Loss of Cytochrome C Oxidase Activity and Acquisition of Resistance to Quinone Analogs in a Laccase-Positive Variant of Azospirillum lipoferum

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Laccase, a p-diphenol oxidase typical of plants and fungi, has been found recently in a proteobacterium, Azospirillum lipoferum. Laccase activity was detected in both a natural isolate and an in vitro-obtained phase variant that originated from the laccase-negative wild type. In this study, the electron transport systems of the laccase-positive variant and its parental laccase-negative forms were compared. During exponential (but not stationary) growth under fully aerobic (but not under microaerobic) conditions, the laccase-positive variant lost a respiratory branch that is terminated in a cytochrome c oxidase of the aa-type: this was most likely due to a defect in the biosynthesis of a heme component essential for the oxidase. The laccase-positive variant was significantly less sensitive to the inhibitory action of quinone analogs and fully resistant to inhibitors of the bc1 complex, apparently due to the rearrangements of its respiratory system. We propose that the loss of the cytochrome c oxidase-containing branch in the variant is an adaptive strategy to the presence of intracellular oxidized quinones, the products of laccase activity.

Polyphenol oxidases are a diverse group of copper proteins that catalyze oxidation of aromatic compounds by molecular oxygen. According to the substrate specificity, two classes of phenol oxidases are recognized: tyrosinasess and laccases. Tyrosinases have monophenol monoxygenase (EC 1.14.16.2) and o-diphenol:oxygen-oxidoreductase (EC 1.10.3.1) activity and are widely distributed throughout the phylogenetic tree, from bacteria to mammals. Laccases have p-diphenol:oxygen-oxidoreductase (EC 1.10.3.2) activity and have been found exclusively in fungi and plants (44). In contrast to current understanding the redox biochemistry (34, 51) and the structure of laccases (10), relatively little is known of their physiological functions. Laccases play a key role in morphogenesis, development, and lignin metabolism in fungi and plants (22, 31, 44) and are virulence-associated factors in pathogenic fungi (49). Laccases are implicated in the biodegradation of a variety of toxic organic pollutants (4) and thus are potential bioremediation agents.

Recently, laccase activity has been demonstrated in an atypical isolate of the proteobacterium Azospirillum lipoferum (17). Azospirillum spp. are plant root-associated bacteria that stimulate the growth and development of many agriculturally important crops (29). Although laccase has been the subject of study for more than 100 years and the enzyme has been identified in a wide variety of plant and fungal species (44), A. lipoferum remains one of only two prokaryotic organisms in which laccase activity has been demonstrated (36). However, evidence has been obtained that laccases may be widespread in bacteria. Using similarity searches with known plant and fungal laccase gene sequences, we have identified putative laccase genes in several completely and partially sequenced genomes of α- and γ-proteobacteria (3). By initializing the oxidation of plant phenolic compounds (12, 13), the laccase may provide an obvious advantage to plant-associated Azospirillum cells. The physiological role of the enzyme in other bacterial species remains to be seen.

Oxidizing aromatic substrates, laccase generates reactive species, such as semiquinones and quinones, that are powerful inhibitors of the electron transport system in both bacteria (5, 20) and mitochondria (11). It appears that plants and fungi circumvent the problem: where it is known, laccases are extracellular enzymes. In contrast, the A. lipoferum enzyme is located intracellularly (13). Its chemical properties are similar to those of fungal laccases (12). For example, phenolic compounds of the syringic type (aldehyde, acid, and acetophenone) that are typical of plant tissues and exudates are oxidized by the laccase to 2,6-dimethoxy-1,4-benzoquinone (13). In Escherichia coli, derivatives of 1,4-benzoquinone inhibit respiration at concentrations as low as 1 μM by competing for electrons with ubiquinone of the electron transport system (5). How do bacterial cells cope with the intracellular presence of laccase and its toxic by-products? We hypothesized that one way in which the laccase-positive cells adapt to endogenous substituted quinones is by rearranging the electron transport system.

In this study, we compared the arrangement of the electron transport system and its sensitivity to substituted quinones in the laccase-positive variant of A. lipoferum (4VII) and laccase-negative parental forms (4B and 4V). The laccase-positive variant 4VII emerges from a typical laccase-negative strain 4B via a two-step phase-variation-like process, with atypical laccase-negative variant 4V being an intermediate form (2).

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** A. lipoferum wild-type strain 4B (laccase negative) and its variants 4V (laccase negative, atypical) and 4VII
(laccase positive, atypical) (2) were used in this study. The bacteria were grown in 1-liter Erlenmeyer flasks containing 200 ml of tryptone-yeast extract medium at 30°C. Flasks were incubated on a rotary shaker to achieve either fully aerobic (240 rpm) or microaerobic (100 rpm) conditions. For analysis of the respiratory system, cells were harvested during the exponential growth phase (optical density at 600 nm [OD600] = 0.5 to 0.7 for aerobic cultures and 0.3 to 0.4 for microaerobic cultures) or the stationary phase (OD600 = 1.4 to 1.8 for aerobic cultures and 0.6 to 0.7 for microaerobic cultures). Wild-type Bacillus subtilis GO1085 (47) and a cyd mutant of E. coli GO1013 (7) were grown inuria-Bertani broth to the mid-exponential growth phase. Wild-type E. coli MM355 (6) cells were grown to the stationary phase to achieve a maximal content of cytochrome d.

Pentapeptide 

Pentapeptide oxygen consumption in bacterial suspensions was measured by using a Clark-type electrode and an oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). The monitor output was collected in a channel of the MacLab data recording system (Model MK-III; Analog Digital Instruments, Boston, Mass.). The data collected were analyzed and stored in a Macintosh computer by using Chart (version 3.3) software (Analog Digital Instruments). For respiratory measurements, cells were washed twice in 50 mM potassium phosphate buffer (pH 7.0) and suspended in the same buffer containing 1 mM transport inhibitors and specific electron donors revealed both cytochrome c oxidases and cytochrome b oxidase (45). Myxothiazol only slightly inhibited respiration of A. lipoferum 4B cells. Maximal inhibition (respiration rate, 1.7 \times 10^{-10} \text{ mol of O}_2 \text{ per min per cell}) was achieved with 100 \mu M myxothiazol. Addition of ascorbate and N,N,N',N'-tetramethyl-p-phenylenediamine (TMPP; 500 and 250 \mu M, respectively) that donate electrons directly to cytochrome c, bypassing the bc complex, significantly increased the oxygen consumption (respiration rate, 2.6 \times 10^{-10} \text{ mol of O}_2 \text{ per min per cell}). Low concentrations of KCN (10 \mu M) that completely inhibited cytochrome c oxidases inhibited respiration only by 20\% (respiration rate, 1.7 \times 10^{-10} \text{ mol of O}_2 \text{ per min per cell}). A quinone analog 2-hp(4-hydroxyquinoline N-oxide (40 \mu M) inhibited respiration in A. lipoferum 4B by 80\% (respiration rate, 0.4 \times 10^{-10} \text{ mol of O}_2 \text{ per min per cell}), and 1 mM KCN inhibited respiration by 85\% (respiration rate, 0.25 \times 10^{-10} \text{ mol of O}_2 \text{ per min per cell}). Taken together, the results are consistent with the presence of the cytochrome c terminated branch and also indicate that quinol oxidase(s) account for most of respiratory activity in aerobically grown A. lipoferum.

Respiration rates of the 4V and 4VII variants were similar to that of the 4B strain (1.9 and 2.2 \times 10^{-10} \text{ mol of O}_2 \text{ per cell, respectively}, in the mid-exponential phase under full aeration). Inhibitory respiratory analysis revealed no significant difference between laccase-negative cells of the 4B strain and the 4V variant. However, respiration in the aerobically grown laccase-positive 4V variant in the mid-exponential phase was completely insensitive to myxothiazol (100 \mu M) and not stimulated by ascorbate plus TMPP (500 and 250 \mu M, respectively), indicating the absence of the bc complex and cytochrome c oxidase for 4V variant. The shoulder at 500 nm attributed to 1 complex, significantly increased the respiration of A. lipoferum (Table 1). The two \alpha peaks at 558 and 562 nm and
the β peak at 528 nm are indicative of b-type cytochromes. An α peak at 552 nm and a β peak at 522 nm are indicative of c-type cytochromes. The highly asymmetric pattern of the α and β regions, typical of the cbb-type cytochrome c oxidases (16, 21, 25, 35, 43), has never been observed in membrane preparations from A. lipoferum. These results indicate that (i) the major cytochrome c oxidase in A. lipoferum is most likely of the aa₃ and not the cbb type and that (ii) the cells utilize different quinol oxidases. CO-bound reduced-minus-reduced absorption spectra confirmed this conclusion (Fig. 2A). First, the trough at 562 nm is indicative of the bo (bb)-type oxidase. Second, the peak at 595 nm and the troughs at 447 and 608 nm (shifts from the peaks of 441 and 601 nm on the reduced-minus-oxidized spectra, respectively) are indicative of the cytochrome oxidase of the aa₃ type.

No differences between the wild-type and laccase-negative 4V1 cells were observed in the difference spectra under any experimental conditions (Fig. 1A to D). A major difference between the wild-type and the laccase-positive 4VII variant cells was observed for the aerobically grown cells at the expo-
wild-type cells, whereas cytochrome positive 4VII variant cells grown exponentially under full aeration appeared under other experimental conditions (Fig. 1B to C). Cells were grown exponentially under microaerobic conditions (Fig. 3C).

Principal differences between the wild-type and 4VII cells were observed under other experimental conditions (Fig. 1A), raising the possibility that there is more than one heme A-containing cytochrome in A. lipoferum. No principal differences between the wild-type and 4VII cells were observed under other experimental conditions (Fig. 1B to C). CO-bound reduced-minus-reduced absorption spectra also revealed a difference between the wild-type and the laccase-positive 4VII variant cells grown exponentially under full aeration (Fig. 2), whereas no difference was observed under other growth conditions (data not shown). The peak at 595 nm and the trough at 608 nm were present in the 4VII variant, confirming the absence of the aa3-type cytochrome in the latter. However, the trough at 447 nm was present in both 4B and 4VII cells (Fig. 2), suggesting the presence of the second heme A-containing terminal oxidase, which has low absorption in the alpha band.

**Heme composition.** All of the membrane preparations from A. lipoferum for which differential spectroscopy analysis has been performed (see above) were subjected to heme analysis. First, the control membranes isolated from B. subtilis (containing hemes B and A), E. coli stationary-phase wild-type cells (containing hemes B and D), and E. coli cytochrome d mutant cells (containing hemes B and O) were analyzed to determine the retention times for known heme compounds (Table 1). Comparative analysis using membrane fractions from wild type A. lipoferum cells grown under different experimental conditions (described in the previous section) revealed the presence of known and unknown heme compounds. Heme B was present abundantly in all fractions, and traces of hemes A and D were also detected (Fig. 3A to D). No evidence for heme O has been found. Therefore, the oxidase identified on the CO difference spectra (the trough at 562 nm on Fig. 2) is likely that of a bb type and not a bo-type cytochrome. Interestingly, three unknown heme compounds were identified in wild-type A. lipoferum that were designated R1, R2, and R3 according to their retention times (Table 1). Apart from heme B, which was present abundantly in all fractions, heme R3 appears to be a dominant component under all conditions tested. Heme R1 was present only under fully aerobic conditions (Fig. 3A and C), and heme R2 was present only under very low-oxygen concentrations (stationary-phase, microaerobic conditions) (Fig. 3D). Heme R1 had a slightly higher hydrophobicity than heme B and was predicted to be a modified heme B. Hemes R2 and R3 had higher hydrophobicity than hemes A and O.

In order to characterize further the unknown heme species, the absolute absorption spectra of individual heme compounds were recorded by using a photodiode array detector (Fig. 4). The spectra confirmed potential relatedness of heme R1 to heme B, and hemes R2 and R3 to heme A based on the maximal absorption peak in the Soret region and a similar pattern of the alpha band. For example, heme R1 had a maximal absorption peak at 397 nm (Fig. 4C); heme B isolated from B. subtilis (Fig. 4A) and A. lipoferum (Fig. 4B) had exactly the same maximal peak. However, both heme R2 (Fig. 4G) and heme R3 (Fig. 4F) had the maximal absorption peak at 406 nm, which is characteristic of heme A isolated from B. subtilis (Fig. 4D) and A. lipoferum (Fig. 4E).

No differences between the laccase-negative 4B and 4VII cells were observed in heme profiles under any experimental conditions (Fig. 3A to D). A major difference between the laccase-negative forms and the laccase-positive 4VII variant was observed for the cells in the exponential phase under full aeration. Two heme species typical of these experimental conditions, namely, R3 and R1, were absent in the 4VII cells, whereas the amount of heme A drastically increased in comparison with laccase-negative wild-type and 4VII cells (Fig. 3A). Also, heme R1, which was present in laccase-negative parental strains only under fully aerobic conditions, appeared in the 4VII variant cells grown exponentially under microaerobic conditions (Fig. 3C).

**Inhibitory effects of substituted quinones on the respiratory system.** Substituted quinones that pass freely across the cytoplasmic membrane are known to be strong respiratory inhibitors for bacteria (5, 20). On the other hand, substituted quinones are products of laccase activity in A. lipoferum (13). The laccase-positive variant apparently lost the cytochrome c oxidase terminated branch of electron transport, which is known to be the most sensitive to respiratory inhibitors in different bacterial species (45). We hypothesized that laccase-positive variant is less sensitive to the inhibitory effect of oxidized quinones. The sensitivity of respiration to substituted quinones of different reduction potentials was tested in the laccase-positive variant and its parental forms. Substituted quinones of high reduction potential decreased the respiration in the strain 4B and the laccase-negative variant 4V, but to a significantly lesser extent in the laccase-positive variant 4VII (Table 2). The 4VII cells were two times more resistant to the most potent inhibitor, 1,4-benzoquinone, than the parental strains. The laccase-positive variant was practically insensitive to the low-reduction-potential quinones at concentrations that caused a sta-
tistically significant inhibitory effect on the laccase-negative strains. As expected (5), there was a direct correlation between the inhibitory effect and the reduction potential (electron affinity) of the quinone: the higher the reduction potential, the more toxic the quinone.

FIG. 3. Reversed-phase HPLC chromatograms of the noncovalently membrane-bound hemes from laccase-negative (4B and 4V I) and laccase-positive (4V II) A. lipoferum. (A) Fully aerated exponentially grown cells. (B) Fully aerated cells, stationary phase. (C) Low-aerated exponentially grown cells. (D) Low-aerated cells, stationary phase. Bacterial cultures: 1, 4B; 2, 4V I; 3, 4V II. Positions of peaks corresponding to known (D, B, and A) and unknown (R1, R2, and R3) heme species are indicated.

DISCUSSION

Prior to this investigation, no information was available on the electron transport system of A. lipoferum. Therefore, our first goal was to obtain sufficient data on the arrangement of
the respiratory system in *A. lipoferum* in order to reveal possible differences in laccase-positive and laccase-negative variants. Previous spectral and polarographic studies suggested the presence of cytochrome aa₃, cytochrome d, and cytochrome o terminal oxidases in *Azospirillum brasilense*, the closest relative of *A. lipoferum* (33). Recent investigation provided genetic and biochemical evidence for the presence of an alternative (cbb₃) cytochrome c oxidase in *A. brasilense* (25). The data obtained during inhibitory respiratory analysis, difference absorption spectroscopy, and heme composition analysis suggest a general scheme for the arrangement of the respiratory system in *A. lipoferum* 4B and its variants 4V and 4V₉ (Fig. 5). Both cytochrome c oxidase and quinol oxidase activities were detected. We refer to the major cytochrome c oxidase of *A. lipoferum* as the aa₃ type based on the spectral characteristics typical of this oxidase. However, without detailed mechanistic studies of the enzyme (which are beyond the scope of this investigation) it is impossible to determine its exact nature. Although the spectral studies did not indicate the presence of the cbb₃-type oxidase in *A. lipoferum*, whereas the cbb₃ signature peaks are present in similar membrane preparations from *A. brasilense* (25), we cannot rule out the presence of an alternative cytochrome c oxidase. The major cytochrome c oxidase terminated branch was present in wild-type *A. lipoferum* 4B and in its laccase-negative variant 4V, under all growth conditions tested, a result consistent with the notion that it is the most efficient energy generating pathway in bacteria (46). However, it is unusual that the bacteria appear to use this respiratory branch under microaerobic conditions in the stationary stage of growth, in which most species it is not active. In addition to the cytochrome c oxidase terminated branch, *A. lipoferum* contains quinol oxidases that account for myxothiazol- and cyanide-resistant respiration. Carbon monoxide difference spectra and heme analysis suggest that several quinol oxidases, including those of the bb and ba types, may be present in *A. lipoferum*.

Only four types of noncovalently bound hemes are usually found in bacteria: heme B (protoheme IX) and its derivatives heme D, heme O, and heme A (15, 42). Archaeabacteria have prenylated hemes, which are modifications of hemes A and O, as components of their terminal cytochromes (23, 24). *A. lipoferum* appears to have three known hemes (B, D, and A) and three unknown hemes, designated R1, R2, and R3 (Table 1). Heme R1 is predicted to be a modification of heme B, whereas hemes R2 and R3 are predicted to be modifications of heme A. The following lines of evidence support our suggestion that compounds designated R1, R2, and R3 are indeed heme components of the respiratory complexes. First, the method of Sone and Fujiwara (38), which was used for heme extraction in our study, has been applied to different microbial species and always yielded no compounds other than noncovalently bound hemes of the respiratory complexes. Second, the presence of at least two novel heme species on the HPLC profiles correlated with the presence of characteristic absorption peaks on the reduced-minus-oxidized spectra obtained from the same membrane preparations. Heme R2 correlated with the 630-nm peak and heme R3 correlated with the 601-nm peak (compare Fig. 1A to D and Fig. 3A to D). Finally, the absolute absorption spectra of R1 and of R2 and R3 compounds were similar to those of heme B and heme A, respectively (Fig. 4).

We have not observed any difference in the respiratory metabolism between the laccase-negative wild-type 4B and the 4V variant under any experimental conditions, although various changes in carbohydrate and secondary metabolism were observed in the 4V variant (2). However, we found dramatic differences between the laccase-negative parental forms and the laccase-positive 4V₉ variant. These changes occurred only under specific growth conditions. During exponential growth under full aeration, the laccase-positive variant cells lost the cytochrome aa₃ oxidase activity, most likely, due to inability to synthesize a heme component essential for the oxidase, and we propose that the novel heme R3 is such a component. The following lines of experimental evidence support this hypothesis.

(i) The absence of the cytochrome c oxidase of the aa₃-type is obvious from the loss of a 601-nm peak from the reduced-minus-oxidized spectrum of the 4V₉ variant grown exponentially under high aeration (Fig. 1A). This peak is characteristic of the high-spin heme A in aa₃-type oxidases (30). Similarly, the peak at 595 nm and the trough at 608 nm that are indicative of the oxidase were lost from the carbon monoxide spectrum of the 4V₉ variant grown under the same conditions (Fig. 2B). Loss of the cytochrome c oxidase activity in the variant grown exponentially under high aeration was also confirmed by respiratory analysis.

(ii) Heme R3 is the only heme compound whose presence or absence in the HPLC profiles was coincident with that of the aa₃-oxidase characteristic (601-nm) peak on the reduced-minus-oxidized spectra of the same membrane preparations (compare Fig. 1A to D and Fig. 3A to D).

(iii) Large amounts of unmodified heme A in the laccase-positive variant cells were detected only under exponential growth under full aeration (Fig. 3B), where aa₃-type oxidase signature peaks are missing from the reduced-minus-oxidized spectra (601 nm, Fig. 1A) and carbon monoxide spectra (peak at 595 and trough at 608 nm, Fig. 2B). This indicates that unmodified heme A is not a component of the aa₃-type cytochrome c oxidase. Most likely, unmodified heme A is a component of another a-type terminal cytochrome (apparently, a quinol oxidase), which is present both in wild type and, to a greater extent (as judged by the difference absorption spectra on Fig. 1A and Fig. 2), in the 4V₉ variant. This oxidase accounts for the presence of the 441 peak on the reduced-minus-oxidized spectra (Fig. 1A) and the corresponding trough (447 nm) on the carbon monoxide spectra (Fig. 2B) in the cells lacking cytochrome c oxidase of the aa₃ type.

(iv) Heme R3 has a higher hydrophobicity than unmodified heme A (Fig. 3); however, its spectral characteristics (absolute absorption spectra) are very similar to those of heme A (Fig. 4D to F), suggesting that R3 is a modified heme A.

Furthermore, our results are supported by previous findings that a modified (prenylated) heme A can substitute heme A as a component of both a cytochrome c oxidase of the aa₃ type and the ba₃ quinol oxidase in *Archeae* and *Bacteria* (23, 24). Spectral characteristics of the aa₃-type cytochromes that contain a modified heme A and unmodified heme A are similar.

<table>
<thead>
<tr>
<th>Heme</th>
<th>Organism</th>
<th>Mean retention time (min) ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td><em>E. coli</em>, <em>A. lipoferum</em></td>
<td>17.1 ± 0.6 (15)</td>
</tr>
<tr>
<td>B</td>
<td><em>E. coli</em>, <em>B. subtilis</em>, <em>A. lipoferum</em></td>
<td>21.1 ± 0.3 (18)</td>
</tr>
<tr>
<td>R1</td>
<td><em>A. lipoferum</em></td>
<td>24.8 ± 0.3 (5)</td>
</tr>
<tr>
<td>A</td>
<td><em>B. subtilis</em>, <em>A. lipoferum</em></td>
<td>29.9 ± 0.4 (14)</td>
</tr>
<tr>
<td>O</td>
<td><em>E. coli</em></td>
<td>31.9 ± 0.3 (3)</td>
</tr>
<tr>
<td>R2</td>
<td><em>A. lipoferum</em></td>
<td>34.7 ± 0.2 (3)</td>
</tr>
<tr>
<td>R3</td>
<td><em>A. lipoferum</em></td>
<td>36.4 ± 0.4 (11)</td>
</tr>
</tbody>
</table>

*The results shown are the mean ± standard deviation as calculated from several (number in parentheses) independent experiments.*
FIG. 4. Absolute visible absorption spectra of hemes from control and *A. lipoferum* membranes in acetonitrile solutions obtained by using a photodiode array detector. Heme compounds: heme B from *B. subtilis* (A) and *A. lipoferum* 4B (B), heme R1 from *A. lipoferum* 4B (C), heme A from *B. subtilis* (D) and *A. lipoferum* 4VII (E); heme R3 from *A. lipoferum* 4VII (F), heme R2 from *A. lipoferum* 4VII (G), and heme D from *A. lipoferum* 4VII (H).
changes have been previously reported in the 4VII variant of some other changes related to oxidative metabolism. Interestingly, a loss of a regulatory protein caused changes in the laccase-positive variant form (2). Genes for enzymes that are involved in heme modification, such as a protoheme IX farnesyl transferase (cyoE) and ctaB), are found in the operons encoding for terminal oxidases in different bacterial species (8, 27, 41). Deficiency in these genes leads to a loss of a correspondent terminal oxidase due to the blockage of the heme O (cyoE) or heme A (ctaA and ctaB) biosynthesis pathway. Where known, expression of genes involved in heme biosynthesis is under environmental control, with oxygen being one of the major factors (9, 18, 26, 52). Interestingly, a loss of a regulatory protein causes changes in B. subtilis that are similar to those observed in the 4VII variant of A. lipoferum. The ResD protein, which is similar to the two-component signal transduction proteins, was shown to be a global regulator of the respiratory system in B. subtilis (40). Mutation in resD leads to a loss of the cytochrome c oxidase of the a3 type due to defects in heme biosynthesis (ctaB) and some other changes related to oxidative metabolism. Similar changes have been previously reported in the 4VII variant of A. lipoferum; moreover, all changes that occur in the variant are related to oxidative metabolism (1, 2). This allows us to propose that an unspecified mutation in the 4VII variant also affects a regulatory system that controls directly or indirectly the expression of both laccase and enzymes involved in heme biosynthesis.

The exact role of the laccase in A. lipoferum is unknown, although utilization of plant phenolic compounds (12, 13) may be advantageous for the bacterial survival in the rhizosphere. Using a variety of membrane-permeable oxidized quinones, we have demonstrated that the laccase-positive variant was up to two times less sensitive to the inhibitory effect of these compounds on the respiratory system. We attribute the resistance of the variant solely to changes in its electron transport system, i.e., the absence of the cytochrome c oxidase and the preferential use of quinol oxidases. Most interestingly, the loss of the major cytochrome c oxidase and acquisition of resistance to exogenous quinones that are described in this study occurred under conditions (exponential phase, full aeration), where laccase activity reached a high level (1). Such changes in the respiratory metabolism may reflect an adaptive strategy of the bacterium to the presence of intracellular laccase and its toxic quinone by-products. Shutoff of the quinone-sensitive cytochrome c oxidase and the preferential use of quinone-insensitive quinol oxidases would be beneficial for quinone-producing laccase-positive cells. At the same time, a laccase-positive quinone-tolerant variant would have a competitive advantage in the rhizosphere in the presence of quinone compounds, such as sorgoleone, which are naturally occurring inhibitors of the be complex/cytochrome c oxidase-containing branches of the electron transport system (32). Taking into account possible widespread distribution of laccases in bacteria (3), future studies on genetic mechanisms and the environmental control of the expression of laccase and components of the respiratory system in A. lipoferum should be productive.

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Reduction potential (mV)a</th>
<th>Conc (μM)</th>
<th>Oxygen consumption rate (μmol/min per cell [10⁻¹¹])b</th>
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<tbody>
<tr>
<td>None</td>
<td>+99</td>
<td>10</td>
<td>21 ± 3, 4B</td>
</tr>
<tr>
<td>1,4-Benzquinone</td>
<td>+73</td>
<td>10</td>
<td>19 ± 2, 4V</td>
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<tr>
<td>2-Methyl-1,4-benzquinone</td>
<td>+23</td>
<td>10</td>
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<td>2,6-Dimethyl-1,4-benzquinone</td>
<td>−20</td>
<td>100</td>
<td>15 ± 1, 4V</td>
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<td>2-Hydroxy-1,4-naphthoquinone</td>
<td>−415</td>
<td>1,000</td>
<td>20 ± 1, 4VII</td>
</tr>
</tbody>
</table>

a Data from Wardman (48).
b The results shown are the mean ± the standard deviations as calculated from two independent experiments, with three replicates in each.

FIG. 5. Putative respiratory pathways in A. lipoferum. In the laccase-positive cells during the exponential growth phase under fully aerobic conditions, the pathway shown in bold outlines is not present. Black rectangles indicate the sites of inhibition. Q, ubiquinone and/or menaquinone.


