

A New Method for the Identification of *Bacillus* spp. and Related Species Involved in Food Poisoning and Spoilage

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Introduction

The aerobic spore-forming Gram Positive Bacilli belonging to the genus *Bacillus*, and other closely related species play an important role in food poisoning and spoilage. However, due to the lack of standardized methods, the identification of these organisms in food testing laboratories has been difficult. Although the use of morphological and physiological tests have provided the best means available for laboratories to identify organisms, these methods have proven to be inconsistent and generally unreliable for this group of organisms. As a result of the absence of reliable and standardized methods for the identification of this group of organisms, investigators have generally focused on the isolation and identification of *B. cereus* as a causative agent of food related illnesses. However, numerous other investigations have demonstrated that a considerably larger range of species (Table 1) can cause food related illness (1,2).

| Bacillus spp. | |
|------------------------------|-------------------------|
| <i>B. cereus</i> | <i>B. subtilis</i> |
| <i>B. mycooides</i> | <i>B. pumilus</i> |
| <i>B. weihenstephanensis</i> | <i>B. thuringiensis</i> |
| <i>B. coagulans</i> | <i>B. sphaericus</i> |
| <i>B. licheniformis</i> | <i>B. circulans</i> |
| Paenibacillus spp. | |
| <i>P. alvei</i> | |
| Brevibacillus spp. | |
| <i>Br. brevis</i> | |

Table 1. Major mesophilic *Bacillus* spp. and related species causing food poisoning or other problems in food.



Figure 1. Inoculation of the Microgen® Bacillus ID test panels

Microgen® Bacillus ID, developed by Microgen Bioproducts, UK is a simple biochemical identification system based on 24 selected substrates for the standardized identification of 23 *Bacillus* spp and closely related organisms involved in both food poisoning and spoilage (Table 2). All substrates included in the identification system have been specifically formulated to ensure optimal performance and consistency (Table 3). The identification system is supported by the Microgen® Identification System Software to assist in the interpretation of the results achieved.

The performance of the Microgen® Bacillus ID was compared with the API Bacillus identification system comprising the API 50CHB and API 20E identification systems from BioMerieux (France).

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

Note: *B. cereus* group consists of *B. cereus*, *B. thuringiensis*, *B. mycooides* and *B. weihenstephanensis*. *B. subtilis* Group consists of *B. subtilis* and *B. amyloliquefaciens*

Table 2. *Bacillus* and closely related genera identified using MID Bacillus.

Materials and Methods

Bacterial Strains

A total of 49 isolates obtained from recognized culture collections, and fully characterised isolates from a diverse range of foods and beverages were identified using both systems. All cultures were maintained in the –80°C culture collection prior to use in this investigation. Immediately prior to investigation, all cultures were subcultured onto sheep blood agar plates and incubated for 18–24 hours at 37°C.

Identification

Sufficient growth of each isolate being identified was inoculated into the suspension medium supplied with the Microgen® Bacillus ID so as to produce an inoculum density equivalent to a MacFarland 2 standard. Two separate suspensions were prepared for use with the API systems, one for the API 20E, the other for the API 50CHB. Both were prepared at a density equivalent to a MacFarland 2 standard. Using sterile pasteur pipettes, approximately 100µl (3 drops) of the organism suspension was inoculated into each well of the Microgen® Bacillus ID (Figure 1). The API systems were inoculated in accordance with the manufacturers instructions. After inoculation, all identification systems were incubated at 30°C for 48 hours (Figure 2). Each system was read after 24 hours incubation, with a final reading being made after 48 hours incubation. Following incubation, each identification system was read in accordance with the manufacturers instructions. All results were recorded on the work sheets provided and interpreted using the database systems provided for each system.



Figure 2. MID Bacillus strips after incubation (BAC1, top, BAC2, bottom)

Results

A total of 49 strains of “*Bacillus* and related genera” comprising strains from recognised culture collections and fully characterised 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 successfully identified all of the isolates tested whilst the API system combination of the 50CHB and 20E was able to identify 95.9% of the isolates (Table 4).

The API Bacillus identification system failed to identify 2 isolates of *B. thuringiensis*. This organism was not included in the API database, however they were identified as *B. cereus*. *B. thuringiensis* is deemed to be a member of the *B. cereus* group according to current taxonomy.

Discussion

The Microgen® Bacillus ID comprising 24 substrates has been specifically designed for the identification of *Bacillus* spp., whilst the API Bacillus identification system requires the use of both the API 20E and the API 50CHB, which have been adapted to the identification of this group of organisms. The combination of the two API identification systems also required the inoculation and interpretation of 70 separate substrates many of which are duplicated between the two systems.

The Microgen® Bacillus ID is designed specifically for the identification of those species of aerobic spore-forming Gram Positive Bacilli associated with food poisoning and food spoilage. The system is simple to use, standardized and provides accurate identification of the target organisms. The database provide to assist in the interpretation of results is extensive and employs current taxonomy.

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

Table 3. Substrates included in the Microgen® Bacillus ID.

| | Total Tested | MID-66 | API |
|-----------------------------|--------------|-----------|-----------|
| <i>B. firmus</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% |

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%

Table 4. Summary of Microgen® Bacillus ID and the API 50CHB + 20E identification results.

References

- 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. pp 21–70.
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