Cytochrome Oxidase Analyses of *Bacillus* Strains: Existence of Oxidase-Positive Species

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Analyses of 173 *Bacillus* strains (representing 30 species) with a newly developed quantitative colorimetric *N,N,N',N'*-tetramethyl-*p*-phenylenediamine oxidase assay revealed the presence of many oxidase-positive species. Of 144 mesophilic strains analyzed, the following were oxidase positive: 16 of 16 *Bacillus alvei* strains, 7 of 9 *Bacillus laterosporus* strains, 5 of 10 *Bacillus circulans* strains, 4 of 5 *Bacillus macerans* strains, 2 of 5 *Bacillus firmus* strains, 1 strain of *Bacillus sphaericus*, and 1 strain of *Bacillus brevis*. The thermophilic organisms *Bacillus stearothermophilus* T-10 and ATCC 8005, "*Bacillus caldotenax*" (one strain), and "*Bacillus thermodenitrificans*" DSM 465 were also oxidase positive. In fact, when the oxidase-positive thermophilic strains were grown at 65°C, they exhibited very high oxidation rates; most of these rates were equivalent to the high rates of reaction recorded for strains of *Pseudomonas aeruginosa* and *Neisseria* spp. Most mesophilic *Bacillus* strains and all of the psychrophiles examined were oxidase negative or oxidase indeterminate (the latter had oxidase rates equivalent to the rates in the *N,N,N',N'*-tetramethyl-*p*-phenylenediamine autooxidation reaction). Spectral absorbance analyses on membranes of oxidase-positive *B. alvei* strain 385 revealed the presence of a type *c* cytochrome with cytochrome oxidases *o* and *a+a₃*; *Bacillus pumilus* strains which could not oxidize *N,N,N',N'*-tetramethyl-*p*-phenylenediamine lacked type *c* cytochromes but possessed these two oxidases. The paper strip Kovacs oxidase test with Marion reagent could be routinely used for detecting oxidase-positive *Bacillus* strains. A distinct class of oxidase-positive strains should be identified, which might eventually be useful for identification studies.

The oxidase test has been used extensively in studies on gram-negative bacteria since it was introduced in 1928 by Gordon and McCleod (5). The use of the oxidase test for gram-positive organisms has been somewhat limited (8-10, 15), but it has been used to examine differences between the staphylococci and micrococci (1, 3, 16). Quantitative *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) oxidase studies have shown that "*Sarcina flava*," "*Sarcina luteus*," and *Sporosarcina urea* strains are quite active, the latter sporeformer being usually active (8, 9, 11). Previous reports have stated that *Bacillus* strains can give "equivocal" oxidase reactions (15), and one report specifically stated that an unexpectedly large number of soil sporeforming isolates were oxidase positive (4). The results of other, more recent studies suggest that TMPD (a redox dye), which is commonly used as the oxidase reagent, may be impermeable in gram-positive microorganisms (3, 6, 8, 9). Thus, the relevance of the oxidase reaction in gram-positive cells has been questioned.

In this report we describe a distinct class of oxidase-positive *Bacillus* spp. which can be detected experimentally. The quantitative capabili-

ty of some of these *Bacillus* strains to oxidize reduced TMPD to Wurster blue (9) approaches the high rates previously reported for *Azotobacter vinelandii*, *Pseudomonas aeruginosa*, and *Neisseria* spp. (8-11). Such oxidase-positive *Bacillus* cultures are readily detected by the qualitative Kovacs paper strip test (13). In our recent studies we found oxidase-positive strains among mesophilic *Bacillus alvei*, *Bacillus circulans*, *Bacillus firmus*, *Bacillus laterosporus*, *Bacillus macerans*, *Bacillus sphaericus*, and *Bacillus brevis*. Of 24 thermophilic strains examined, 18 were found to be highly oxidase positive; among these were *Bacillus stearothermophilus* strains T-10 and ATCC 8005, "*Bacillus thermodenitrificans*" strain DSM 465 and one "*B. caldotenax*" strain.

(Preliminary reports of the results have been presented elsewhere [Jurtshuk and Liu, Abstr. XIII Int. Congr. Microbiol., Boston, Mass., p. 62, 1982; Liu and Jurtshuk, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, K122, p. 197].)

For the standard colorimetric oxidase assay, bacteria were grown for 20 h on tryptic soy agar (Difco Laboratories, Detroit, Mich.) at the appropriate temperature. Colonies were directly

washed off petri plates with the aid of sterile glass rods, and whole cells were harvested by centrifugation at 6,000 rpm ($4,340 \times g$) and 25°C . The pellets were washed three times in 0.02 M KH_2PO_4 buffer (pH 6.0), and a 3.0-ml bacterial suspension (in 0.02 M KH_2PO_4 buffer, pH 6.0) was prepared and turbidimetrically standardized to 0.20 ± 0.02 optical density unit at 610 nm with a Bausch & Lomb Spectronic 20 colorimeter. TMPD oxidation was initiated by adding 20 μl of a 1.2% (wt/vol) TMPD-hydrochloride solution. Wurster blue formation ($\Sigma \text{ mM} = 12.0$) was monitored at 610 nm at 5-min intervals for 15 min; specific activities were calculated by using the initial 5-min reaction rate and are reported on the basis of cellular dry weight. Analysis of 107 bacterial cultures having the standard turbidity given above revealed a dry weight mean $\pm$ standard error of $0.194 \pm 0.007 \text{ mg/ml}$ (standard deviation, 0.0729 mg/ml). The type of spectrophotometric TMPD oxidase assay described above was first described by Lieberman and Lanyi in 1971 for use in studies on bacterial electron transport systems (14). The use of this assay with bacterial whole cells has been documented previously by Jurtshuk et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C191, p. 306), who described the parameters and precautions necessary for rapidly quantifying bacterial TMPD oxidase reactions.

Figure 1 shows the TMPD oxidation rate patterns for three representative *Bacillus* strains, *B. alvei* strain 385, *Bacillus megaterium* strain UH, and *Bacillus pumilus* strain UH. Similarly, standardized suspensions of a laboratory strain of *P. aeruginosa* and *Escherichia coli* strain B (Fig. 1, dashed lines) served as the oxidase-positive and -negative controls, respectively. TMPD oxidation in the absence of bacterial cells (autooxidation control) is also plotted in Fig. 1 (dotted line); this reaction started at "true" zero. For all specific activity calculations (Table 1) the TMPD autooxidation control rate was corrected for by subtraction.

As shown in Fig. 1, the rate of TMPD oxidation by *B. alvei* strain 385 cells approached the high rate observed for the oxidase-positive *P. aeruginosa* strain. In fact, analyses of 15 other *B. alvei* strains showed that strain 385 cells gave the lowest specific activity (Table 1) of all the *B. alvei* strains examined. *B. megaterium* strain UH had a TMPD oxidation rate that approached the rate of the autooxidation control; this reaction rate was typical of organisms having oxidase-indeterminate reactions. TMPD oxidation by cells by *B. pumilus* strain UH was essentially negative, much like the oxidation observed for *E. coli* strain B. The negative base-line reaction observed with cells of *E. coli* (and somewhat with cells of *B. pumilus*) suggests that such

FIG. 1. Oxidation of TMPD by standardized resting cell suspensions of *P. aeruginosa* strain UH, *B. alvei* strain 385, *B. megaterium* strain UH, *B. pumilus* strain UH, and *E. coli* strain B. The oxidation rates for *P. aeruginosa* and *B. alvei* cells are characteristic of oxidase-positive strains. The rate shown for *B. megaterium* cells is oxidase indeterminate; the overall TMPD oxidation rate is equivalent to the rate of the autooxidation control (dotted line). Cells of *B. pumilus* and *E. coli* are the least active, and the reaction patterns shown for these two organisms are characteristic of oxidase-negative strains. For the colorimetric TMPD oxidase assay, the bacterial cells were turbidimetrically standardized at 610 nm to 0.20 ± 0.02 optical density unit in 3.0 ml of 0.02 M KH_2PO_4 buffer (pH 6.0). The TMPD oxidation reaction was then initiated by adding 1.0 μmol of reduced TMPD (final concentration, $3.3 \times 10^{-4} \text{ M}$). Wurster blue formation was monitored at 610 nm and was plotted as a function of time. For this study, a prolonged 25-min reaction interval was used to observe particularly the reaction patterns for the oxidase-indeterminate (*B. megaterium*) and oxidase-negative (*B. pumilus* and *E. coli*) strains. The autooxidation control (dotted line), which starts at true zero, optically represents the rate of TMPD oxidation that occurred in the absence of bacterial cells.

oxidase-negative organisms have functional oxidases with high affinities for binding molecular O_2 yet are not TMPD dependent. Such cytochrome oxidases would remove oxygen from solution (via normal respiration) so that O_2 would no longer be available for autooxidation of TMPD. Quantitative TMPD oxidase analyses

TABLE 1. Oxidase-positive *Bacillus* strains exhibiting high TMPD oxidase activities

Organism	Source ^a	Growth temp (°C)	Sp act ^b	Organism	Source ^a	Growth temp (°C)	Sp act ^b
<i>B. stearothermophilus</i> T-10	P. Saunders	55	2.636	<i>B. alvei</i> White colony	UH	32	1.062
" <i>B. thermodenitrificans</i> " DSM 465	F. Pinchoty	55	2.443	<i>B. circulans</i> No. 4	Pasteur Institute	32	1.044
<i>Bacillus</i> sp. strain NGB 101	SHSU	55	1.856	<i>B. macerans</i> NCTC 6355	L. Seldin	32	1.043
<i>B. alvei</i> B-4300	NRRL	32	1.672	<i>Bacillus</i> sp. strain RS 173	R. Sharp	55	1.025
<i>B. alvei</i> B-387	NRRL	32	1.562	<i>B. alvei</i> B-386	NRRL	32	1.014
<i>Bacillus</i> sp. strain RS 20	R. Sharp	55	1.501	<i>B. macerans</i> No. 2	Pasteur Institute	32	1.004
<i>B. alvei</i> B-397	NRRL	32	1.483	" <i>B. caldotenax</i> "	R. Sharp	55	0.962
<i>B. alvei</i> 29	DSM	32	1.453	<i>B. circulans</i> No. 3	Pasteur Institute	32	0.960
<i>Bacillus</i> sp. strain NGB 301	SHSU	55	1.442	<i>B. alvei</i> B-384	NRRL	32	0.946
<i>B. brevis</i> B-335	NRRL	32	1.402	<i>B. alvei</i> 385	UH	32	0.937
<i>B. macerans</i> No. 3	Pasteur Institute	32	1.401	<i>B. firmus</i> No. 1	Pasteur Institute	32	0.922
<i>B. alvei</i> B-383	NRRL	32	1.362	<i>B. laterosporus</i> SHSU	SHSU	32	0.916
<i>B. alvei</i> B-4010	NRRL	32	1.356	<i>B. sphaericus</i> No. 2	Pasteur Institute	32	0.885
<i>B. alvei</i> F	G. S. Byng	32	1.340	<i>Bacillus</i> sp. strain RS 43	R. Sharp	55	0.862
<i>B. circulans</i> No. 7	Pasteur Institute	32	1.290	<i>B. laterosporus</i> NRS 590	R. Gordon	32	0.839
<i>B. alvei</i> B-385	NRRL	32	1.242	<i>B. stearothermophilus</i> ATCC 8005	R. Sharp	55	0.664
<i>Bacillus</i> sp. strain NC 1101	SHSU	55	1.242	<i>B. firmus</i> No. 2	Pasteur Institute	32	0.648
<i>B. alvei</i> B-4186	NRRL	32	1.231	<i>B. laterosporus</i> NCTC 6357	L. Seldin	32	0.615
<i>Bacillus</i> sp. strain NC 902	SHSU	55	1.222	<i>Bacillus</i> sp. strain EP 136	R. Sharp	55	0.558
<i>Bacillus</i> sp. strain MHS 1601	SHSU	55	1.220	<i>B. laterosporus</i> No. 2	Pasteur Institute	32	0.515
<i>B. circulans</i> No. 5	Pasteur Institute	32	1.176	<i>B. laterosporus</i> NRS 1647	R. Gordon	32	0.465
<i>B. alvei</i> B-4185	NRRL	32	1.166	<i>Bacillus</i> sp. strain RS 241	R. Sharp	55	0.465
<i>B. alvei</i> B-382	NRRL	32	1.151	<i>B. laterosporus</i> No. 3	Pasteur Institute	32	0.464
<i>Bacillus</i> sp. strain FH 2301	SHSU	55	1.139	<i>B. laterosporus</i> No. 1	Pasteur Institute	32	0.430
<i>B. circulans</i> No. 2	Pasteur Institute	32	1.137	<i>Bacillus</i> sp. strain RS 93	R. Sharp	55	0.428
<i>Bacillus</i> sp. strain FH 2101	SHSU	55	1.130				
<i>B. macerans</i> No. 1	Pasteur Institute	32	1.129				
<i>B. alvei</i> B-4009	NRRL	32	1.121				
<i>Bacillus</i> sp. strain MHS 1701	SHSU	55	1.118				

^a SHSU, Sam Houston State University; NRRL, U.S. Department of Agriculture Northern Regional Research Center; DSM, Deutsche Sammlung von Mikroorganismen; UH, University of Houston.

^b Micromoles of TMPD oxidized per hour per milligram (dry weight).

of 173 *Bacillus* strains, representing 30 species, showed this same degree of diversity. In this report we focus on (i) those *Bacillus* spp. which were highly oxidase positive (Table 1) and conversely (ii) those species which were distinctly oxidase negative and gave zero TMPD oxidation reaction rates, similar to the rate observed for *B. pumilus* or *E. coli* or both.

Table 1 lists representative *Bacillus* strains (and their sources) having high oxidase specific activities. Of the 144 mesophilic *Bacillus* strains

tested, all 16 strains of *B. alvei*, as well as 7 of 9 strains of *B. laterosporus*, were found to be highly oxidase positive. Also highly active were 4 of 5 *B. macerans* strains, 5 of 10 *B. circulans* strains, 2 of 5 *B. firmus* strains, 1 of 3 *B. sphaericus* strains and one strain of *B. brevis*. Interestingly, two strains that were received originally as *B. alvei* cultures were consistently oxidase negative. These strains were subsequently found to be misidentified; one was a strain of *Bacillus licheniformis*, and the other

FIG. 2. Difference spectra of a partially purified TMPD-dependent cytochrome oxidase (protein concentration, 1.6 mg/ml) isolated from respiratory membranes of *B. alvei* strain 385. The carbon monoxide-dithionite-reduced spectrum (a) shows large concentrations of cytochrome *o* (0.65 nmol/mg of protein). (b) Dithionite-reduced minus oxidized spectrum, with alpha peaks of cytochromes *a*, *o*, and *c* at 604, 557, and 550 nm, respectively. The dotted line shows the absorbance changes after bubbling CO. For further details see reference 12.

was a strain of *B. megaterium* (Ruth E. Gordon, personal communication). Without exception, all 16 *B. alvei* strains which we tested were oxidase positive. Conversely, only 2 of 32 cultures originally received as *Bacillus polymyxa* were highly oxidase positive. These two *B. polymyxa* strains were examined by R. E. Gordon; one was found to be a strain of *B. laterosporus*, and the other was most probably a *B. brevis* strain. In one respect, the heterogeneity and diversity of TMPD oxidation by *Bacillus* strains are quite surprising. Most gram-positive heterotrophs exhibit a "homogenous" cytochrome pattern (namely, cytochromes *b*, *c*, *a*+*a*₃, and *o*); thus, in theory, the TMPD oxidase reactions of *Bacillus* spp. should depend on whether type *c* cytochrome is present and functionally integrated with type *o* and *a* cytochrome oxidases (7, 11). Since most *Bacillus* species analyzed were oxidase negative, if any of these organisms contained both type *c* and type *o*

cytochromes, this finding would suggest that there are cellular impermeability problems that would affect TMPD oxidation.

The following mesophilic *Bacillus* strains were oxidase negative, as determined by the quantitative colorimetric oxidase test: 4 of 8 *B. circulans* strains, including strain ATCC 9500; *B. licheniformis* strain SHSU; five *B. megaterium* strains, including strain Starka 899; 30 of 30 *B. polymyxa* strains; *B. pumilus* strains LMD 68.28, F.8, F.10, 48.24, and UH; and "*Bacillus thuringiensis* subsp. *pakistani*" strain HD-395.

Most of the thermophilic sporeforming strains tested were unidentified hot spring isolates. In our survey, the highest rate of TMPD oxidase activity was observed in *B. stearothermophilus* T-10 cells grown at 65°C; specific activities of more than 5.0 were obtained, whereas specific activities of 2.636 $\mu\text{mol/h}$ per mg (dry weight) were found for cells grown at 55°C (Table 1). All sporeforming thermophiles showed this same activity pattern; substantially higher TMPD oxidase rates were found in cells grown at 65°C than in cells grown at 55°C. All thermophilic strains grew poorly at 43°C, and such cells had even lower specific activities (range, 1.4 to 1.9). In a study by Wolf and Sharp (17), 8 of 12 thermophilic *Bacillus* strains examined for oxidase activity were found to be oxidase positive with a qualitative paper strip test.

All 11 psychrophilic *Bacillus* strains analyzed (at 23°C) were either oxidase negative or oxidase indeterminate. The least active were "*Bacillus psychrophilus*" strains W5 and W10A and *Bacillus globisporus* subsp. *globisporus* strain T26A; the most active strain was "*B. psychrophilus*" strain W16A, whose specific activity was approximately 0.40 (oxidase indeterminate).

Analyses of 11 mesophilic *B. megaterium* strains also produced interesting results; 5 strains were oxidase negative. One *B. megaterium* strain exhibited an oxidase value of 0.260, thus falling in the oxidase-indeterminate group. A similar observation was made previously by Downs and Jones (2), who noted that respiratory membranes isolated from *B. megaterium* strain M (which had type *c:o* and *a* cytochromes) had TMPD oxidation values that were 5 to 10 times greater than the values for comparable membranes isolated from *B. megaterium* strain D440 (which lacked type *c* cytochrome). This finding could explain the metabolic diversity of oxidase activities observed for the 11 *B. megaterium* strains which we tested. Similar observations were made for 24 strains of *B. thuringiensis*. TMPD oxidase activities ranged from 0 μmol of TMPD/h per mg (dry weight) for "*B. thuringiensis* subsp. *pakistani*" to 0.351 μmol of TMPD/h per mg (dry weight) for "*B. thuringiensis* subsp. *entomocidus*."

We attempted to identify the components responsible for TMPD oxidase activity in *B. alvei* strain 385 and *B. laterosporus* strain SHSU. Respiratory membranes were isolated from *B. alvei* strain 385, and a partial purification of the TMPD oxidase enzyme complex was carried out by using the procedure developed for *A. vinelandii* (12). An active solubilized cytochrome oxidase complex was isolated, and this complex was similar to the one described previously for *A. vinelandii*. The *B. alvei* strain 385 oxidase consisted of a cytochrome *c:o* complex containing a small concentration of cytochrome *a+a₃*; the absorption spectrum is shown in Fig. 2. The carbon monoxide-dithionite difference spectrum (Fig. 2a) shows the major CO-reacting component(s), and the presence of a large concentration of type *o* cytochrome (0.65 nmol/mg of protein) was observed, with major Soret peak at 412 nm and a prominent sharp trough at 430 nm. Alpha and beta peaks reacting with CO were found at 572 and 583 nm, respectively, and there was a minor but characteristic carbon monoxide-cytochrome *o* trough at 557 nm. The presence of the type *c* cytochrome was marked by the peak at 550 nm (Fig. 2b). The dithionite-reduced components of the oxidase (Fig. 2b, dashed line) revealed a type *a* cytochrome at 604 nm and the alpha peak of cytochrome *o* at 557 nm. The dotted line in Fig. 2b shows the extent of CO reactivity of the cytochrome components in this partially purified oxidase preparation. The results of spectral analyses of the cytochrome components in the respiratory membranes of *B. alvei* strain UH and *B. laterosporus* strain SHSU (Table 1) were similar to the results obtained with *B. alvei* strain 385. However, cytochrome analyses of membranes isolated from *B. pumilus* strain UH revealed that type *c* cytochrome was absent but both oxidases (cytochromes *o* and *a+a₃*) were present. The results of our spectral studies on the cytochrome components of *Bacillus* strains are consistent with the observation that bacterial TMPD oxidations still require type *c* cytochrome(s) integrated with a terminal oxidase of cytochrome *o* or *a+a₃* (8-12).

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LITERATURE CITED

1. Boswell, P. A., G. F. Batstone, and R. G. Mitchell. 1972. The oxidase reaction in the classification of the *Micrococcaceae*. J. Med. Microbiol. 5:267-269.
2. Downs, A. J., and C. W. Jones. 1975. Energy conservation in *Bacillus megaterium*. Arch. Microbiol. 105:159-167.
3. Faller, A., and K. H. Schleifer. 1981. Modified oxidase and benzidine tests for separation of staphylococci from micrococci. J. Clin. Microbiol. 13:1031-1035.
4. Goodfellow, M. 1968. Properties and composition of the bacterial flora of a pine forest soil. J. Soil Sci. 19:154-167.
5. Gordon, J., and J. W. McLeod. 1928. The practical application of the direct oxidase reaction in bacteriology. J. Pathol. Bacteriol. 31:185-190.
6. Hollander, R. 1977. The influence of permeability of the cell membrane on TMPD oxidase activity. Zentralbl. Bakteriell. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 237:351-357.
7. Jones, C. W. 1980. Cytochrome patterns in classification and identification including their relevance to the oxidase test, p. 127-138. In M. Goodfellow and R. G. Board (ed.), Microbiological classification and identification. Academic Press Inc., New York.
8. Jurtshuk, P., Jr., and D. N. McQuitty. 1976. Survey of oxidase-positive and -negative bacteria using a quantitative Kovacs oxidase test. Int. J. Syst. Bacteriol. 26:127-135.
9. Jurtshuk, P., Jr., and D. N. McQuitty. 1976. Use of a quantitative oxidase test for characterizing oxidative metabolism in bacteria. Appl. Environ. Microbiol. 31:668-679.
10. Jurtshuk, P., Jr., T. J. Mueller, and W. C. Acord. 1975. Bacterial terminal oxidases. Crit. Rev. Microbiol. 3:399-468.
11. Jurtshuk, P., Jr., T. J. Mueller, D. N. McQuitty, and W. H. Riley. 1978. The cytochrome oxidase reaction in *Azotobacter vinelandii* and other bacteria, p. 99-121. In H. Degen, D. Lloyd, and G. C. Hill (ed.), Functions of alternative terminal oxidase. Proceedings of the 11th FEBS Copenhagen Meeting. Pergamon Press, Oxford.
12. Jurtshuk, P., Jr., T. J. Mueller, and T. Y. Wong. 1981. Isolation and purification of the cytochrome oxidase of *Azotobacter vinelandii*. Biochim. Biophys. Acta 637:374-382.
13. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178:703.
14. Lieberman, M. M., and J. K. Lanyi. 1971. Studies on the electron transport chain of extremely halophilic bacteria. Biochim. Biophys. Acta 245:21-33.
15. Steel, K. J. 1961. The oxidase reaction as a taxonomic tool. J. Gen. Microbiol. 25:297-306.
16. Steel, K. J. 1962. The oxidase activity of staphylococci. J. Appl. Microbiol. 25:445-447.
17. Wolf, J., and R. J. Sharp. 1981. Taxonomic and related aspects of thermophiles within the genus *Bacillus*, p. 251-296. In R. C. W. Berkeley and M. Goodfellow (ed.), The aerobic endospore forming bacteria. Academic Press, Inc., London.