SHORT ARTICLE

EFFECT OF MICROWAVE ENERGY ON THE METABOLISM OF ENTEROBACTERIACEAE

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SUMMARY. The effect of microwave irradiation on the metabolism of 94 strains of Enterobacteriaceae was studied. Sixteen substrates were used and the results obtained with microwave irradiation were compared with those given by conventional biochemical tests. There was good correlation between the methods but not sufficient to enable accurate identification of unknown species. However, microwave irradiation considerably increased the enzymatic activity of bacterial suspensions and the results obtained could form the basis for a rapid method of microbial identification.

INTRODUCTION

Microwave energy is a type of high frequency radio wave, familiar to many as 'Microwave ovens'. It causes water molecules to vibrate at extremely high speeds producing friction which in turn produces heat. Dreyfuss and Chipley (1980) found that sublethal microwave irradiation of *Staphylococcus aureus* increased certain enzyme activities such as α -ketoglutarate dehydrogenase and cytochrome oxidase. Conventional biochemical tests used in the identification of bacteria depend upon such enzyme activity and the increased activity offered the possibility of more rapid results. Therefore we examined the effect of microwave radiation on the metabolism of Enterobacteriaceae in a series of tests normally used for their identification.

MATERIALS AND METHODS

Microwave oven. A domestic oven, Sharp Model 9410E with temperature and timer control, was used.

Bacterial strains. A total of 94 strains of Enterobacteriaceae were studied: *Escherichia coli* (40 strains), *Salmonella* spp. (10), *Klebsiella* spp. (10), *Proteus mirabilis* (7), *Citrobacter* spp. (6), *Enterobacter* spp. (6), *Shigella* spp. (5) *Providencia* spp. (5) and *Serratia* spp. (5). All were isolated in the Diagnostic Microbiology Laboratory, Royal Hallamshire Hospital, Sheffield, and identified by the laboratory's standard technique (Pease, 1983). Pure subcultures were incubated overnight at 37°C on blood agar.

Substrates. Stock solutions, 10% w/v in distilled water, of glucose, lactose, sucrose, mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, arabinose, raffinose and rhamnose were made and sterilised by filtration through a cellulose acetate membrane filter, pore size 0.45 μ m (Oxoid). Other tests were prepared and interpreted as described by Cowan (1974)—indole production (Method 4, Medium 2), urease (Method 2), methyl red and citrate utilisation (Method 2).

Buffer and indicators. The buffer, pH 7.2, consisted of dipotassium hydrogen orthophosphate 0.4g, potassium dihydrogen orthophosphate 0.1g and potassium chloride 0.8g (BDH Analar grade) in 1L of distilled water. Two indicators were used for evaluation—phenol red, 0.04 ml of a 1% w/v solution in distilled water and bromo-cresol purple 0.04 ml of a 0.2% w/v solution in Sorensens Phosphate Buffer 0.025 mol/L, pH 7.2.

Tests. A thick suspension, c. 10^8 cfu/ml, of the test organism was made in 3 ml of buffer and homogenised by a vortex mixer. A mixture of 100 μ l of suspension with 50 μ l of substrate was put

Received 3 May 1984; accepted 23 Jul. 1984

into each well of a microtitration plate. The microtitration plates had previously been sterilised by exposing them to microwave energy at full power (640 watts) for 5 min. The plates were sealed with 'cling film' and exposed to microwave energy at a constant temperature of 40°C for 30 min with constant radiation of 2450 MHz. An alternative method was to set the oven at Defrost (150 watts) for 5 min with no temperature control (but found not to exceed 45°C). The plates were then left at room temperature for 30 min before the results were read. The reactions observed after microwave incubation were compared with those listed in standard texts (Edwards and Ewing, 1972).

RESULTS

The results obtained are shown in the table. There is close correlation between the results obtained after microwave irradiation and those expected from standard methods; for standard reactions that are listed as positive we usually found a positivity rate > 90% although this was not true for all species. With *Serratia* spp., sucrose and sorbitol reactions were either completely negative or a minority of strains gave positive results. For those expected results listed as 'd' the irradiated suspensions in most cases gave a lower proportion of positive results (10%–40%). Some tests were positive with the majority of strains by the microwave method but negative by conventional tests: *Serratia* with raffinose, rhamnose, dulcitol; *Enterobacter* with adonitol, inositol; *Shigella* with sucrose.

The results of certain reactions could be compared with those obtained by the standard technique which had already been used to identify the bacterial strains (Pease, 1983). The percentage of strains which gave the same reaction with the two methods were: indole 94%, sorbitol 89%, inositol 77%, urease 73%, citrate 71% and rhamnose 62%. Again there was good overall correlation.

Of the two indicators used in the fermentation tests, bromo-cresol purple gave more satisfactory results because the difference between positive (yellow) and negative (purple) was easier to read than the yellow to red change of phenol red. Both methods of exposure to microwave radiation, either full power with temperature control or defrost without temperature control, gave similar results.

DISCUSSION

Various studies of the action of microwave radiation on bacteria have shown either no observable effect on bacterial growth rates (Hamrick and Butler, 1973), or a decrease in growth rate (Webb and Dodds, 1968; Webb and Booth, 1969). In each instance the temperatures of the media were controlled at $37 \pm 0.5^\circ\text{C}$, $25.5 \pm 0.5^\circ\text{C}$ and 25°C respectively. The different effects appear to depend upon the frequency and intensity of the microwave radiation. Webb and Booth (1969) believed that bacterial cells absorb microwaves of specific frequency and that the absorbed energy alters the metabolic processes and cell growth. Dreyfuss and Chipley (1980) studied the effects of sublethal microwave radiation on *S. aureus*. After exposure to 2450 MHz for periods of 10–40s, cell lysates and cell walls were assayed for enzymatic activity. Malate and α -ketoglutarate dehydrogenases, cytochrome oxidase and cytoplasmic adenosine triphosphatase activities were all increased. Membrane adenosine triphosphate, alkaline phosphatase and lactate dehydrogenase were unaffected. Other workers have examined the effect of microwave radiation on various enzymatic activities from sources other than microorganisms. These studies have included glucose-6-phosphate dehydrogenase in human blood (Belkhode, Johnson and Muc, 1974); horseradish peroxidase (Henderson, Hergenroeder and Stuchly, 1975) and human lysozyme and trypsin (Yeagers *et al.*, 1975). In each case, no effect of microwave radiation could be demonstrated.

Our results show that with a variety of substrates, microwave irradiation of bacteria caused increased enzymatic activity. It reduced considerably the time needed to obtain results in carbohydrate fermentation and other tests. This suggests that the application of microwave radiation to tests based upon pre-formed enzyme activity could provide a rapid method of microbial identification. At the moment our studies are not sufficiently reproducible to give an accurate prediction of species type, as some tests differ too much from conventional results with

TABLE
Effects of microwave irradiation on substrate metabolism

Test	Number of positive results (and expected result*) with strains of								
	<i>E. coli</i> (40)†	<i>Salmonella</i> (10)	<i>Klebsiella</i> (10)	<i>Proteus</i> (7)	<i>Citrobacter</i> (6)	<i>Enterobacter</i> (6)	<i>Shigella</i> (5)	<i>Providencia</i> (5)	<i>Serratia</i> (5)
<i>Fermentation of:</i>									
Glucose	40(+)	10(+)	10(+)	7(+)	6(+)	6(+)	5(+)	5(+)	5(+)
Lactose	40(+)	1(-)	10(+)	0(-)	1(d)	6(d)	4(w)	0(-)	1(-/+)
Sucrose	18(d)	0(-)	10(+)	3(d)	3(d)	5(d)	3(-)	1(d)	1(+)
Mannitol	40(+)	10(+)	10(+)	1(-)	6(+)	6(+)	2(+/-)	1(d)	5(+)
Dulcitol	12(d)	1(d)	3(-/+)	0(-)	2(d)	1(d)	1(d)	0(-)	5(-)
Salicin	6(d)	0(-)	9(+)	0(d)	3(d)	5(d)	0(-)	0(-)	0(-)
Adonitol	2(-)	0(-)	8(+/-)	0(-)	0(-)	4(-)	0(-)	2(d)	5(d)
Inositol	1(-)	5(d)	10(+)	1(-)	0(-)	3(-)	0(-)	2(d)	0(d)
Sorbitol	39(+)	10(+)	10(+)	1(-)	6(+)	5(+)	2(d)	0(-)	0(+)
Arabinose	40(+)	9(+)	10(+)	0(-)	6(+)	6(+)	0(d)	0(-)	0(-)
Raffinose	14(d)	1(-)	10(+)	0(-)	3(d)	5(d)	3(d)	0(-)	4(-)
Rhamnose	15(d)	10(+)	10(+)	1(-)	6(+)	6(+)	4(d)	0(-)	3(-)
Indole production	40(+)	0(-)	3(+/-)	1(-)	0(-)	0(-)	0(-/+)	5(+)	0(-)
Urease production	0(-)	0(-)	0(+/-)	6(+)	0(d)	6(d)	0(-)	0(-)	0(-)
Citrate utilisation	0(-)	0(d)	9(+)	6(+)	5(+)	3(+)	0(-)	3(+)	5(+)
Methyl Red test	40(+)	10(+)	0(-)	7(+)	6(+)	3(+)	0(+)	5(+)	1(-/+)

*According to standard text (Edwards and Ewing, 1972); (+), 90% or more positive; (-), 90% or more negative; (d), different biochemical types; (w), delayed positive; (-/+), majority negative and (+/-), majority positive.

†Number of isolates tested.

respect to *Serratia*, *Enterobacter* and *Shigella* species. It will be necessary to examine larger numbers of strains of more species. For full identification it will be necessary to expand the range of tests to include for example, the Voges-Proskauer reaction and to determine if these can also be performed successfully in the shorter time. The mechanism(s) of these alterations in substrate utilisation are debatable. Microwave radiation seems to affect metabolic activities in a manner which cannot be explained by thermal effects alone (Dreyfuss and Chipley, 1980). Preliminary studies (unpublished observations) with transmission and scanning electron microscopy, show that the bacterial cell walls are damaged by exposure to microwave irradiation allowing the intracellular contents to escape. Therefore, the enzymes may be able to react with the substrates in a much shorter time. Our own investigations have shown that the supernates from bacterial suspensions exposed to the microwaves have increased enzyme activity compared with those from control cells, which suggest the escape of enzymes into the extracellular medium. The damage to the cell walls could also explain why some reactions were positive with microwave irradiation when a negative result was expected, as the test then does not depend on substrate uptake by the cell.

We thank Professor B. I. Duerden for much helpful discussion in drafting this manuscript.

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