

Microgen Bacillus-ID: New Users Guide

Technical Bulletin No. 118 is a guide to assist new users in achieving accurate results from the Microgen Bacillus-ID.

Ensure that the isolate being identified belongs to the *Bacillus* genus by always performing the following basic confirmatory tests before attempting to identify any isolate:

- Gram-stain: gram-positive bacilli
- Catalase: positive
- Endospore forming
- Optimal growth temperature of the isolate is between 25 and 45°C (mesophilic). Isolates growing at =25°C (psychrophiles) or isolates growing at >45°C (thermophiles) are not identified by this product.

Selection of colonies for identification:

The suspension used to inoculate the Microgen Bacillus-ID must be prepared to turbidity equivalent to a MacFarland 2 Standard. In order to achieve this multiple colonies will need to be selected.

Isolates must be tested from a pure culture on non-selective medium i.e. Blood Agar: in order to achieve this sub-culturing from a primary plate will be required.

Inoculum preparation:

Colonies should be selected from an 18-24 hour pure culture with the use of a sterile swab. Several sweeps of the swab may be required. Some species grow down into agar and removal of some agar with the colonies may occur. Also some colonies may be 'sticky' and difficult to dispense. The use of a vortex mixer is recommended to mix thoroughly and allow particulates to settle prior to inoculating the strips.

Interpretation:

Many *Bacillus* species strongly metabolise some sugars and/or proteins which can result in some biochemical reactions exhibiting initial positive reactions but which may revert back to negative reactions after further incubation (alkaline reversion). Other sugars may be weakly or slowly metabolized and may take longer to develop strong positive reactions.

For this reason we advise that the test results be read and recorded after both 24 hours and 48 hours incubation. This ensures that:

1. Organisms which may strongly metabolise sugars and exhibit a positive reaction after 18 – 24 hours incubation, may exhibit alkaline reversion i.e. appear negative or weak positive after 48 hours incubation. These reactions will be correctly recorded.
2. Where organisms weakly metabolise sugars, these slower reactions will be correctly recorded.

After the microwell strips have been incubated for 24 hours at 30°C, peel back the adhesive strip and record the results of the carbohydrate fermentation tests (well 1 to 18) in reference to the control well (well 24, strip 2): anything more orange or yellow compared to the control well should be scored as positive. The Arginine, ONPG and Citrate results should be read against the colour chart and recorded.

After microwell strips have been incubated for 48 hours at 30°C, add the appropriate reagents to the following microwells in the second microwell test strip:

1. Add 2 drops of Kovac's reagent to well 19. Read and record the results after 60 seconds.
2. Add 1 drop each of the VP I and VP II reagents to well 23 and read after 15-30 minutes.
3. Perform the nitrate reduction test on well 20 after reading and recording the ONPG result. Add 1 drop each of Nitrate A and Nitrate B reagents to the well and read after 60 seconds.

Record these additional results on the forms provided.

Occasionally some sugar fermentation tests may be positive after 18 – 24 hours incubation but negative after 48 hours incubation.

When the final Octal Code is calculated, all tests which showed a positive reaction after either 18 – 24 hours incubation or 48 hours incubation should be scored as **POSITIVE**. Only reactions negative at both readings should be scored as **NEGATIVE**.

Identification:

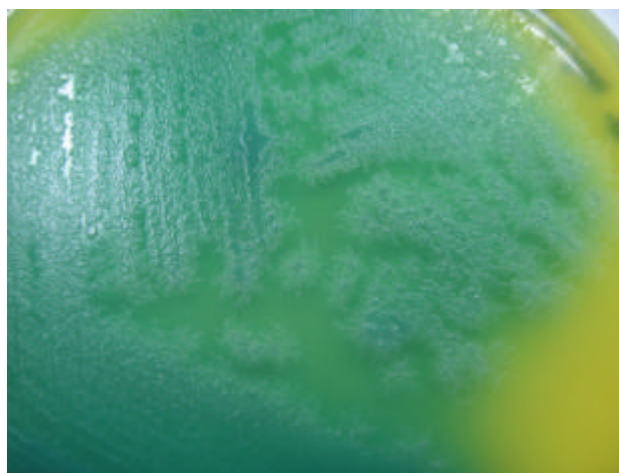
On the basis of routinely employed biochemical tests, *B. cereus* group consists of *B. cereus*, *B. thuringiensis* and *B. mycoides* and *B. weihenstephanensis*. These species are very difficult to distinguish based on commonly available phenotypic tests. Normally molecular methods are required to fully differentiate these species. However, the following information may assist further in achieving satisfactory differentiation.

Organism	MOT	TOX	RHZ	GR47	ADH
<i>B. cereus</i>	+	-	-	-	v
<i>B. thuringiensis</i>	+	+	-	-	+
<i>B. mycoides</i>	-	-	+	-	v
<i>B. weihenstephanensis</i>	?	-	-	+	v

(MOT = Motility, TOX = Toxin Crystals, RHZ = Rhizoidal colony morphology, GR47 = Growth at 4 - 7°C (No Growth at 43°C), ADH = Arginine Dihydrolase)



B. cereus on PEMBA Agar plates



B. mycoides on PEMBA Agar plates



B. thuringiensis on PEMBA Agar plates