Mutants of *Bacillus* Species Isolated on the Basis of Protonophore Resistance Are Deficient in Fatty Acid Desaturase Activity

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The fatty acid desaturase activity in cell extracts of *Bacillus subtilis* was characterized and found to be O₂ dependent, NADH dependent, and cyanide sensitive. In cell fractionation studies, only 10% of the desaturase activity was recovered in the membrane fraction; the addition of cytosolic factors, which by themselves were devoid of activity, restored membrane activity to the level found in the unfractionated cell extracts. NADH was preferred over NADPH as an electron donor, and palmitoyl-coenzyme A was used preferentially over stearyl-coenzyme A as the straight-chain fatty acid substrate. An increase in desaturase activity was observed when either the growth or the assay temperature was lowered from 37 to 20°C, although the assay temperature appeared to be the more important parameter. Three protonophore-resistant mutants of *B. subtilis* and a comparable mutant of *Bacillus megaterium* had been found to possess reduced levels of unsaturated fatty acids in their membrane phospholipids; their protonophore resistance was abolished when grown in the presence of an unsaturated fatty acid supplement. All of these strains were found to be either significantly deficient in or totally lacking desaturase activity in comparison with their wild-type parent strains. Full, protonophore-sensitive revertants of the mutants had levels of desaturase activity comparable to those of the wild-type. Temperature-sensitive revertants of two of the mutants, which grew at 32°C but not at 26°C in the presence of protonophore, exhibited desaturase activity comparable to that of the wild type at 26°C but lacked activity at 32°C. These results indicate that the biochemical basis for protonophore resistance in these *Bacillus* mutants is a fatty acid desaturase deficiency.

Three protonophore-resistant strains of *Bacillus subtilis* that were isolated in our laboratory grew on nonfermentable carbon sources in the presence of protonophore concentrations that completely inhibited growth of the wild type (15). The mutants did not, however, inactivate or exclude the protonophore (15). Like a similar mutant strain of *Bacillus megaterium* described by Decker and Lang (4, 5), these strains synthesized more ATP than the wild type at submaximal levels of the proton motive force (5, 15, 17). All of the mutants had reduced unsaturated fatty acid content in their membrane phospholipids relative to wild-type *B. subtilis* and *B. megaterium*. In the *B. subtilis* mutants, the sole monounsaturated fatty acid, C_{16:1} was reduced by approximately 50% in each mutant. More dramatically, the entire monounsaturated fatty acid content drops from 12% of the total fatty acid in wild-type *B. megaterium* phospholipids to 0% in the protonophore-resistant mutant C8 (17). We therefore sought to examine whether the primary mutational event in the protonophore-resistant mutants of *Bacillus* species was in the fatty acid desaturase.

The most extensive studies of the biosynthesis of unsaturated fatty acids in bacteria have been conducted in *Escherichia coli*. However, this pathway is oxygen independent (6). In contrast, studies using whole cells of *B. megaterium*, conducted by Fulco et al., indicated that the activity involved an oxygen-dependent pathway (12, 13). Detailed in vitro and molecular studies of the eukaryotic O₂-dependent desaturase have been conducted in mammals (25, 26, 28, 29), plants (20, 23), and yeast cells (21, 27), from which a well-characterized pattern has emerged. The fatty acid de-

saturation system of eukaryotes is membrane associated, O₂ dependent, NADH dependent, and cyanide sensitive and is composed of cytochrome *b*₅, cytochrome *b*₅ reductase, and the cyanide-sensitive desaturase (10, 16). In vitro studies of the O₂-dependent pathway in prokaryotes have been less extensive, with work reported for *Alcaligenes faecalis* (18), *Mycobacterium phlei* (14), and *Pseudomonas* sp. (33) and for a cyanobacterium (30-32). In this work, we characterize the O₂-dependent pathway in cell extracts of *B. subtilis* and then compare this activity in the wild-type strains of both *B. subtilis* and *B. megaterium* with that of their protonophore-resistant counterparts.

MATERIALS AND METHODS

Strains and growth conditions. Wild-type *B. subtilis* BD99, protonophore-resistant AG1A3, AG2A, and AG3A, full revertants AG1A3rev8 and AG2Arev35, and temperature-sensitive revertants AG1A3Ts47 and AG2ATs1 were grown on 50 mM DL-malate (pH 7) in Spizizen salts (24) supplemented with 0.1% yeast extract and 5 µg each of tryptophan, threonine, and histidine per ml at 30°C to late logarithmic phase. Wild-type *B. megaterium* ATCC 19213 and its uncoupler-resistant mutant derivative C8 (4, 5) were grown in the same medium without the amino acid supplements. In some experiments, wild-type *B. subtilis* BD99 was grown on the above media at temperatures of 20 or 37°C. Temperature-sensitive revertants were selected by growth on plates at either 26 or 32°C in the presence or absence of 5 µM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP).

Preparation of cell extracts and membranes. One liter of cells was harvested and resuspended in 40 ml of 0.1 M potassium phosphate buffer (pH 7.2) containing 0.5 M su-

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crose. The protease inhibitors phenylmethylsulfonyl fluoride and *p*-aminobenzamidine were added to final concentrations of 1 and 5 mM, respectively. After French pressure cell lysis at 20,000 lb/in², the mixture was centrifuged for 15 min at 12,000 × *g* to remove debris and unbroken cells. The supernatant was the crude extract used in most of the assays. In fractionation studies, the crude extract was centrifuged for 60 min at 200,000 × *g* to obtain the membrane fraction (pellet) and the cytosol fraction (supernatant).

Assay of the fatty acid desaturase. The conversion of radioactive palmitoyl-coenzyme A (palmitoyl-CoA) to a monounsaturated product has been used previously for assays of the fatty acid desaturase of alkaliphilic *Bacillus* extracts (8). The assay contained 60 mM potassium phosphate buffer (pH 7.2), 1.25 mM NADH, 150 nmol of [¹⁴C]palmitoyl CoA, and 0.25 to 2 mg of the enzyme source in a total volume of 0.5 ml (8) unless otherwise indicated. The protein content was determined by the method of Lowry et al. (19) by using lysozyme as the standard. The assay components were preincubated for 5 min at the assay temperature. The reaction was initiated by the addition of crude extract and stopped at time points, between 8 and 30 s, by the addition of 0.25 ml of 3 M KOH–25% methanol. The mixtures were then saponified by incubation under nitrogen for 20 min at 80°C. A modified procedure of Bligh and Dyer (1) was used for the extraction of the fatty acids. After cooling, the mixtures were titrated to pH 4 by the addition of concentrated HCl. The addition of a 3-ml volume of chloroform-methanol (1:2) was followed by the addition of 1 ml of chloroform and 1 ml of water. After the phase separation, the lower (chloroform) layer was taken and dried under nitrogen. Methyl esters of the fatty acids were made by the addition of 1 ml of boron trifluoride, under N₂, for 1 h at 90°C and extracted with 1 ml of chloroform and 1 ml of H₂O. After phase separation, the lower (chloroform) layer was taken, dried under N₂, and resuspended in 100 µl of chloroform. The fatty acid methyl esters were then plated on Analtech thin-layer chromatography plates containing either 5% AgNO₃ or Silica Gel G (1,000 µm) and separated by development in benzene-hexane (60:40) or acetone-water (90:10). Commercial fatty acid methyl esters from Sigma were used as the standards, and the *R_f* values of the methyl esters were as described by others (18, 33). Plates were developed by iodine vapors, and the visible spots were scraped off with an efficiency of recovery of 90%. Radioactivity was measured by suspension of the scrapings in a toluene cocktail and counted in a liquid scintillation counter.

Isolation of temperature-sensitive revertants. *B. subtilis* AG1A3 and AG2A were grown to late log phase, and 10 ml of these cultures was subjected to ethyl methanesulfonate mutagenesis (15) in standard growth medium (24). After 90 min, the cells were washed with Spizizen salts, allowed to grow for one generation, plated onto solid standard medium containing 5 µM CCCP, and incubated at 32°C overnight. Colonies which grew overnight were replica plated on identical plates which were incubated at 26°C. Temperature-sensitive revertants were chosen for their ability to grow at 32°C and inability to grow at 26°C in the presence of 5 µM CCCP. Phenotypic rescreening was performed in broth medium identical to that of the plates.

Locant of the double bond. ¹³C nuclear magnetic resonance and gas chromatography-mass spectroscopy was used to determine the double-bond positions of the monounsaturated fatty acids (11). ¹³C nuclear magnetic resonance proton-decoupled spectra were obtained by using a computer-controlled system based on a Varian XL-100 spectrome-

TABLE 1. Effect of various inhibitors and cofactors on the fatty acid desaturase activity of *B. subtilis*

Assay system	% Activity of complete system ^a
Complete ^b	100
–NADH.....	11
–O ₂	0 ^c
+50 mM NaCN.....	0 ^c
+2 mM EDTA.....	0 ^c
+CO ^d	145

^a Values are the means from at least three independent experiments with standard deviations less than 20%.

^b The complete assay contains 60 mM potassium phosphate (pH 7.2), 1.25 mM NADH, 0.5 mg of crude extract, and 150 µM ¹⁴C-labeled palmitoyl-CoA in a total reaction volume of 0.5 ml.

^c Activity below limits of detection, i.e., below 0.7 nmol/min/mg of protein.

^d In carbon monoxide studies, reaction tubes were purged with CO for 1 min, and 50% of the atmosphere was replaced with O₂. Control experiments were purged with N₂ instead of CO.

ter operating at 25.2 MHz, with a data memory size of 16 kilobytes for free induction decay. The spectra were obtained in CDCl₃ solutions. The table of Bus and Marcel (2) was used for assignment of carbonyl carbon atoms. Gas chromatography-mass spectroscopy analysis was carried out on a JEOL JCG-20K gas chromatograph coupled directly to a JEOL JMS D-100 mass spectrometer operating at an electron bombardment energy of 20 eV. The mass spectra were obtained with the machine operating at 70 eV. The column used for chromatography was made of stainless steel, 6 ft by 1/8 in. (inside diameter) (ca. 2 m by 0.3 cm) packed with 10% SE-30 on Chromosorb WAW, with helium as the carrier gas. Fatty acids were converted to the methyl derivative by the addition of 6 mmol of dimethyl disulfide and 0.05 mmol of iodine (Sigma) and stirring under nitrogen for 24 h at room temperature. Standard methyl (Z)-9-hexadecenoate was obtained from Supelco, Inc.

RESULTS

Characterization of the fatty acid desaturase of *B. subtilis*. The assay conditions described in Materials and Methods were optimized with respect to components of the reaction mixture, protein concentration, and time. Under optimal conditions, the rate of formation of unsaturated fatty acid from fatty acyl-CoA was linear from 0 to 30 s at concentrations between 0.25 and 2 mg of protein per ml; after 30 s, the rate of desaturation decreased dramatically. The fatty acid desaturase activity of *B. subtilis* BD99, as shown in Table 1, was O₂ dependent, NADH dependent, and CN[–] sensitive, albeit that somewhat high CN[–] concentrations were needed for complete inhibition. The reaction was also inhibited by EDTA, suggesting the involvement of a divalent cation in the reaction. Dialyzed cell extracts were devoid of desaturase activity. The activity was not inhibited by carbon monoxide, which was added 1:1 (vol/vol) with O₂. The addition of flavin adenine dinucleotide or flavin mononucleotide had an inhibitory effect on undialyzed samples and no stimulatory effect on dialyzed samples (data not shown). The requirement for reduced pyridine nucleotide was studied in detail. As shown in Fig. 1, activity increased from 0 to 1.25 mM NADH and then steadily decreased until the reaction was completely inhibited at 5 mM NADH. No activity was found when NADPH was substituted for NADH at a concentration of 1.25 mM. The equimolar addition of NADH plus NADPH to a final concentration of 1.25 mM resulted in an activity which

FIG. 1. The effect of increasing NADH concentration on the activity of the fatty acid desaturase. Crude extracts were prepared as described in Materials and Methods from wild-type *B. subtilis* BD99 and assayed at 30°C in the standard assay mixture in the presence of 0 to 5 mM NADH and 0.5 mg of crude extract (○). In one assay, NADPH was substituted for NADH at a concentration of 1.25 mM (□), and NADH and NADPH were combined in another assay in an equimolar ratio to a final concentration of 1.25 mM (△). Units of activity are nanomoles per 10 min per milligram.

was less than that with 0.62 mM NADH alone. The activity seen when no NADH was added to the assay was probably due to the presence of residual NADH or another endogenous reductant in the preparations.

Palmitoyl-CoA was preferred to stearoyl-CoA as a substrate. Under the standard assay conditions, the rates of product formation when palmitoyl-CoA or stearoyl-CoA was used were 9.5 and 4.0 nmol/min/mg of protein, respectively. Phosphatidylethanolamine, a major phospholipid in *B. subtilis* and the site of esterification of the major portion of monounsaturated fatty acid (7), was not utilized as a substrate. With palmitoyl-CoA as the substrate, the activity of the desaturase appeared to approach a maximal velocity at about 1 mM (Fig. 2). At substrate concentrations below 50 μ M, activity was not linear with concentration, but the assays were not sufficiently reproducible at these concentrations to define the kinetic properties more specifically.

Cells of *B. subtilis* were fractionated, and the fractions were assayed for desaturase activity. The activity was associated with the membrane fraction, but the amount was less than 10% of the total activity measured in the crude extract (Table 2). Recombination of the membrane with untreated cytosol restored the activity to that of the unfractionated extracts. Dialyzed or boiled cytosol added separately to the membrane fraction restored only 12 and 5%, respectively, of the activity but together restored 40% of the activity.

The effects of growth and assay temperatures on desaturase activity were examined (Table 3). Raising the assay temperature led to lower desaturase activity in cultures grown at any of the three temperatures. Raising the growth temperature also led to lower desaturase activity, as anti-

FIG. 2. The effect of increasing palmitoyl-CoA concentration on the activity of the fatty acid desaturase. Crude extracts were prepared as described in Materials and Methods from wild-type *B. subtilis* BD99 and assayed at 30°C in the standard assay mixture in the presence of 0.05 to 1 mM palmitoyl-CoA and 0.5 mg of crude extract. Units of activity are nanomoles per 10 min per milligram.

pated. However, cultures grown at 37°C had substantial activity when assayed at 20°C, indicating that a functional desaturase system was present in these cells.

Comparison of the wild-type activity with that of the protonophore-resistant mutants. All of the protonophore-resistant mutants exhibited a substantial reduction in fatty acid desaturase activity in both membrane and crude fractions when compared with their wild-type parents (Table 4). *B. subtilis* mutant AG1A3 exhibited approximately 10% of the wild-type activity, whereas AG2A and AG3A exhibited no detectable activity. Experiments were conducted in which mixtures of crude extracts of the wild type and each of the three mutant strains (1:1) were assayed; no inhibitory effect

TABLE 2. Fatty acid desaturase activity of *B. subtilis* BD99

Fraction ^a	Total units ^b (nmol/min)	Sp act ^b (nmol/min/mg of protein)
Crude extract	1,858	10.6
Membrane	177	16.3
Cytosol	ND ^c	ND
Membrane plus:		
No cytosol	ND	ND
Untreated cytosol	2,142	22.1
Dialyzed cytosol	258	3.9
Boiled cytosol	116	4.6
Boiled cytosol + dialyzed cytosol	819	22.0

^a Cells were grown at 30°C to late log phase, harvested, and lysed in a French pressure cell at 20,000 lb/in². The supernatant resulting from low-speed (12,000 $\times$ g) centrifugation is defined as the crude extract. Membrane and cytosol fractions are the pellet and supernatant, respectively, of high speed (100,000 $\times$ g) centrifugation. Cell fractions at a concentration of 1 mg/ml were assayed from 0 to 20 s. In recombination studies, the total protein concentration was 1 mg/ml with a membrane/cytosol ratio of 1:9, which is representative of the unfractionated crude extract.

^b Values are the means from at least five independent experiments, with standard deviations less than 30%. Numbers represent conversion of palmitoyl-CoA to a monounsaturated derivative.

^c ND, not detectable.

TABLE 3. Effect of growth and assay temperatures on the fatty acid desaturase activity of crude extracts of *B. subtilis*

Growth temp ^a (°C)	Sp act (nmol/min/mg of protein) at assay temp ^b of:		
	20°C	30°C	37°C
20	28.6	13.3	7.8
30	14.3	11.1	1.7
37	18.1	4.2	<0.7 ^c

^a Cells of wild-type *B. subtilis* BD99 were grown at the temperature indicated to late log phase.

^b All components of the reaction mixture were incubated for 2 min at the assay temperature prior to initiation of the assay.

^c Activity below the limits of detection.

of the mutant extracts upon the wild-type activity was found (data not shown). Similarly, strain C8, the protonophore-resistant mutant of *B. megaterium*, was totally deficient in desaturase activity. Since previous studies (15) had shown that all three of the protonophore-resistant strains of *B. subtilis*, but not strain C8 of *B. megaterium* (3), had residual C_{16:1} in the membranes, it was of interest to determine the position of the double bond in these strains. Studies of the locant of the double bond (Fig. 3 and Table 5) indicated that the wild type, AG2A, and AG3A only had the double bond in the 5-6 position in C_{16:1}. Interestingly, AG1A3 had the double bond in the 5-6 position and in the 9-10 position in its C_{16:1}. Quantitatively, it appeared that the Δ9 fatty acid was a minor component (data not shown).

Assays of wild-type *B. subtilis*, two protonophore-resistant derivatives, two temperature-sensitive revertants, and two full revertants were conducted. When assayed at 26°C, both AG1A3Ts47 and AG2ATs1 exhibited substantial desaturase activity which was comparable to that of the wild type (Table 6). At 32°C, however, the temperature-sensitive revertants had markedly reduced desaturase activity approximating that of the resistant strains AG1A3 and AG2A. These temperature-sensitive, protonophore-resistant strains thus showed temperature-sensitive desaturase activity that correlated inversely with their pattern of protonophore resistance. Full revertants, which had previously been shown to have restored levels of C_{16:1} fatty acids (15), also exhibited restored levels of desaturase activity at both temperatures and had lost the ability to grow at either temperature in the presence of the protonophore.

TABLE 4. Comparison of the fatty acid desaturase activities of wild-type and uncoupler-resistant *Bacillus* strains

Strain ^a	Sp act ^b (nmol/min/mg of protein)	
	Crude extract	Membranes
<i>B. subtilis</i>		
BD99, wild type	11.4	15.2
AG1A3	1.8	2.7
AG2A	<0.7 ^c	<0.7 ^c
AG3A	<0.7 ^c	<0.7 ^c
<i>B. megaterium</i>		
Wild type	7.4	7.6
C8	<0.7 ^c	<0.7 ^c

^a All strains were grown at 30°C to late log phase.

^b Values are the means from at least three independent experiments with standard deviations less than 20%.

^c Activity below the limits of detection.

DISCUSSION

The fatty acid desaturase of *B. subtilis* exhibited many properties that were characteristic of previously described aerobic desaturase systems, e.g., it was membrane associated, oxygen dependent, and cyanide sensitive. The specific activity was higher than that observed in *Mycobacterium phlei* (14), *Alcaligenes faecalis* (18), and *Pseudomonas* sp. (33). However, the linear time frame for activity was 30 s, considerably shorter than that of the other prokaryotic systems. This could be the result of rapid turnover of the enzyme or of cytochrome P-450-mediated degradation of the monounsaturated product. Evidence for the latter possibility came from the observation that the addition of CO, an inhibitor of cytochrome P-450, modestly increased the activity (Table 1). Cytochrome P-450s are known to be capable of desaturation (16) as well as epoxidation of double bonds. Since carbon monoxide did not inhibit the desaturase activity and in fact gave some stimulation, insertion of a double bond into palmitoyl-CoA was probably due not to P-450 activity but rather to fatty acid desaturase activity. The preference for NADH over NADPH had been noted in the insect system (34), although NADPH was preferred in the rat liver system (25). The potent inhibition by EDTA pointed to a possible requirement for divalent cations. Ferrous iron and flavins were presumably required for desaturase activity (14); however, these requirements were not directly demonstrable, probably because of bound cation and cofactor.

Fatty acid desaturases of eukaryotes are membrane-bound systems composed of cytochrome *b*₅, cytochrome *b*₅ reductase, and the desaturase (9, 10). However, the fractionation experiments indicated that soluble factors are required in the *Bacillus* system. The fractionation experiments showed that, although the activity was confined to the membrane, both dialyzable and nondialyzable cytosolic factors were required to restore the membrane activity to a level comparable to that observed in the crude extract. This result is similar to results in *Mycobacterium phlei* (14), in which both a heat-labile factor and a dialyzable factor were found to be required for activity.

Palmitoyl-CoA was preferred to stearoyl-CoA as the substrate for the fatty acid desaturase of *B. subtilis*. Consistently, the only monounsaturated fatty acid esterified in the membrane phospholipids is C_{16:1} (15). This contrasts with the observation of the stearyl-CoA desaturase of eukaryotes (25), for which stearyl-CoA is the preferred substrate. The fact that fatty acid desaturation did not take place on the phospholipid, as has been observed in plants (23), indicated the requirement for an activated fatty acid substrate. Assays using the free fatty acid as a substrate were not feasible because of the insolubility of palmitic acid in an aqueous environment.

In vivo studies of the effect of temperature on the activity of the fatty acid desaturase of *B. megaterium* by Fulco indicated that elevated growth temperatures inhibited the synthesis of the enzyme and implicated the involvement of a protein modulator (12). The current results with *B. subtilis* suggest that the desaturase activity is also directly affected by lowering or raising the temperature. Desaturase activity of cultures grown at 20°C was significantly higher than that of cultures grown at 37°C. However, increasing the assay temperature led to a reduction in activity regardless of the growth temperature, and substantial activity was observed from a culture grown at 37°C when assayed at 20°C. These results indicated that, while growth temperature modulated desaturase activity, the in vitro desaturase activity was also

FIG. 3. Mass spectra for locant of the double bond for wild-type *B. subtilis* BD99 and uncoupler-resistant strains AG1A3, AG2A, and AG3A. Total lipids were prepared by the procedure of Bligh and Dyer (1), and the monounsaturated fatty acids were derivatized by alkylthiolation (11) for purposes of the spectra. (A) Wild-type *B. subtilis* BD99; (B) AG2A and AG3A; (C) AG1A3.

directly sensitive to temperature. It would be of interest to determine whether this property would persist in a solubilized preparation. A major finding in the current study was the absence of O_2 -dependent fatty acid desaturase activity in two protonophore-resistant strains, AG2A and AG3A, of *B. subtilis* and strain C8 of *B. megaterium* and the presence of low desaturase activity in protonophore-resistant strain AG1A3. However, while membrane composition studies of the wild-type and mutant strains showed that strain C8 of *B. megaterium* totally lacked unsaturated fatty acids, the three *B. subtilis* mutants retained 50% of the wild-type content of monounsaturated fatty acid esterified into the phospholipid. There must be an alternate pathway for the biosynthesis of unsaturated fatty acids in *B. subtilis* which is active in AG2A and AG3A in the absence of the O_2 -dependent fatty acid

desaturase activity assayed here. The mutation in AG1A3 probably leaves that alternate pathway intact and changes both the activity of the O_2 -dependent enzyme and its specificity with respect to the placement of the double bond. The desaturase systems of the wild-type *B. subtilis* apparently desaturate exclusively at the 5-6 position, since that is the only species found in the membranes. In contrast to the *B. subtilis* mutants, C8, a protonophore-resistant mutant of *B. megaterium*, had no detectable levels of unsaturated fatty acids; this species may thus have no alternate pathway for the biosynthesis of unsaturated fatty acids.

The inverse correlation between desaturase activity and uncoupler resistance was most clearly shown in the assays of the temperature-sensitive revertants. At a temperature which allowed growth of the temperature-sensitive strains in the

TABLE 5. Carbon chemical shifts of palmitoleic acid methyl ester (in $CDCl_3$) from *B. subtilis* wild type and protonophore-resistant mutant strains^a

Lipid sample	Carbon chemical shift (ppm) in locant of C atom															
	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1 OCH ₃
Standard (Δ^9)	14.05	22.65	31.60	29.40	27.30	27.80	120.00	129.50	28.50	26.80	26.50	26.00	25.10	24.95	34.10	174.25
Wild type	14.05	22.60	31.60	30.80	27.30	27.00	26.80	26.50	26.40	27.0	129.0	130.0	27.0	24.95	34.10	174.25
AG2A	14.04	22.60	31.50	29.60	27.30	27.20	26.80	26.50	26.40	27.0	129.0	130.0	27.0	24.95	34.10	174.25
AG3A	14.05	22.60	31.50	29.60	27.30	27.20	26.80	26.50	26.40	27.0	129.0	130.0	27.0	24.95	34.10	174.25
AG1A3	14.05	22.60	31.50	29.60	27.30	28.40	125.80	130.50	28.40	27.0	128.40	128.60	27.0	24.95	34.10	174.25

^a Internal reference tetramethylsilane.

TABLE 6. Effect of temperature on growth in the presence of CCCP and fatty acid desaturase activity of *B. subtilis* wild type, uncoupler-resistant mutants, full revertants, and temperature-sensitive revertants

Strain	Growth on 5 μ M CCCP at:		Sp act (nmol/ min/mg of protein) when assayed at ^a :	
	25°C	32°C	25°C	32°C
Wild type	No	No	13.9	11.6
CCCP resistant				
AG1A3	Yes	Yes	1.6	<0.7 ^b
AG2A	Yes	Yes	<0.7 ^b	<0.7 ^b
Full revertant				
AG1A3rev8	No	No	18.7	3.4
AG2Arev35	No	No	12.6	8.1
Temperature-sensitive revertant				
AG1A3Ts47	No	Yes	5.8	<0.7 ^b
AG2ATs1	No	Yes	8.6	<0.7 ^b

^a Cells were grown at 30°C to late logarithmic phase and assayed at the temperatures shown. Values are the means from at least two independent experiments with standard deviations less than 20%.

^b Activity below the limits of detection.

presence of the uncoupler, the desaturase activity was like that of the parent uncoupler-resistant strains. At the nonpermissive temperature, the activity of both temperature-sensitive strains was significantly higher, indicating that temperature-sensitive desaturase activity correlated inversely with temperature-sensitive uncoupler resistance. Full revertants, which had desaturase activity resembling that of the wild type, were sensitive to the uncoupler at both temperatures.

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