

## Unraveling the role of *Bacillus* spp. Involved in Food Poisoning and Food Spoilage

### Introduction

The aerobic spore-forming Gram Positive Bacilli belonging to the genus *Bacillus*, and other closely related genera are playing an increasingly important role in the food and beverage industries. However, the significance of these organisms is poorly understood.

The extent to which *Bacillus* and closely related genera may be responsible for outbreaks of food associated illness is most likely understated in many regions of the world. This in most cases may be due to the emphasis being on alternative foodborne organisms such as *Salmonella* spp. and *Campylobacter* spp.

- In the UK, USA and Australia – *Bacillus* spp. is reported to be responsible for 5% of food poisoning cases. (1)
- Scandinavia and Canada report rates as high as 10 – 47%, whilst (1)
- Asian countries have reported incidences as high as 42%. (2)

The reported cases of food poisoning contributed to “*Bacillus* and closely related genera” has also resulted in a wide range of food types being implicated (Table 1.)

In addition, historically all investigations of food poisoning where *Bacillus* is suspected have focussed on the isolation and identification of *B. cereus*.

Meat (pies, stews, curries, sandwiches)  
Chinese food  
Poultry and Poultry products  
Seafood and Seafood products  
Bakery products  
Cereal products  
Rice  
Asian Boxed meals  
Dairy products (milk, UHT milk, yoghurt, cream)

Table 1. Confirmed sources of *Bacillus* related food poisoning.

However, numerous reports are now available to suggest that in many of these cases species other than *B. cereus* (Table 2.) may be the cause ( 3,4)

*Bacillus* investigations should not be restricted to *B. cereus* but should be extended to include all species including those in the closely related groups.

### Why is the role of *Bacillus* understated?

The role of *Bacillus* as a food poisoning agent is most likely understated due to a combination of factors which may include:

- The role of *Bacillus* spp. other than *B. cereus* not being recognised.
- A lack of recognition of the potential range of food types that may be involved.
- Confusion over the taxonomy of “*Bacillus* and closely related genera”.
- Difficulties with the identification of “*Bacillus* and closely related genera”.

**Bacillus spp.**  
*B. cereus*  
*B. mycoides*  
*B. weihenstephanensis*  
*B. coagulans*  
*B. licheniformis*  
*B. subtilis*  
*B. pumilus*  
*B. thuringiensis*  
*B. sphaericus*  
*B. circulans*

**Paenibacillus spp.**  
*P. alvei*

**Brevibacillus spp.**  
*Br. brevis*

**Table 2. Major mesophilic *Bacillus* spp. and related species causing food poisoning or other problems in food.**

## Taxonomy

In 1952, Smith *et. al.* (2) developed a diagnostic scheme for the identification of *Bacillus* spp. which was based on sporangial morphology and selected biochemical and physiological tests. This scheme recognised 18 species which were able to be divided into 3 groups based upon their sporangial morphology. This scheme, although effective was limited by the availability of specialized culture media. In 1973, this scheme was further updated by Gordon *et. al.* (3) and again by Logan and Berkeley in 1984 (4). At this point in time, based on phenotypic characteristics, 38 species were recognised. Using 16S rDNA studies, at least 128 species including an additional 16 genera have been described which embraces “*Bacillus* and closely related genera”.

## Identification

Due to the diverse ranges in optimal growth conditions, the identification of *Bacillus* spp. and closely related genera has been difficult. These difficulties have been worsened by a lack of standardization in the methods employed.

In terms of practicality, the use of physiological and morphological methods are the most practical for food and beverage testing laboratories. Unfortunately however, many of

the physiological tests commonly employed for bacterial identification have been found to produce incorrect or inconsistent results when applied to the identification of “*Bacillus* and closely related genera”. Many species are strongly proteolytic and will produce false positive urease reactions when certain formulations of urease test media are used. Similarly, when peptone based carbohydrate fermentation media are used, results may initially present as positive and then change to negative after further incubation.

The use of these tests is further complicated by the similarities in the physiological characteristics of many species. Based on these characteristics, the “*Bacillus* and closely related genera” may be divided into 2 groups, the reactive species which will give positive results in many of the routine biochemical tests available, and the nonreactive species which have few if any positive results in such tests. The nonreactive isolates are often members of the genus *Brevibacillus*.

When food and other industrial laboratories are required to identify “*Bacillus* and closely related genera” the usual concern is that the isolate is *B. cereus* or another species, by implication, the other species being non-pathogenic. Although *B. cereus* and *B. subtilis* are more easily recognisable, strains of other species including *Brevibacillus* and *Paenibacillus* are more difficult. The easily recognisable species tend to be reactive in a range of commonly employed biochemical tests, whilst *Brevibacillus* is inactive in these media. *Paenibacillus* however is the opposite, being highly reactive in a wide range of tests resulting in very few tests being available to differentiate between the species.

## Introducing Microgen Bacillus ID

Microgen<sup>®</sup> Bacillus ID (MID 66) is the newest addition to the Microgen ID product range (currently Gram Negative Bacilli and *Listeria* spp.). This identification system has been specifically designed to enable the easy identification of these “*Bacillus* and closely related genera” commonly implicated as causes of food poisoning and food spoilage. By focussing on these key species which have both

<b>Bacillus spp.</b>	<i>B. alvei</i>
<i>B. cereus</i> group	<i>B. pumilus</i>
<i>B. firmus</i>	<i>B. licheniformis</i>
<i>B.adius</i>	<i>B. megaterium</i>
<i>B. laevolacticus</i>	
<i>B. coagulans</i>	<b>Vergibacillus spp.</b>
<i>B. lentus</i>	<i>V. pantothenicus</i>
<i>B. amyloliquefaciens</i>	
<i>B. subtilis</i>	<b>Paenibacillus spp.</b>
<i>B. circulans</i>	<i>P. polymyxa</i>
<i>B. insolitus</i>	<i>P. macerans</i>
<i>B. thiaminolyticus</i>	
<i>B. freudenreichii</i>	<b>Brevibacillus spp.</b>
<i>B. globisporus</i>	<i>B. brevis</i>
<i>B. sphaericus</i>	<i>B. laterosporus</i>

Note: *B. cereus* group consists of *B. cereus*, *B. thuringiensis*, *B. mycoides* and *B. weihenstephanensis*.

**Table 3. *Bacillus* and closely related genera identified using MID Bacillus.**

BAC 1	BAC2
Arabinose	Adonitol
Cellobiose	Galactose
Inositol	Methyl-D-Mannoside
Mannitol	Methyl-D-Glucoside
Mannose	Inulin
Raffinose	Melizotose
Rhamnose	Indole
Salicin	ONPG
Sorbitol	Nitrate
Sucrose	Arginine Dihydrolase
Trehalose	Citrate Utilization
Xylose	Voges Proskauer
	Control

**Table 4. Substrates included in the MID Bacillus.**

public health and economic implications (Table 3), Microgen Bioproducts has been able to develop an identification system based on a total of 24 conventional biochemical substrates (Table 4.). These substrates have been specifically formulated to ensure that those

problems associated with conventional substrates (discussed earlier) do not occur.

The 24 substrates included in this identification system are housed in the standard microwell format employed in all of our identification systems. To achieve this, 2 separate strips are included for each identification (BAC 1 and BAC 2).

Sufficient colonies of the isolate to be identified are selected from a pure culture plate and emulsified in the 3ml *Bacillus* suspending media provided, to produce a suspension whose turbidity is equivalent to a MacFarland 2.0 standard. Approximately 100µl of this suspension is inoculated into each microwell of the 24 well test system which is incubated at 30°C. After 24 hours incubation the 18 carbohydrate reactions, ONPG and citrate results are recorded and the strips returned to 30°C for a further 24 hours. After a total of 48 hours incubation the strips are re-read after the addition of the appropriate reagents, the Indole, Nitrate and VP tests are read (see Figure 1), and the results are recorded on the report form provided and the 8 digit Octal Code is calculated. The code is entered in to the Microgen® Identification System Software and a result generated.

### Performance

A total of 49 strains of “*Bacillus* and related genera” comprising strains from recognised culture collections and isolates from various food and beverage sources were identified using the Microgen® *Bacillus* ID (MID - 66) and the API System comprising the API 50CHB and the API 20E. The Microgen® *Bacillus* ID comprising 24 substrates and the API system combination



**Figure 1. MID Bacillus strips after incubation (BAC1, top, BAC2, bottom)**

requiring the inoculation of 70 separate substrates. Both systems were inoculated and incubated according to the manufacturers recommendations.

The Microgen® Bacillus ID successfully identified all of the isolates tested (Table 5.). The API system combination of the 50CHB and 20E was also able to identify 100% of the isolates however some taxonomy employed was found to be out of date (see Notes, Table 5).

## References

1. Opinion of the Scientific Committee on Animal Nutrition on the Safety of Use of Bacillus Species in Animals. 2000. European Commission, Health and Consumer Protection Directorate-General. pp 3- 4.
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3. Kramer J.M. and R.J. Gilbert (1989) *Bacillus cereus* and other *Bacillus* species In M.P. Doyle (Ed) Food borne Bacterial Pathogens. Marcel Dekker, New York. pp21 – 70.
4. Gilbert R.J., P.C.B Turnbull, J.M. Parry and J.M. Kramer (1981) *Bacillus cereus* and other *Bacillus* species: their part in food poisoning and other clinical infections. In The Aerobic Endospore-forming Bacteria: Classification and Identification. Academic Press, London pp 297 – 314.

	Total Tested	MID-66	API
<i>B. firmius</i>	2	2	2
<i>B. cereus</i>	11	11	11
<i>B. licheniformis</i>	11	11	11
<i>P. macerans</i>	1	1	1
<i>B. megaterium</i>	1	1	1
<i>B. pumilus</i>	4	4	4
<i>B. sphaericus</i>	3	3	3
<i>B. subtilis</i> (1)	11	11	11
<i>B. thuringiensis</i> (2)	2	2	0
<i>P. alvei</i>	1	1	1
<i>B. circulans</i>	2	2	2
Total	49	49	47
Percent Correct		100.0%	95.9%

**Table 5. Summary of Microgen ID Bacillus and API 50CHB + 20E identification results.**

1. 2 isolates identified a *B.amyloliquefaciens* - a member of the *B.subtilis* group
2. 2 *B.thuringiensis* not included in API database - isolates identified a *B.cereus*. *B.thuringiensis* a member of the *B.cereus* group. If the ID of *B.cereus* considered correct, API = 100%

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