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 aerobes or facultative anaerobes and are catalase positive. The various species can be found in many environmental reservoirs including soil, decaying vegetables, 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 24 and 48 hours and the results recorded and analysed using a dedicated software programme.

Bacillus species can be sub-divided into three groups based on their optimal growth conditions. Thermophiles grow optimally above 45ºC, Mesophiles grow optimally between 45 – 25ºC and Psychrophiles grow optimally below 25ºC. This new Bacillus identification system is designed to identify 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. badius
B. laevolacticus
B. coagulans
B. lentus
B. amyloliquefaciens
B. subtilis
B. circulans
B. insolitus
B. thiaminolyticus
B. freundenreichii
B. globisporus
B. sphaericus
B. pumilus
B. licheniformis
B. megaterium

Vergibacillus species

V. pantothenticus

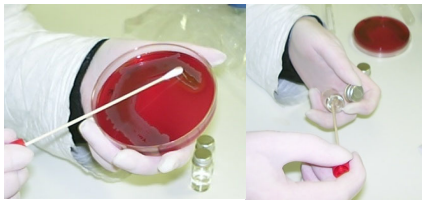
Paenibacillus species

P. alvei
P. polymyxa
P. macerans

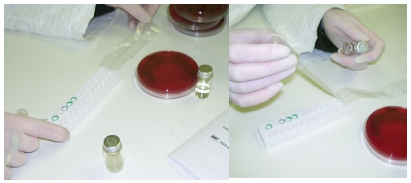
Brevibacillus species

Br. brevis
Br. laterosporus

MICROGEN™ BACILLUS ID METHOD



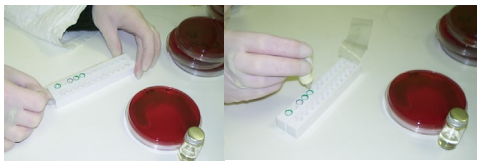
Prepare a McFarland 2.0 suspension in the suspending broth provided



Peel back the adhesive strip (do not remove)

Add 4 drops of 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

MICROGEN BIOPRODUCTS

Lab. No. F4560

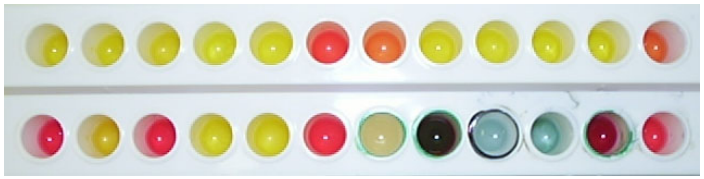
Specimen Type Cooked Rice

Date 12-10-04

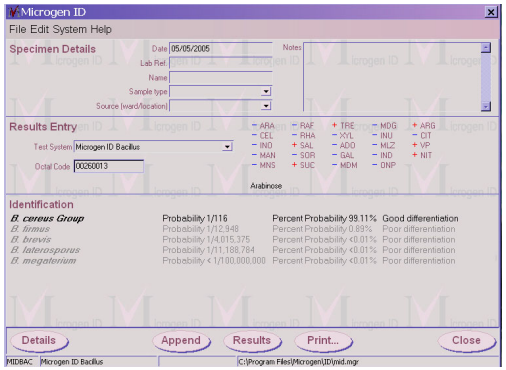
	Bacillus Strip 1 (BAC 1)												Bacillus Strip 2 (BAC 2)												
Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	IND	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	NO	ONPG	ARG	CIT	VP	NIT	NEG
24 hours	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	+	–			
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

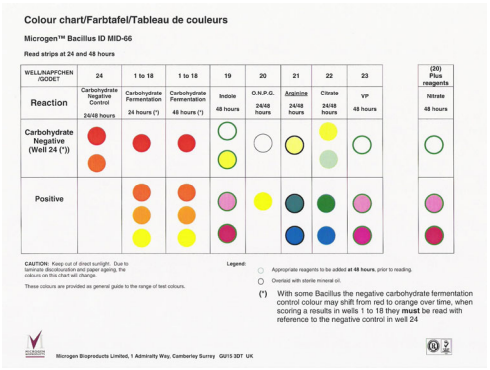
Microgen™ Bacillus ID Typical 48H Result 30 Minutes Post Addition of Reagents



MICROGEN™ BACILLUS ID DEDICATED SOFTWARE ANALYSIS OF IDENTIFICATION RESULTS



MICROGEN™ BACILLUS ID COLOUR CHART



MICROGEN™ BACILLUS ID SUBSTRATE SYSTEM

Well	Substrate	Reaction	Positive	Negative
1	Arabinose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow	Yellow	Red
2	Cellobiose			
3	Inositol			
4	Mannitol			
5	Mannose			
6	Raffinose			
7	Rhamnose			
8	Salicin			
9	Sorbitol			
10	Sucrose			
11	Trehalose			
12	Xylose			
13	Adonitol			
14	Galactose			
15	Methyl-D-Mannoside			
16	Methyl-D-Glucoside			
17	Inulin			
18	Melezitose			
19	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added.	Pink / Red	Colourless / Yellow
20	ONPG	Hydrolysis - ONPG hydrolysis by B-galactosidase results in the production of yellow ortho-nitrophenol.	Yellow	Colourless
20 Plus reag	Nitrate	Nitrate is reduced to nitrite which forms a deep red complex after the addition of α-Naphthylamine and Sulphanilic Acid	Red	Colourless
21	Arginine Dihydrolase	Arginine is converted to ornithine, ammonia and CO_2 by arginine dihydrolase resulting in an increase in pH and a change in colour of the bromothymol blue from green to blue. At 48 hours green reactions are negative.	Green/ Blue Blue	Yellow Yellow / Green
22	Citrate Utilisation	Utilisation of citrate (only carbon source) leading to a pH increase giving a colour change in bromothymol blue from yellowy green to blue.	Blue	Yellow / Light Green
23	Voges Proskauer	Acetoin production from glucose is detected by the formation of a pink / red complex after the addition of alpha naphthol and creatine in the presence of KOH.	Pink / Red	Straw colour
24	Control	Carbohydrate Control	Red	Red

PRODUCT PERFORMANCE

Bacillus species	Total Tested	Microgen Bacillus ID MID-66	API 50CHB + 20E (see note 3)
B.firmus	2	2	2
B.cereus	11	11	13
B.licheniformis	11	11	11
P.macerans	1	1	1
B.megatarium	1	1	1
B.pumilus	4	4	4
B.sphaericus	3	3	3
B.subtilis(see note 1)	11	11	11
B.thuringensis(see note 2)	2	2	0
P.alvei	1	1	1
B.circulans	2	2	2
Total	49	49	49

Notes:

1. Two isolates identified a B.amyloliquefaciens - a member of the B.subtilis group
2. Two B.thuringiensis no 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%
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**

ACKNOWLEDGEMENTS

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