitant with an increase of pellicle on the surface of the static culture. In contrast, reappearance of cytochrome a_1 in *A. aceti* was attained only after serial successive shaking cultures of an original static culture; cytochrome a_1 predominated after the culture was repeated five times. In the culture of *A. aceti*, two different types of cells were observed; one forms a rough-surfaced colony, and the other forms a smooth-surfaced colony. Cells of the former type predominated in the static culture, while the cells of the latter type predominated in the shaking culture. Thus, data suggest that a change of the culture conditions, from static to shaking or vice versa, results in a change of the cell type, which may be related to the change in the terminal oxidase from cytochrome a_1 to cytochrome *o* in *A. aceti*.**

Acetic acid bacteria are obligate aerobes and are well known as oxidative bacteria able to oxidize various sugars and sugar alcohols such as ethanol, acetaldehyde, glucose, and so on. Such oxidation reactions are carried out by their respective dehydrogenases linked to the respiratory chain located in the cytoplasmic membrane of the organism. Acetic acid bacteria are classified into two genera, *Gluconobacter* and *Acetobacter*. The respiratory chain of *Gluconobacter* species has been characterized relatively well; *Gluconobacter suboxydans* has a respiratory chain consisting of cytochrome *c*, ubiquinone, and a terminal cytochrome *o* ubiquinol oxidase (3, 6, 9, 11, 14), and the respiratory chain also contains a KCN-insensitive alternative oxidase (3, 9). Furthermore, it has recently been shown that *G. suboxydans* subsp. α strains incapable of oxidizing ethanol lack a quinoprotein alcohol dehydrogenase, a large amount of cytochrome *c*, and the alternative oxidase activity all together; it has also been shown that all such characteristics are restored at the same time by reconstituting the alcohol dehydrogenase to the membrane of *G. suboxydans* subsp. α strains (10).

The respiratory chain of *Acetobacter* species has not been well characterized so far except for some knowledge for the terminal oxidase. *Acetobacter pasteurianus* is shown to contain cytochrome a_1 as a terminal oxidase by classic work (for a review, see references 13, 15, and 16), and thereafter *Acetobacter* strains were shown to be divided into two groups on the basis of the terminal oxidase: one group including *Acetobacter aceti* contains only cytochrome a_1, but the other group contains cytochrome *d* (4). *Acetobacter* strains are able to grow, forming a pellicle on the surface, on static cultures as well as on shaking cultures, in contrast to *Gluconobacter* strains, which are unable to grow on static cultures. Recently, we have purified a terminal oxidase from *A. aceti* grown on a shaking culture and characterized it to be a cytochrome *ba* ubiquinol oxidase (13).

Thus, in this study, we compare the respiratory chains of *A. aceti* grown on shaking and static cultures. The results show that the terminal oxidase of the organism is changed from cytochrome a_1 to cytochrome *o* by the change of the culture conditions from shaking to static. Data also suggest that the change of the terminal oxidase of the strain is related to the change of cell type adapted to the culture condition.

MATERIALS AND METHODS

Materials. Ubiquinone-1 and -2 (Q_1 and Q_2, respectively) were kindly supplied by Eizai (Tokyo, Japan), and ubiquinol-2 (Q_2H_2) was prepared as described by Rieske (17). All other materials were of analytical grade and were obtained from commercial sources.

Bacterial strain and seed culture. *A. aceti* IFO 3284 was used throughout this study. The strain was maintained on an agar slant whose medium was prepared by adding 2% agar and 0.5% $CaCO_3$ to a potato medium consisting of 5 g of glucose, 10 g of yeast extract, 10 g of polypeptone, and 150 ml of potato extract in 1 liter of tap water (8). The cells maintained on the agar slant were inoculated in 5 ml of the potato medium and cultivated with shaking for 24 h as a seed culture.

Culture condition. Shaking culture was performed at 30°C with rotary shaking (200 rpm) in a medium consisted of 10 g of glycerol, 3 g of yeast extract, 2 g of polypeptone, 1 g of KH_2PO_4, and 1 g of K_2HPO_4 in 1 liter of tap water. Preculture was performed by inoculating the seed culture into 100 ml of the medium in a 500-ml flask and shaking for 24 h. Then the preculture was transferred to 1 liter of the

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same medium in a 3-liter flask and incubated with shaking for 16 h. Static culture was done at 30°C without shaking, in the same medium as the shaking culture. The seed culture was inoculated into 50 ml of the medium in a 500-ml flask and incubated statically for 2 days. Then the preculture was transferred into 500 ml of the same medium in a 3-liter Erlenmeyer flask and incubated statically for 7 days.

Preparation of membrane and solubilization of cytochromes. Cells were harvested by centrifugation at 9,000 × *g* for 10 min and washed first with distilled water and then with 50 mM potassium phosphate buffer (pH 6.5). The washed cells were resuspended in the same buffer at a concentration of 5.5 ml/g of wet cells and passed twice through a French pressure cell at 16,000 lb/in^2. After centrifugation at 9,000 × *g* for 10 min to remove intact cells, the membrane fraction was collected by centrifugation at 80,000 × *g* for 60 min. In order to solubilize some cytochrome components, the membrane was solubilized with Triton X-100. Membrane fractions were suspended at a protein concentration of 10 mg/ml in 10 mM potassium phosphate buffer (pH 6.0), and Triton X-100 was added to a final concentration of 0.3%. After standing on ice for 30 min, the mixture was centrifuged at 68,000 × *g* for 60 min to separate the solubilized supernatant and precipitate. The precipitate was resuspended in 50 mM potassium phosphate buffer (pH 6.5) and centrifuged again to remove the detergent contaminated.

Enzyme assays. Oxidase activities for Q_2H_2 and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) were measured spectrophotometrically at 25°C by monitoring the increase in A_{275} and A_{520}, respectively. The reaction mixture (total volume of 1 ml) contained 50 mM potassium phosphate buffer (pH 6.5), 50 μM Q_2H_2 or 2 mM TMPD, 0.04% Tween 20, and the enzyme. Activity was calculated by using a millimolar extinction coefficient of 12.25 for quinol and 6.1 for TMPD. Oxidase activities for glucose, ethanol, aldehyde, lactate, NADH, and Q_1H_2 were measured at 25°C polarographically with an oxygen electrode. The reaction mixture (total volume of 3 ml) contained 50 mM potassium phosphate buffer (pH 6.5), the enzyme, and 10 mM each substrate, except for Q_1H_2 oxidase, in which 2 mM dithiothreitol and 50 μM Q_1 were added as the substrates. Alcohol or glucose dehydrogenase activity was measured spectrophotometrically with phenazine methosulfate and dichlorophenol indophenol as electron acceptors (8).

Other analytical procedures. Absorption spectrophotometry was performed with a Hitachi 557 dual-wavelength spectrophotometer. Low-temperature difference spectra were taken in liquid nitrogen in a cuvette with a 2-mm light path. Samples were reduced with sodium dithionite in the absence or presence of 1 mM cyanide and were oxidized with ferricyanide. CO reduced-minus-reduced difference spectra were taken at room temperature. Samples were reduced with sodium dithionite, and portions of them were bubbled with carbon monoxide. CO-binding cytochrome content was calculated by using a millimolar absorption coefficient of 190 (6). Cytochrome *c* content was determined by using a millimolar extinction coefficient of 19.1 (553 to 540 nm) in the redox spectrum measured at room temperature. Heme *a*, *b*, and *c* contents were determined by measuring the reduced-minus-oxidized difference spectra of their pyridine hemochromes by using millimolar absorption coefficients of 21.7, 20.7, and 24.3, respectively (13). Protein content was determined by the modified Lowry method (7).

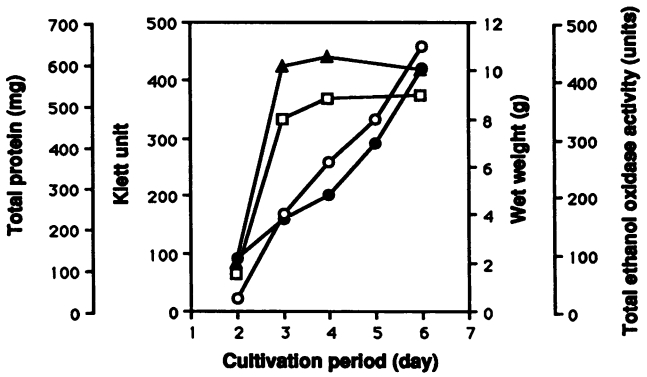

FIG. 1. Time course of cell growth and ethanol oxidase activity during static culture of *A. aceti*. Static culture was performed as described in Materials and Methods, and the growth was followed by measuring Klett units (●) after mixing the culture completely. After harvesting from 500 ml of the culture, the wet weight (○), total protein content (▲), and total ethanol oxidase activity (□) of the cells were measured.

RESULTS

Cytochromes of *A. aceti* grown on shaking and static cultures. *A. aceti* is able to grow on static culture as well as normal shaking submerged culture. When preculture grown on a shaking culture is transferred to a static culture, some of the cells slowly form a pellicle on the surface of the medium while a large part of the cells sink to the bottom and probably stop growing. As shown in Fig. 1, the cells grow very slowly on the static culture and it takes about 1 week to form a pellicle spread over the surface of a 500-ml culture. The wet weight of the cells collected from the static culture continued to increase over a week, but the protein content in the cells stopped increasing after 3 days. Ethanol oxidase activity of the intact cells also increases after 3 to 4 days of culture. Therefore, the cells may continue to produce cellulosic materials even after cell growth ceases. On the other hand, on the shaking culture, the cells grow rapidly and reach stationary phase by 24 h (see Fig. 7).

Thus, we initially prepared both types of *A. aceti* cells grown on shaking and static cultures and compared their low-temperature difference spectra. As shown in Fig. 2, both types of cells showed almost the same spectra in the absence of KCN, having a peak at 553 nm and shoulders at 548 and 562 nm in the α absorption band. However, the spectrum of the cells on shaking culture was largely changed by the addition of KCN; a broad peak at 580 to 600 nm shifted to a sharp peak at 587 nm, while such a change was not seen with the cells on static culture. Therefore, the change of the low-temperature difference spectra of the cells was traced during the static culture. After 2 days of culture, as shown in Fig. 3, the sharp peak at 587 nm in the presence of KCN was still detected to some degree. The peak gradually decreased day by day and finally disappeared after 6 days. Thus, the peak observed at 587 nm in the presence of KCN on the shaking culture decreases and disappears as the number of cells forming pellicle increases on the static culture.

Furthermore, by using the membrane fractions prepared from both types of cells, low-temperature difference spectra and CO difference spectra were measured. Although data are not shown, the low-temperature difference spectra of the membranes of each cell type were almost the same as those of the respective cells. A critical difference was observed in the CO difference spectra of the membranes prepared from

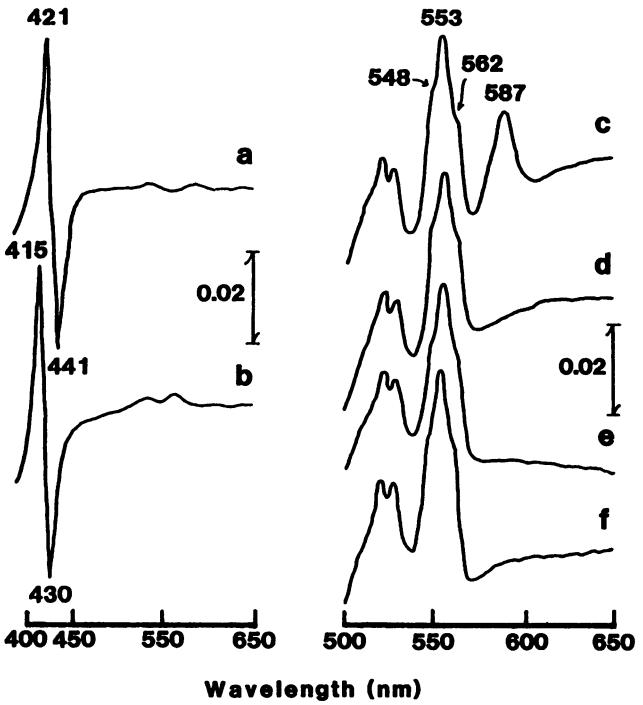

FIG. 2. Low-temperature reduced-minus-oxidized difference spectra of the cells of *A. aceti* grown on shaking and static cultures and CO reduced-minus-reduced difference spectra of the membranes (a and b) prepared from both types of the cells. Low-temperature difference spectra and CO difference spectra were measured as described in Materials and Methods. CO difference spectra were measured with the membranes prepared from each cell type grown on shaking (a; 4.0 mg of protein per ml) and static (b; 3.8 mg of protein per ml) cultures. Dithionite-reduced-minus-ferricyanide-oxidized spectra of the cells grown on shaking culture (c and d; 12.8 mg of protein per ml) and on static culture (e and f; 8.5 mg of protein per ml) were taken in the presence (c and e) and absence (d and f) of cyanide.

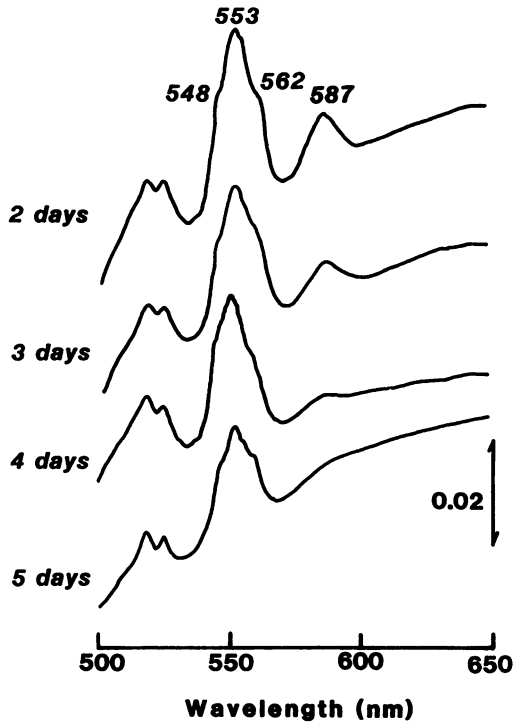

FIG. 3. Low-temperature reduced-minus-oxidized difference spectra of each type of *A. aceti* cells during static culture. Low-temperature redox spectra were taken, in the presence of cyanide, with the cells grown statically for 2, 3, 4, and 5 days at protein concentrations of 15.9, 15.1, 9.5, and 4.0 mg/ml, respectively, as described in Materials and Methods.

both types of cells (Fig. 2). The spectrum of the shaking culture had a peak at 420 nm and a trough at 440 nm, while that of the static culture showed a peak and a trough at 415 and 430 nm, respectively. The contents of the cytochrome components in the membranes prepared from both types of cells are summarized in Table 1. As shown, there is little difference in heme *b* and *c* contents between both membranes, while heme *a* was detected only in the membrane from the shaking culture.

Enzyme activities in the respiratory chain of the membranes prepared from the cells grown on shaking and static cultures. Several oxidase activities were compared between the membranes from two types of cells, those grown on shaking and those grown on static cultures. As shown in Table 1, both membranes exhibited comparable oxidase activities for glucose, ethanol, acetaldehyde, lactate, and NADH, of which ethanol and acetaldehyde oxidase activities are involved in vinegar production and are thus characteristic for acetic acid bacteria. Neither membrane has glycerol oxidase activity specific for *Gluconobacter* species (data not shown). Furthermore, as shown in Table 1, both membranes showed a high ubiquinol oxidase activity but a very low TMPD oxidase activity. The terminal oxidase activity was higher in the membrane prepared from the cells on shaking culture than in that from cells on static culture. Although data are not shown, no cytochrome *c* oxidase activity was detected in either membrane. Since the spectral data suggested the presence of different types of terminal oxidase, the effects of KCN on ethanol and Q_1H_2 oxidase activities of both membranes were compared. As shown in Fig. 4, the membrane from the static culture shows a higher sensitivity for KCN than that from the shaking culture. The K_i values for KCN

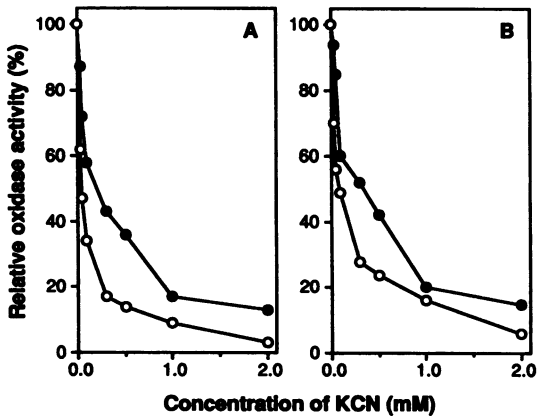

FIG. 4. Effect of KCN on ethanol (A) and ubiquinol (B) oxidase activities of the membranes prepared from *A. aceti* grown on shaking and static cultures. Oxidase activities were assayed with the membranes prepared from the cells grown on shaking (●) and static (○) cultures, as described in Materials and Methods.

TABLE 1. Respiratory chain-linked enzyme activities and cytochrome contents of the membranes from *A. aceti* grown on shaking and static cultures.[a]

Culture	Oxidase activity (units/mg of protein)							Content (nmol/mg of protein) of:				
	Glucose	Ethanol	Aldehyde	Lactate	NADH	Q_2H_2	TMPD	CO-cyt.	Cyt. *c*	Heme *a*	Heme *b*	Heme *c*
Shaking	0.28	0.79	0.36	0.76	0.66	5.0	0.066	0.24	0.60	0.12	0.55	0.41
Static	0.23	0.45	0.18	0.48	0.29	1.2	0.028	0.25	0.60	ND[b]	0.51	0.44

[a] All oxidase activites were measured as described and are expressed as micromoles of substrate oxidized per minute per milligram of protein. Contents of CO-binding cytochrome (CO-cyt.), cytochrome *c* (Cyt. *c*), and hemes were determined as described in Materials and Methods.
[b] ND, not detected.

with membranes from both the shaking and static cultures were calculated to be 74 and 38 μM, respectively.

Effect of partial solubilization of the membranes with Triton X-100 on the respiratory chain of *A. aceti*. As mentioned above, *A. aceti* exhibits no cytochrome *c* oxidase activity but does exhibit ubiquinol oxidase activity as the terminal oxidase in spite of having relatively high levels of cytochrome *c* in the respiratory chain. By analogy to the respiratory chain of *G. suboxydans* (3, 9), this can be explained by the notion that cytochrome *c* is functioning only in the segments preceding ubiquinone, i.e., primary dehydrogenases. Actually, it is known that alcohol dehydrogenase and aldehyde dehydrogenase, the primary dehydrogenases of ethanol and aldehyde oxidase respiratory chains of *A. aceti*, contain 3 and 1 mol, respectively, of cytochrome *c* (1, 2). Since the alcohol dehydrogenase can be solubilized with relatively low concentrations of detergent (1), we tried to fractionate the membranes with 0.3% Triton X-100 to selectively delete cytochrome *c* from the respiratory chain of *A. aceti*. The detergent at this concentration seemed enough to solubilize only cytochrome *c* but not cytochromes *b* and *a*, since the low-temperature difference spectra showed only two α peaks at 548 and 551 nm in the solubilized supernatants of the membranes prepared from both types of cells while the spectra of the residual membranes exhibited peaks at 556 and 562 nm and, in the case of the shaking culture, an additional peak at 587 nm with KCN (Fig. 5). The solubilization of the membranes prepared from the shaking culture is summarized in Table 2. As shown, almost all cytochrome *c* was solubilized from the membranes, while almost all terminal ubiquinol oxidase together with heme *b* and heme *a* moieties were retained in the membrane residues. Furthermore, almost all alcohol dehydrogenase activity was solubilized from the membranes, which led to a total defect of ethanol oxidase activity. In contrast, fair amounts of glucose dehydrogenase were retained in the solubilized membrane residues, which also exhibited a relatively high glucose oxidase activity. Thus, glucose oxidase system at least does not require any cytochrome *c* in the respiratory chain. Although data are not shown, no striking difference in the solubilization of the membranes from the static culture could be seen.

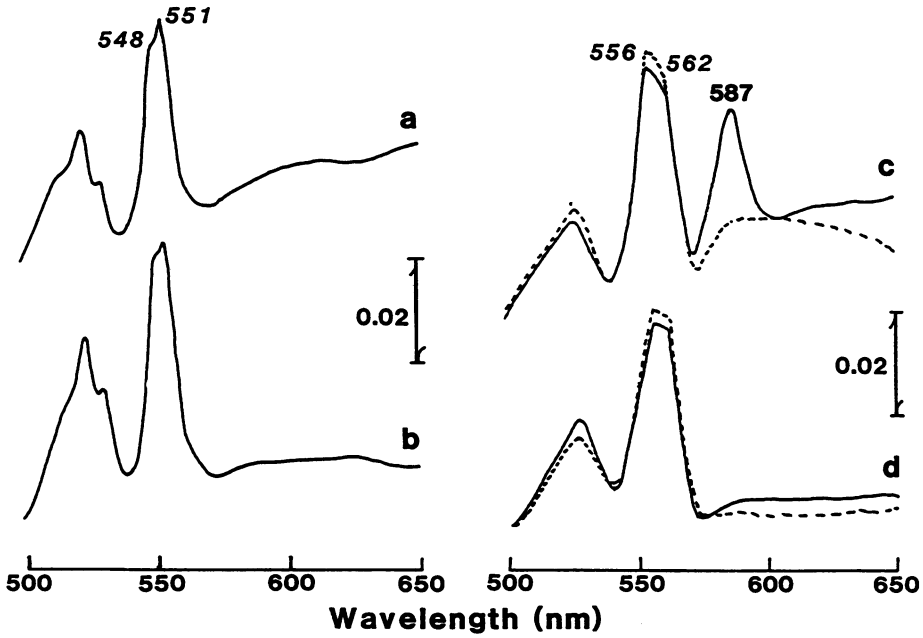

FIG. 5. Low-temperature reduced-minus-oxidized difference spectra of Triton X-100-solubilized supernatants and the residual membranes. Membranes prepared from *A. aceti* cells grown on shaking (a and c) and static (b and d) cultures were solubilized with 0.3% Triton X-100, as described in Materials and Methods. Low-temperature redox spectra were taken with the supernatants (a and b) at protein concentrations of 5.0 and 6.2 mg/ml, respectively, and with the residual membranes (c and d), in the presence (solid lines) and absence (dashed lines) of cyanide, at protein concentrations of 9.6 and 16.3 mg/ml, respectively.

TABLE 2. Separation of enzyme activities and cytochrome components from the membranes of *A. aceti* by solubilization with Triton X-100[a]

Fraction	Protein (mg)	Dehydrogenase[b]		Oxidase[b]			Content of[c]:			
		Glucose	Ethanol	Glucose	Ethanol	Q_2H_2	CO-cyt.	Heme *a*	Heme *b*	Heme *c*
Membrane	68.3	22.8 (0.34)	165 (2.40)	18.8 (0.28)	51.0 (0.76)	219.0 (3.3)	12.2 (0.18)	10.3 (0.15)	40.0 (0.59)	26.0 (0.39)
Supernatant	5.8	3.0 (0.51)	132 (22.8)	1.0 (0.17)	0.7 (0.11)	1.1 (0.2)	ND	ND	ND	30.0 (5.10)
Precipitate	48.8	14.7 (0.30)	5.8 (0.10)	14.0 (0.23)	1.7 (0.03)	146.0 (3.0)	13.6 (0.28)	11.0 (0.20)	31.0 (0.63)	ND

[a] Membranes were prepared from the cells grown on shaking culture and solubilized with 0.3% Triton X-100 as described in Materials and Methods. Enzyme activities and cytochrome contents were determined as described in Table 1.
[b] Total activities (units) of each enzyme are shown, and the specific activities (units per milligram of protein) are shown in parentheses.
[c] Total contents (nanomoles) of CO-binding cytochrome (CO-cyt.) and hemes are shown, with the specific content (nanomoles per milligram of protein) in parentheses. ND, not detected.

Alteration in cytochrome components by the change of the culture condition from static to shaking. To see how *A. aceti* regulates the synthesis of the cytochrome that exhibits an α peak at 587 nm in the presence of KCN and may contain heme *a* moiety, the appearance of the cytochrome was examined by changing the culture conditions from static to shaking. In these experiments, a fresh medium (100 ml) was inoculated with 5 ml of static culture and cultivated with shaking for 24 h. Then 5 ml of this broth was transferred to 100 ml of another fresh medium, and it was cultivated with shaking for another 24 h. The shaking culture was carried out five times. To count the viable cells and also to see the colony state during cultivation, each culture was spread on a potato agar plate. As shown in Fig. 6, two different colonies were observed in the shaking culture of *A. aceti*. Interestingly, almost all cells on the static culture formed a rough-surfaced colony, while others formed smooth-surfaced ones; then the cells forming a smooth-surfaced colony increased gradually during the repeated shaking culture, finally predominating after five repetitions (Fig. 7). At the same time, low-temperature difference spectra and CO difference spectra were measured with the membranes prepared from the cells of each shaking culture. As shown in Fig. 8, the peak at 587 nm in the presence of KCN does not simply appear by a single shaking culture but gradually increases by repeating the shaking culture. Parallel to the change, the peak and the trough in the CO difference spectra shifted gradually from 415 to 420 nm and from 430 to 440 nm, respectively. Thus, the results indicate that the spectral change in the terminal oxidase of each culture may correspond to the change in the rate of rough- and smooth-surfaced colonies.

DISCUSSION

It is well known that several *Acetobacter* species have an ability to float on the surface of a static culture, on which they form a pellicle in order to maintain the floating state. Such characteristics are observed most remarkably with *Acetobacter xylinum*, for which the cellulose biosynthesis involved in the pellicle formation has been extensively studied (for a review, see reference 18). However, the meaning of such floating and pellicle formation seen on static cultures of *Acetobacter* species remains to be clarified from a biochemical point of view.

A. aceti used in this study is able to grow well on static culture as well as on normal shaking culture. Thus, two types of cells were obtained from shaking and static cultures of *A. aceti*, and the cytochrome components and enzyme activities of each cell type and its membrane fraction were compared in this study. Although both types of cells exhibited different cytochrome spectra, data obtained here suggest that *A. aceti* has almost the same respiratory chain, except for the terminal oxidase, regardless of the culture conditions. As shown in this study, the respiratory chain of *A. aceti* looks peculiar, similar to that of *G. suboxydans* (3), since it has a ubiquinol oxidase activity but not cytochrome *c* oxidase activity as the terminal oxidase in spite of having cytochrome *c*. To see how the respiratory chain of *A. aceti* is organized, therefore, the components in the membrane-bound respiratory chain were fractionated with Triton X-100. As shown in Table 2, almost all cytochrome *c* and alcohol dehydrogenase was solubilized from the membranes with a relatively low concentration of the detergent, which led to a total defect of ethanol oxidase activity. However, the solubilized membrane residues retained almost all glucose oxidase activity besides ubiquinol oxidase activity, irrespective of depletion of cytochrome *c* from the membrane. Furthermore, recently it has been shown that alcohol dehydrogenase of *G. suboxydans* or *A. aceti* (unpublished data) and glucose dehydrogenase of *G. suboxydans* (12) are able to react directly with ubiquinone in a phospholipid bilayer. Therefore, it is conceivable that cytochromes *c* of *A. aceti* are not electron donors to the terminal oxidase but constituents of alcohol and aldehyde dehydrogenases in the

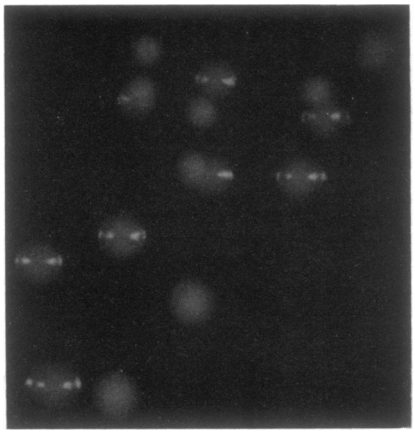

FIG. 6. Rough- and smooth-surfaced colonies which appeared during repeated shaking cultures of *A. aceti*. Five milliliters of static culture with *A. aceti* were transferred to 100 ml of fresh medium prepared as described in Materials and Methods, and it was incubated with shaking for 24 h. The 5 ml of the shaking culture was further inoculated into another 100 ml of fresh medium and incubated with shaking for 24 h. The culture was diluted and spread on a plate prepared with the same composition as the agar slant, whose composition was described in Materials and Methods, except that $CaCO_3$ was not added.

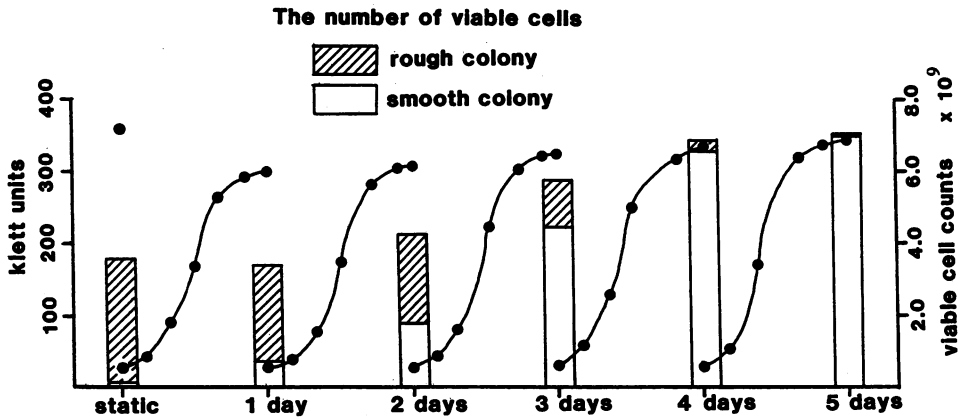

FIG. 7. Growth curve and viable cell count of repeated shaking cultures of *A. aceti*. Shaking culture was repeated five times as described in the legend to Fig. 6, and the cell growth was monitored by using Klett units (●). Then, at the end of each cultivation, each culture was spread on an agar-potato plate in order to count viable cells. The cell numbers of rough- and smooth-surfaced colonies were counted separately.

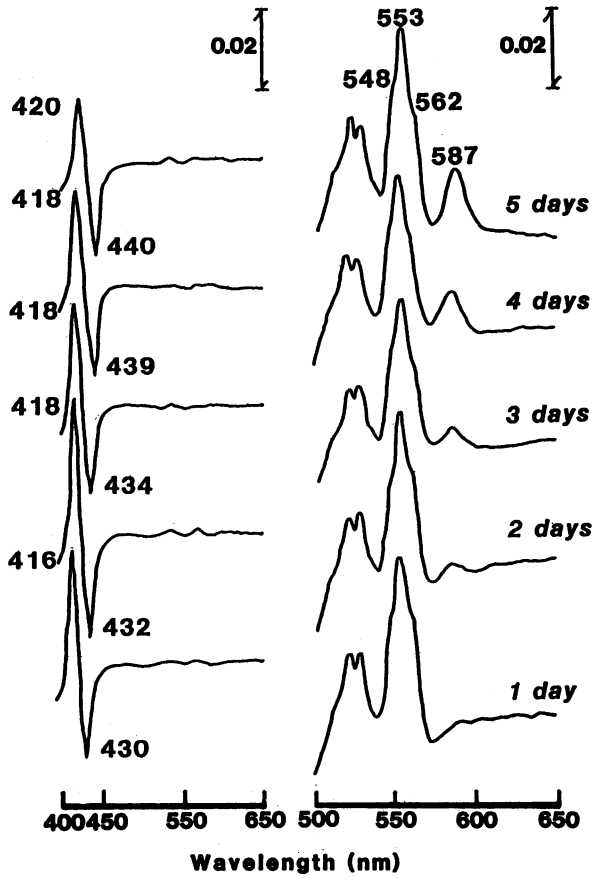

FIG. 8. Low-temperature reduced-minus-oxidized difference spectra and CO reduced-minus-reduced difference spectra of the membranes from each cell type of *A. aceti* during repeated shaking culture. Static culture of *A. aceti* was inoculated into fresh medium and the shaking culture was repeated five times (5 days) as described in the legends to Fig. 6 and 7. Low-temperature redox spectra in the presence of KCN and CO difference spectra were taken with the membranes prepared from the cells of each repeated shaking culture. Protein concentrations of low-temperature redox and CO difference spectra were adjusted at about 4.0 and 1.0 mg of protein per ml, respectively.

respiratory chain. Thus, the respiratory chain of *A. aceti* is constituted of primary dehydrogenases, ubiquinone (Q_9), and terminal oxidase(s), which is remarkably similar to the respiratory chain of *G. suboxydans* (3, 9), which belongs to another genus of acetic acid bacteria.

The data presented here indicate that the terminal oxidase of *A. aceti* changes from *b*-type or *a*-type cytochromes depending on the culture conditions, static or shaking cultures, respectively. The terminal oxidase present in the shaking culture can be concluded to be cytochrome a_1 on the basis of the following observations. (i) The membranes show the redox spectrum of an α peak at 587 nm in the presence of KCN, which is consistent with the earlier findings with *A. pasteurianus* (for a review, see references 15 and 16). (ii) The CO difference spectrum shows a peak at 420 nm and a trough at 440 nm, suggesting the presence of *a*-type cytochrome reacting with CO. (iii) The membranes contain heme *a* besides hemes *b* and *c*. (iv) Finally, cytochrome a_1 has been purified from the membranes of a shaking culture of *A. aceti* and characterized to be cytochrome *ba* functioning as ubiquinol oxidase (13). On the other hand, the terminal oxidase of the static culture could be cytochrome *o* judging from the CO difference spectrum exhibiting a peak at 415 nm and a trough at 430 nm and also the presence of only heme *b* in the Triton X-100-washed membranes retaining ubiquinol oxidase activity. This cytochrome *o* has also been purified and shown to be a typical cytochrome *bo* ubiquinol oxidase (unpublished data). Thus, the respiratory chain of *A. aceti* can be depicted as shown in Fig. 9, in which the terminal oxidase can be changed from cytochrome a_1 to cytochrome *o* by the change of the culture conditions from shaking to static.

The exchange mechanism of the terminal oxidase was also examined preliminarily in this study. In the static culture, after inoculation of preculture, on which a large number of cells are unable to float and to grow rapidly but on which some are able to float and to form a pellicle, cytochrome a_1 is gradually decreased and cytochrome *o* appears. Conversely, when the static culture is transferred to a shaking culture, no change of cytochrome components is detected after a single culture, but it is produced by repeating the shaking culture, by which cytochrome *o* is decreased but cytochrome a_1 is increased. Thus, the terminal oxidase component may not be changed simply from cytochrome a_1

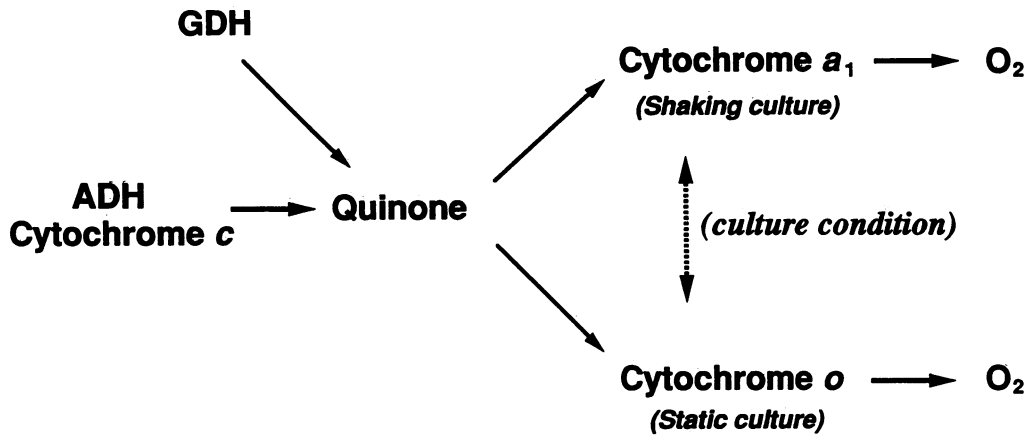

FIG. 9. Schematic diagram of the respiratory chain of *A. aceti*. Alcohol dehydrogenase (ADH) containing cytochrome *c* and glucose dehydrogenase (GDH) are shown to donate electrons directly to ubiquinone (Q_9), and cytochrome a_1 and cytochrome *o* are shown to oxidize ubiquinol directly.

to cytochrome *o* by the change of culture conditions from shaking to static and vice versa. Data obtained in this study suggest that there are two different types of cells, one of which forms a smooth-surfaced colony and one of which forms a rough-surfaced colony, and that the cells forming the rough-surfaced colony may grow to form a pellicle on the surface of static culture, while cells forming the smooth-surfaced colony may not grow on the static culture but predominate on the shaking culture. Since the change of the colony state seems to be parallel to the change of the terminal oxidase, the cells able to grow on the static culture may produce cytochrome *o* as the terminal oxidase while the cells grown only on the shaking culture may produce cytochrome a_1. Although data are not shown, the notion described above has been confirmed with the isolated rough-surfaced and smooth-surfaced colonies. Since each colony seems not to be interconvertible after isolation as tested so far, one may speculate that the culture of *A. aceti* can be contaminated with some other strain or contain mutant cells of the wild-type strain. The former possibility can be easily ruled out by the following observations. (i) There is no remarkable difference in ethanol and acetaldehyde oxidase respiratory activities, which are characteristic of acetic acid bacteria, among both types of cells and the original culture. (ii) Both types of cells are able to grow as much the same as the original culture in the presence of 1% acetic acid (data not shown), and such an acetic acid resistance is characteristic for *Acetobacter* species. (iii) The membranes prepared from both types of cells have an antigen that cross-reacts at the same level with the antibody raised against alcohol dehydrogenase purified from the original culture (unpublished data). Furthermore, such a colony change is not so surprising among *Acetobacter* species. The kind of colony change observed in this study has already been obtained in an industrial application of bacterial cellulose production by *Acetobacter* species; the cells grown on static culture segregate into two types, one of which forms a rough-type colony and shows higher production of cellulosic material than the other, which forms a smooth-type colony (5). It has also been reported that laboratory maintenance of *Acetobacter* species by serial transfer gives rise to different "species" (19). Thus, *Acetobacter* species appear to be easily changed by a certain adaptation to environmental conditions. Although the mechanism of such a colony change remains unsolved to date, the occurrence of two types of *A. aceti* in the static and shaking cultures would result from spontaneous mutation or a related phenomenon adapted to the culture condition. If that is the case, how does the change of colony situation affect the change of terminal oxidase in *A. aceti*? There are mainly two possibilities to be considered: (i) some mutation occurs at the gene sites, correlating to biosynthesis of both the cellulosic material and the terminal oxidase, or (ii) cellulosic material coating the cells may create specific environmental conditions which may regulate expression of the terminal oxidase, e.g., by affecting the ability of nutrients or dissolved oxygen to permeate.

Thus, it is reasonably explained that *A. aceti* cells able to form cellulosic material and therefore able to grow on static culture produce cytochrome *o*, while the cells grown only on shaking culture produce cytochrome a_1 as the terminal oxidase. The exchange mechanism of the terminal oxidase is now under investigation in relation to the elucidation of the floating ability of two different types of *A. aceti*.

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