

Comparison of Effects of Sublethal Microwave Radiation and Conventional Heating on the Metabolic Activity of *Staphylococcus aureus*†

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This study was conducted in an attempt to characterize some of the effects of sublethal microwave radiation on cells of *Staphylococcus aureus*. Cultures were exposed to microwave radiation for 10, 20, 30, and 40 s. The effects of a conventional heat treatment were also compared by placing flasks containing cultures in a boiling water bath for the amount of time required to reach temperatures equivalent to those found in cultures exposed to microwave radiation. Control, microwave-treated, and conventionally heat-treated cultures were centrifuged, pellets were resuspended in distilled water, and the resulting suspensions were passed through a French pressure cell. Cell lysates and walls were then isolated and assayed for enzymatic activity. Thermonuclease production was also determined at various levels of exposure of cells to microwave radiation. Activities of malate and α -ketoglutarate dehydrogenases, cytochrome oxidase, and cytoplasmic adenosine triphosphatase were higher in microwave-treated cells than in control cells. Membrane adenosine triphosphatase, alkaline phosphatase, and lactate dehydrogenase activities were unaffected when cells were exposed to microwave radiation. The activity of glucose-6-phosphate dehydrogenase was decreased by exposure of cells to microwave radiation. In conventionally heated cells, activities of glucose-6-phosphate and malate dehydrogenases and cytoplasmic adenosine triphosphatase increased activities of α -ketoglutarate and lactate dehydrogenases decreased, and alkaline phosphatase activity remained unaffected. Increased levels of thermonuclease activity were observed when cells were exposed to microwave radiation for 10 or 20 s. Data indicate that microwave radiation affects *S. aureus* in a manner which cannot be explained solely by thermal effects.

There has been considerable disagreement as to whether microwave radiation per se or heat generated by microwave radiation is the cause of lethality to microorganisms. Decreases have been shown in the survival of microorganisms exposed to microwave radiation once the thermal death point has been reached (8, 11, 16). Some reports have indicated that when temperature was controlled, no effects of microwave radiation could be seen (4, 9, 21), whereas others have indicated injury to cells regardless of temperature (17, 22).

A study has been reported recently (19) in which various bacteria, actinomycetes, fungi, and bacteriophages were exposed to microwave radiation in the presence and absence of water. Microorganisms were inactivated only when in the presence of water. Dry or lyophilized organisms were not affected even by extended ex-

posures. The authors stated that their data prove that microorganisms are killed by "thermal effect" only and that, most likely, there is no "nonthermal effect."

The purpose of the present study was to attempt to characterize some of the effects of sublethal microwave radiation on cells of *Staphylococcus aureus*. The specific activities of selected enzymes from cells exposed to microwave radiation and from cells conventionally heated were compared. Heat-stable deoxyribonuclease production was also determined at various levels of exposure to microwave radiation, since the production of this enzyme is closely correlated with enterotoxin production by this organism.

MATERIALS AND METHODS

A culture of enterotoxigenic *S. aureus* OSU 701 was obtained from the Culture Collection of the Department of Microbiology, The Ohio State University. A 5-ml inoculum was placed into 1 liter of nutrient broth (Difco Laboratories) at 34°C in a rotary incubator (model G25; 250 rpm; New Brunswick Scientific Co.)

† Ohio Agricultural Research and Development Center Journal Article Series No. 150-79.

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for 14 h. Portions (50 ml) of this culture were transferred to 500-ml Nephelo flasks (Bellco) and diluted with 150 ml of nutrient broth to a reading of 30 Klett units, as measured with a Klett-Summerson colorimeter (660-nm filter). Cultures were then either immediately centrifuged at $16,300 \times g$ in a Sorvall RC-2B centrifuge (Dupont-Ivan Sorvall, Inc.) for 10 min (control) or irradiated for various times and centrifuged. Test cultures in flasks were exposed to microwave radiation (2,450 MHz) in an Amana Radarange oven (model RR-4D) for 10, 20, 30, and 40 s. Experiments were timed by using a stopwatch independent of the timing system of the oven. For conventional heat treatment, flasks with cultures were placed in a boiling water bath for the amounts of time required to reach temperatures equivalent to those found in cultures heated in the microwave oven (M. S. Dreyfuss, J. R. Chipley, and B. J. Kolodziej, Appl. Environ. Microbiol., in press) and then centrifuged.

Pellets of control, irradiated, and heated cells were resuspended in a maximum of 5 ml of distilled water and were twice passed through a French pressure cell (Carver Press, Menemonee Falls, Wis.) at a pressure of 24,000 lb/in². The resulting slurries were then centrifuged three times at $3,020 \times g$ for 10 min to remove whole cells. Supernatants were then centrifuged at $48,200 \times g$ for 20 min to separate cell wall fragments from cell lysates.

Cell lysates and walls were assayed for enzymatic activity. Commercial enzyme preparations, cofactors, and substrates were obtained from Calbiochem and Worthington Biochemicals Corp. All other chemicals were of reagent grade. Assays were performed for the following enzymes: lactate dehydrogenase (L-lactate:nicotinamide adenine dinucleotide oxidoreductase) (14), α -ketoglutarate dehydrogenase (α -ketoglutarate:nicotinamide adenine dinucleotide oxidoreductase) (20), and malate dehydrogenase (L-malate:nicotinamide adenine dinucleotide oxidoreductase) (Worthington Biochemicals Corp.). One unit of each of these enzymes causes an initial oxidation of 1 μ mol of reduced nicotinamide adenine dinucleotide per min. In addition, glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:nicotinamide adenine dinucleotide phosphate oxidoreductase) (Worthington Biochemicals Corp.), cytochrome oxidase (ferrocytochrome *c*-oxygen oxidoreductase) (24), alkaline phosphatase (orthophosphoric monoester phosphohydrolase) (Worthington Biochemicals Corp.), and adenosine triphosphatase (ATPase) (adenosine triphosphate phosphohydrolase) (1, 7, 10, 12) were also assayed. One unit of glucose-6-phosphate dehydrogenase reduces 1 μ mol of nicotinamide adenine dinucleotide per min at 30°C, 1 U of cytochrome oxidase oxidizes 1 mol of ferrocytochrome *c* per mole of enzyme hematin, 1 U of alkaline phosphatase hydrolyzes 1 μ mol of *o*-carboxyphenyl phosphate per min, and 1 U of ATPase liberates 1 μ mol of inorganic phosphate from adenosine 5'-triphosphate per min at 37°C. Total protein was assayed by using a commercial protein assay procedure (5). Assays for enzymatic activity were conducted with lysates and walls from cells exposed to 20 s of microwave radiation since it was observed that a significant enhancement ($P < 0.01$) in cell numbers occurred after 20 s of radiation when cultures were grown in nutrient broth (Dreyfuss et al., in press).

Because of the high correlation of heat-stable deoxyribonuclease (thermonuclease) activity with toxin production by *S. aureus*, assays for determination of thermonuclease activity were conducted according to the procedures outlined by Lachica et al. (15). A 9-ml amount of toluidine blue O-deoxyribonucleic acid agar was placed in a disposable petri dish (100 by 10 mm) and allowed to solidify. Cultures of *S. aureus* were grown as described above. Samples (2 ml) were taken before radiation, and then the flasks were irradiated as described above. Samples (2 ml) from irradiated flasks were then taken every 0.5 h for 3 h. All samples were placed in a boiling water bath for 10 min, and 2- μ l portions of the boiled samples were removed and placed in wells (1 mm wide; made with a Pasteur pipette) in the toluidine blue O-deoxyribonucleic acid agar. Plates were then incubated for 4 h at 37°C and examined for the presence of pink zones surrounding the wells.

RESULTS AND DISCUSSION

It is of interest to note that some of the physicochemical characteristics of the microwave-treated cells used in the enzyme studies appeared to be different than those of control and heat-treated cells (data not shown). Control and heat-treated cells did not form pellets as readily when centrifuged as did microwave-treated cells. Also, there appeared to be more breakage of microwave-treated cells with the French press than of control and heat-treated cells, resulting in higher protein levels in cell-free extracts of microwave-treated cells.

The effects of microwave radiation on the enzymatic activities of control and treated cells are shown in Table 1. Compared with control cells, microwave-treated cells had higher malate dehydrogenase, α -ketoglutarate dehydrogenase, cytochrome oxidase, and cytoplasmic ATPase activities. To insure that these effects were not from heat generation (internal temperatures of 46°C were reached in microwave-treated flasks), conventionally heat-treated cells were also examined. The data indicate that malate dehydrogenase activity increased, but not to the extent found in microwave-treated cells (ratio of 1.84 versus 2.72). The activity of α -ketoglutarate dehydrogenase decreased (ratio of 0.24 versus 1.76). Lactate dehydrogenase activity remained approximately the same in cells exposed to microwave radiation, but decreased when cells were heated conventionally (ratio of 0.97 versus 0.52). Membrane-bound ATPase activity did not appear to be affected by microwave radiation, whereas the activities of cytoplasmic fractions were increased both by microwave radiation and by conventional heat treatment (ratios of 1.55 and 1.66, respectively). The activity of glucose-6-phosphate dehydrogenase was decreased by microwave radiation but was increased by conventional heat treatment (ratio of 0.74 versus

TABLE 1. Effects of microwave radiation and conventional heating upon enzymatic activities of *S. aureus*

Enzyme	Sp act ^a			Ratios	
	Control ^b	Irradiated ^c	Heat treated ^d	Irradiated/control	Heat treated/control
Glucose-6-phosphate dehydrogenase	6.16	4.57	8.51	0.74	1.38
Malate dehydrogenase	3.60	9.82	6.64	2.72	1.84
α -Ketoglutarate dehydrogenase	29.34	51.82	7.10	1.76	0.24
Lactate dehydrogenase	2.89	2.81	1.50	0.97	0.52
Alkaline phosphatase	16.27	16.31	14.61	1.00	0.90
Cytochrome oxidase	0.45	0.55	— ^e	1.23	—
ATPase					
Cell lysate	0.81	1.26	1.35	1.55	1.66
Cell walls	0.28	0.29	—	1.06	—

^a Specific activities for all enzymes except ATPase are expressed as milliunits per milligram of total protein; ATPase specific activities are expressed as moles of inorganic phosphate liberated per gram of total protein.

^b Cells were incubated at 34°C.

^c Cells were irradiated for 20 s; the internal temperature of the media was 46°C.

^d Cells were conventionally heat treated for 39 s; the internal temperature of the media was 46°C.

^e —, Not assayed.

1.38). Alkaline phosphatase activity remained approximately equal whether cells were exposed to microwave radiation or given conventional heat treatment.

The effects of microwave radiation on the thermonuclease activity of *S. aureus* are presented in Table 2. Increased levels of activity of this enzyme could be observed when cells were exposed to microwave radiation for 10 s and then assayed at 0.5 and 1.5 to 2 h post-radiation or when they were exposed for 20 s and assayed at 1.5 h. The data also indicate that little or no enzymatic activity was present after cells were irradiated for 30 or 40 s. This corresponded to the decreases in survival observed previously (Dreyfuss et al., in press).

Previous studies involving metabolic injury by sublethal heat treatment have shown large decreases in the specific activities of malate, lactate, and oxoglutarate dehydrogenases of *S. aureus* (18). Activities of other enzymes from cells sublethally heat treated remained unaffected (6, 18). Studies involving microwave radiation of various enzymes from sources other than microorganisms have shown no effects of this treatment upon enzymatic activities (2, 3, 13, 23).

Data from the present study indicate that microwave radiation affects the metabolic activity of *S. aureus* in a manner which cannot be explained by thermal effects alone. Increases in the specific activities of several key enzyme systems from cell lysates and walls tend to verify this hypothesis.

On the other hand, thermonuclease activity correlates closely with a stimulation of growth of cultures until temperatures approach the terminal death point, where cell numbers decrease

TABLE 2. Effects of microwave radiation on thermonuclease activity of *S. aureus*

Time (h)	Diam of zones (mm)				
	Control	10 s ^a	20 s	30 s	40 s
0	0	0	0	0	0
0.5	3.25	3.50	3.00	0	0
1	4.75	4.40	4.25	0	0
1.5	5.50	6.25	5.75	2.00	0
2	6.20	6.50	6.00	2.00	0
2.5	6.75	6.75	6.25	3.00	0
3	7.50	7.25	7.25	3.75	0

^a Length of time of microwave radiation.

significantly (Dreyfuss et al., in press). Because higher levels of enzymatic activity correlated with higher cell numbers and little or no activity correlated with low cell numbers, the amount of cell biomass appears to be directly responsible for the amount of thermonuclease production and activity.

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