

P-061 Evaluation of a New Biochemical Identification System for the Rapid Speciation of mesophilic *Bacillus* Species Isolates Associated with Food Spoilage and Food Poisoning.

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ABSTRACT

Bacillus species are ubiquitous gram positive spore-bearing bacilli some of which are commonly associated with food poisoning and food spoilage. They tend to be aerobic or facultative anaerobes and are found in a wide range of environments including soil, decaying vegetable plants and some are insect pathogens. As some of these species affect the quality of various food and beverage products they are routinely isolated in food testing laboratories. Currently the only commercial biochemical typing scheme to differentiate the individual *Bacillus* species isolates recovered from food samples requires a heavy inoculum and a minimum of 70 individual biochemical tests to achieve an adequate speciation.

In an attempt to simplify the biochemical identification of commonly isolated *Bacillus* species from food and beverage samples we have designed a 24 biochemical test identification system. The Microgen™ Bacillus ID kit includes 18 carbohydrate and six non-carbohydrate substrates which are presented in 2 x 12 well micro-titre strips. The bacteria are suspended in an appropriate suspension broth then seeded into each well of the 2 x 12 well strips. The strips are read at 48 hours and the results recorded and analysed using a dedicated computer system.

Bacillus species can be sub-divided into three groups based on their optimal growth conditions. Thermophiles grow optimally above 45°C, Mesophiles grow optimally between 45 – 25°C and Psychrophiles grow optimally below 25°C. This new Bacillus identification system is designed to identify mesophilic *Bacillus* species which are the most commonly isolated species from food and environmental samples.

The new Bacillus ID system is compact easy to use and requires only a MacFarland 2.0 inoculum to seed the substrate micro-wells. This compares very favourably to the existing commercial system. The performance of the various substrates has been confirmed by testing *Bacillus* species isolates from our culture collection and it is clear from the profiles produced that our results deliver much more definitive, higher percentage probability, identifications than the existing commercial system giving the operator more confidence in the results achieved.

BACILLUS & RELATED SPECIES IDENTIFIED

Bacillus species

- B. cereus* group
- B. firmus*
- B.adius*
- B. leuvalacticus*
- B. coagulans*
- B. lentus*
- B. amyloliquefaciens*
- B. subtilis*
- B. insolitus*
- B. thiaminolyticus*
- B. freudenreichii*
- B. globisporus*
- B. sphaericus*
- B. pumilus*
- B. licheniformis*
- B. megaterium*

Vergibacillus species

- V. pantothenicus*

Paenibacillus species

- P. alvei*
- P. polymyxa*
- P. macerans*

Brevibacillus species

- Br. brevis*
- Br. laterosporus*

MICROGEN™ BACILLUS ID METHOD



Prepare a McFarland 2.0 suspension in the suspending broth provided



Peel back the adhesive strip (do not remove)
Add 2-3 drops suspension to each well.
Add 2-3 drops mineral oil to the arginine well



Reseal the strip and incubate at 30°C for 24 hours.
Take an initial reading and return to the 30°C incubator
After a total of 48 hours incubation, add the chemical reagents to the appropriate wells.
Record the results on the Report Form

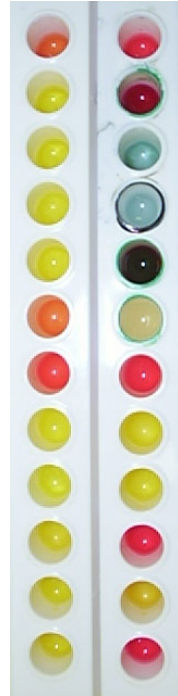
MICROGEN BACILLUS-ID 24 TEST REPORT FORM

Lab. No. F4560 Specimen Type Coarse Rice Date 12-10-04

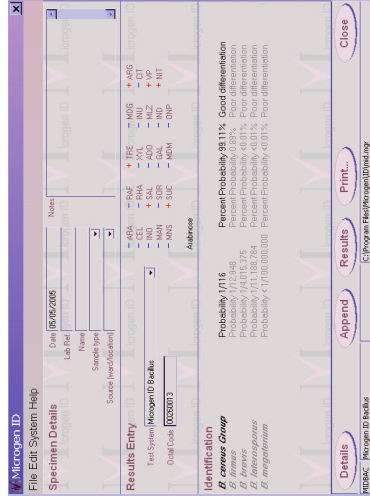
Well Number	Bacillus Strip (BAC 2)																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Reaction	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
48 hours	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Sum of Positive Reactions	0	0	2	6	0	0	1	3																

Profile No. 00260013 Final Identification: *Bacillus cereus* group

Microgen™ Bacillus ID Typical 48H Result 30 Minutes Post Addition of Reagents



MICROGEN™ BACILLUS ID DEDICATED SOFTWARE ANALYSIS OF IDENTIFICATION RESULTS



MICROGEN™ BACILLUS ID SUBSTRATE SYSTEM

Well	Substrate	Reaction	Positive	Negative
1	Arabinose			
2	Cellobiose			
3	Inositol			
4	Mannitol			
5	Mannose			
6	Raffinose			
7	Sorbitose			
8	Sucrose			
9	Sorbitol			
10	Sucrose			
11	Trehalose			
12	Xylose			
13	Adonitol			
14	Inositol			
15	Methyl-D-Mannoside			
16	Methyl-D-Glucoside			
17	Inulin			
18	Mellicose			
19	Inedible			
20	ONPG			
Plus reagent	Nitrate			
21	Arginine Dihydrolyse			
22	Citrate Utilisation			
23	Voges-Proskauer			
24	Control			

ACKNOWLEDGEMENTS

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MICROGEN™ BACILLUS ID COLOUR CHART

MICROGEN™ BACILLUS ID COLOUR CHART

Colour chart (Farbtafel)/Tableau de couleurs
Microgen™ Bacillus ID MUD-66
Read after 24 and 48 hours

REACTION INDEX	24 Hours (1)	48 Hours (2)	19	20	21	22	23
Reaction	Red	Red	Yellow	Yellow	Yellow	Yellow	Yellow
Carbohydrate (MUD-66)	Red	Red	Yellow	Yellow	Yellow	Yellow	Yellow
Positive	Red	Red	Yellow	Yellow	Yellow	Yellow	Yellow

Legend:
 ○ Arginine response to nitrate at 48 hours after incubation
 ○ Positive
 ○ With some *Bacillus* spp. negative carbohydrate fermentation control colour may still form due to change over time, when colour is negative at 48 hours, refer to the negative control in well 24.

PRODUCT PERFORMANCE

Bacillus species	Total Tested	Microgen Bacillus ID	API 50CHB + 20E (see note 3)
<i>B. firmus</i>	2	2	2
<i>B. cereus</i>	11	11	13
<i>B. licheniformis</i>	1	1	1
<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> (see note 1)	11	11	11
<i>B. thuringiensis</i> (see note 2)	2	2	0
<i>P. alvei</i>	1	1	1
<i>B. circulans</i>	2	2	2
<i>B. circulans</i>	2	2	2
Total	49	49	49

Notes:
 1. Two isolates identified as *B. amyloliquefaciens* - a member of the *B. subtilis* group
 2. Two *B. thuringiensis* not included in API database - isolates identified as *B. cereus*. *B. thuringiensis* a member of the *B. cereus* group. If the ID of *B. cereus* considered correct, API = 100%
 3. Using API 50CHB + 20E and API Database

CONCLUSIONS:

1. The Microgen™ Bacillus ID is the first dedicated test for the biochemical identification of mesophilic *Bacillus* species
2. The 24 test system is easy to use and reproducible
3. Mesophilic *Bacillus* species are difficult to differentiate biochemically so some isolates can only be identified to the group level e.g. the *Bacillus cereus* group
4. The colour chart and dedicated software make analysis of the results easy to perform
5. The kit produces results comparable to the 75 test API CH50 + 20E combination