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Studies on the heat resistance of *Bacillus cereus* spores and growth of the organism in boiled rice

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SUMMARY

A comparison was made of the heat resistance of *Bacillus cereus* spores at 95 °C. Spores of serotype 1 strains were more resistant than those of the other types tested. However, there was little difference in the growth rate of the various serotypes in boiled rice at 22 °C. Most samples of uncooked rice contained multiple serotypes of *B. cereus*.

These results indicate that the cooking procedure used for the preparation of cooked rice is likely to be selective for certain serotypes, and this is the most likely reason why type 1 is the most common serotype implicated in outbreaks of food poisoning and can be isolated from many routine samples of cooked rice.

INTRODUCTION

Bacillus cereus causes two distinct types of food poisoning characterized by diarrhoea and abdominal pain or by nausea and vomiting. The first type occurs 8–16 h and the second type 1–5 h after ingestion of contaminated food. Incidents reported from Great Britain have usually been of the vomiting type and mainly associated with the consumption of cooked rice from Chinese restaurants and 'take-away' shops. More than 100 such incidents have been reported and similar accounts have been described in Australia, Canada, Finland, Japan, the Netherlands and the U.S.A. (see Gilbert & Parry, 1977; Gilbert, 1979).

Taylor & Gilbert (1975) described the application of a serotyping scheme to the investigation of *B. cereus* food poisoning. Serotype 1 either alone or with other types is responsible for about 70% of the rice-associated outbreaks in this country and overseas.

Gilbert & Parry (1977) studied the distribution of serotypes among 400 cultures of *B. cereus* isolated from various foods. For boiled and fried rice 23% of the cultures were type 1 compared with only 3% for uncooked rice. In contrast 15% of the cultures from uncooked rice were type 17 compared with only 1% for cooked rice. It was suggested that factors such as heat resistance or growth rate might be selective for certain serotypes and the aim of this paper was to extend some earlier work (Gilbert, Stringer & Peace, 1974) with a further study of these properties.

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MATERIALS AND METHODS

Strains

The strains of *B. cereus* and their source and serotype are given in Table 1. Serotyping was carried out using the scheme of Taylor & Gilbert (1975) extended to include a further five serotypes.

Table 1. *Sources and serotypes of* B. cereus *and heat resistance of their spores in aqueous suspension*

Strain	Source		Serotype	Decimal reduction time (min) at 95 °C
4810/72	Vomitus	Food poisoning outbreaks*	1	9·5
3556/73	Fried rice		1	24·0
4089/73	Faeces		1	29·1
3011/74	Fried rice		1	27·8
3982/75	Fried rice		1	24·7
4174/75	Fried rice		1	36·2
4621/75	Fried rice		1	26·1
2057A/76	Boiled rice		1	25·8
2423/76	Cooked chicken		1	22·4
3463/76	Cooked chicken		1	29·4
3642/76	Faeces		1	32·0
3605/73	Boiled rice		3	5·4
4431/73	Indonesian rice dish		8	5·4
210/76	Indonesian rice dish		20	2·5
2146B/74	Samples of uncooked rice		1	2·9
2739/74			1	16·2
536A/76			1	19·7
530A/76			3	3·7
535C/76			5	2·2
3390/74			8	4·2
378E/76			12	6·0
3565/73			17	2·3
2737/74			17	2·5
529A/76			17	4·1
6833/71			18	1·5
3389/74			18	2·1
654A/76			20	2·1

* Vomiting type syndrome.

Preparation of spore suspensions

The sporulation medium distributed in 500 ml medical flats was nutrient broth containing 1·2 % agar, 0·025 % KH_2PO_4 and 0·003 % $MnSO_4$. After incubation at 30 °C for 10 days the growth was scraped from the surface of the agar and suspended in sterile distilled water. Vegetative cells were removed from the suspension by washing three times and separating by differential centrifugation (Long & Williams, 1958). Aqueous spore suspensions, all containing > 95 % and usually > 99 % phase-bright spores, were stored at 4 °C.

Diluent and colony plate count method

All dilutions were made in quarter-strength Ringer's solution. Colony plate counts were made using a surface drop technique on blood agar containing 5% defibrinated horse blood with incubation for 18–24 h at 35 °C.

Determination of heat resistance of B. cereus *spores in aqueous suspension*

Samples (0·2 ml) of each suspension containing between *ca.* 10^6 and 10^7 spores/ml were distributed into 2 ml freeze-drying ampoules which were sealed under air. The ampoules were heated at 95 °C by total immersion in a thermostatically controlled water bath. At appropriate time intervals an ampoule was removed and immediately cooled in an ice-water mixture. Each ampoule was opened, the contents washed out into 1·8 ml of diluent and tenfold dilutions prepared and plated.

Survivor curves of log percentage surviving organisms against time were constructed using the mean count from three unheated ampoules as the 100% level. Decimal reduction times (D), the time required to reduce the number of surviving organisms by 90% at a constant temperature, were calculated from regression analysis of $\log_{10}$ colony plate counts for various intervals of time, using the digital computer program of Navani, Scholefield & Kibby (1970).

Growth of B. cereus *in boiled rice*

A mixture of long grain rice and cold water in a 1:2 ratio was brought to the boil and allowed to simmer with occasional stirring until all the water was absorbed, *ca.* 20 min. The boiled rice was rinsed once in boiling water to facilitate separation of the grains and after cooling 10 g samples were distributed into 450 g screw-capped jars.

Dilutions of spore suspensions representing serotypes 8, 17 and 18 and five strains of serotype 1 were prepared and 0·2 ml volumes distributed onto the surface of the rice to give an initial inoculum of *B. cereus* spores between $3{\cdot}2 \times 10^2$ and $2{\cdot}7 \times 10^4$/g of rice. Sets of jars were incubated at 22 °C for periods of time up to 40 h. Jars were removed at suitable time intervals and 90 ml of diluent added to each to give a 1/10 dilution. After thorough mixing, further tenfold dilutions were prepared and plated on blood agar for counts. The experiments were repeated using spore suspensions which had received a heat-shock treatment for 10 min at 80 or 90 °C.

Isolation of B. cereus *from uncooked rice*

Three 25 g portions from each of 10 samples of uncooked long grain rice received for routine examination were mixed with:

(1) 100 ml of nutrient broth.

(2) 50 ml of quarter-strength Ringer's solution at 80 °C; the temperature was maintained for 10 min, the mixture cooled rapidly and 50 ml of double-strength nutrient broth added.

(3) 50 ml of quarter-strength Ringer's solution at 95 °C, then proceeding as described for (2).

After incubation for 18 h at 35 °C, subcultures were made onto blood agar. The identity of *B. cereus* colonies was confirmed by subculture on Kendall's B.C. medium (Gilbert & Taylor, 1976) and fermentation tests with glucose, arabinose, mannitol and xylose ammonium salt sugars. Fifteen colonies, five for each treatment, were selected for serotyping.

RESULTS AND DISCUSSION

Results of heat resistance studies on spores of the various *B. cereus* strains were linear when plotted as log percentage survivors against time of heating. All the correlation coefficients were greater than tabulated values at $P = 0{\cdot}05$ for the appropriate degrees of freedom; the curves are therefore exponential. Table 1 shows that, with the exception of 2146B/74, strains of serotype 1 had greater calculated D values (range 9·5–36·2 min, mean 24·8 min) than strains representing the other serotypes tested (range 1·5–6·0 min, mean 3·3 min). Spores of 10 of the 11 serotype 1 strains from separate outbreaks of food poisoning had D values between 22·4 and 36·2 min.

Table 2. *Growth from unheated and heat treated* spores of* B. cereus *in boiled rice stored at 22 °C*

Strain	Serotype	Heat treatment	Log count of *B. cereus* in boiled rice at 22 °C after storage (h)					
			0	6	9	18	24	40
4810/72	1	None	3·50	4·00	5·93	8·18	7·74	8·00
		80 °C	3·15	3·50	5·00	7·09	7·54	7·81
		90 °C	3·04	3·75	4·65	7·54	7·40	7·65
3556/73	1	None	3·45	4·09	5·81	8·30	7·70	8·18
		80 °C	3·20	3·40	4·18	7·09	6·84	7·18
		90 °C	3·15	3·43	4·78	7·40	7·40	8·00
4174/75	1	None	2·90	3·34	5·00	6·60	6·48	7·00
		80 °C	3·08	3·30	4·00	7·24	7·30	7·65
		90 °C	3·18	3·20	4·70	6·87	7·30	7·54
2146B/74	1	None	3·60	5·18	5·98	6·30	6·00	7·81
		80 °C	3·50	3·70	5·00	7·30	6·70	6·65
		90 °C	3·48	3·20	4·40	7·30	6·40	6·70
536A/76	1	None	3·70	4·18	5·60	7·00	7·30	7·54
		80 °C	2·70	2·84	3·72	6·48	7·48	8·18
		90 °C	3·00	2·90	3·85	6·65	6·70	7·48
4431/73	8	None	4·40	5·30	6·74	7·40	7·00	8·30
		80 °C	2·90	3·08	4·15	7·30	6·84	7·18
		90 °C	2·70	3·11	3·80	6·81	6·78	6·48
2737/74	17	None	3·65	4·70	6·30	7·40	7·54	7·65
		80 °C	3·30	3·79	5·00	7·18	7·70	8·18
		90 °C	3·25	3·70	5·00	7·65	8·18	8·18
3389/74	18	None	3·60	4·54	5·48	7·30	7·54	7·74
		80 °C	2·84	2·90	4·70	7·00	7·60	7·74
		90 °C	2·48	< 200	2·30	4·00	7·18	7·40

* Heat treatment of spores at 80 or 90 °C for 10 min before inoculation onto surface of rice.

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Table 3. *Distribution of serotypes of* B. cereus *isolated from 10 samples of uncooked rice*

Sample	Serotypes isolated from uncooked rice after: No heat treatment*	Heating 80 °C/10 min*	Heating 95 °C/10 min
1	17 (4) NT	20 (2) NT (3)	23 NT (4)
2	NT (5)	17 20 NT (3)	20 (5)
3	20 NT (4)	NT (5)	20 (2) NT (3)
4	17 (5)	12 20 NT (3)	18 NT (4)
5	NT (5)	3 (3) NT (2)	20 NT (4)
6	17 (4) NT	18 NT (4)	NT (5)
7	17 (5)	17 (5)	NT (5)
8	13 (2) NT (3)	2 8 20 NT (2)	13 (4) 17
9	17 (2) NT (3)	17 (4) NT	NT (5)
10	NT (5)	13 NT (4)	NT (5)

NT = not typable.
* Five colonies were selected for serotyping; number of each type in parentheses.

Table 2 shows that all the strains tested grew well in boiled rice stored at 22 °C. A heat-shock treatment of 10 min at 80 or 90 °C on spores of *B. cereus* had little effect on subsequent vegetative cell growth in boiled rice.

B. cereus has been isolated, usually at levels of $< 100/g$, from *ca.* 90 % of samples of uncooked rice examined in this laboratory and can be considered as part of the normal flora. Table 3 confirms our previous findings that most samples contain multiple serotypes (Gilbert & Parry, 1977); between two and six types were isolated from each sample of rice. Of the 150 colonies tested, 31 were type 17, 14 were type 20 and 89 were not typable: other serotypes isolated were 2, 3, 8, 12, 13, 18 and 23. Serotype 17 is much less common in cooked rice than in uncooked rice because of its relatively low resistance to heat. In the present study type 17 was isolated from six of the 10 samples of uncooked or heat treated (80 °C/10 min) rice tested, but only once from rice heated at 95 °C for 10 min and even then only one of the five colonies tested was this type.

These studies indicate that the cooking procedure used for the preparation of boiled rice is likely to be selective for relatively heat-resistant spores and that heat

resistance is a property of certain serotypes. This probably explains why type 1 is the most common serotype in routine samples of cooked rice (Gilbert & Parry, 1977). Outbreaks of the vomiting type syndrome are often associated with serotype 1 strains and feeding experiments in monkeys have confirmed that illness is due to a very heat-stable, low molecular weight enterotoxin which is produced in boiled rice (Melling *et al.* 1976, 1978; Melling & Capel, 1978).

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