
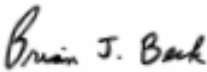


## APPENDICES

**Appendix 1A:** Novel soil bacteria *Paenibacillus haemolyticus* strain 139SI  
(ATCC BAA-2268)

 BACTERIOLOGY PROGRAM	A global bioscience nonprofit Organization dedicated to biological Standards and biodiversity  American Type Culture Collection 10801 University Boulevard Manassas, Virginia 20110-2209 USA Internet <a href="http://www.atcc.org">http://www.atcc.org</a>
<b>CERTIFICATE OF DEPOSIT</b>	
June 15, 2011	
<i>Paenibacillus haemolyticus</i> Strain 139SI (ATCC BAA-2268) was deposited in the ATCC general collection of bacteria by Salmah Ismail, University of Malaya, Kuala Lumpur, Malaysia, on May 11 <sup>th</sup> , 2011;	
The strain was confirmed to be viable and pure. The genotypic/phenotypic characteristics of this strain were determined to be consistent with those provided by the depositor.	
This culture is available for immediate distribution. Researchers may request the organism using the ATCC catalog number. Information on ordering the strain can be accessed through our website at <a href="http://www.atcc.org">www.atcc.org</a> .	
	
Brian J. Beck, Ph.D. Bacteriology Program Manager American Type Culture Collection (ATCC) 10801 University Boulevard Manassas, VA 20110-2209	
Phone: (703) 365-2700 ext. 2676 Fax: (703) 365-2730 Internet: <a href="http://www.atcc.org">http://www.atcc.org</a>	

**Appendix 1B:** Novel soil bacteria *Paenibacillus haemolyticus* strain 139SI with  
Accession No. F825470.1

### Paenibacillus sp. 139SI 16S ribosomal RNA gene, partial sequence

GenBank: JF825470.1

[FASTA](#) [Graphics](#) [PopSet](#)

[Go to:](#)

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LOCUS       JF825470                1496 bp    DNA     linear   BCT 24-OCT-2011
DEFINITION Paenibacillus sp. 139SI 16S ribosomal RNA gene, partial sequence.
ACCESSION  JF825470
VERSION    JF825470.1  GI:350285761
KEYWORDS   .
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  ORGANISM Paenibacillus sp. 139SI
            Bacteria; Firmicutes; Bacillales; Paenibacillaceae; Paenibacillus.
REFERENCE  1 (bases 1 to 1496)
  AUTHORS  Salmah,I., Saad,A.M., Siti-Fatimah-Zahra and Rahmat,O.
  TITLE    DNA Sequence of 16S rRNA from a novel 139SI strain of Paenibacillus
            sp.
  JOURNAL  Unpublished
REFERENCE  2 (bases 1 to 1496)
  AUTHORS  Salmah,I., Saad,A.M., Siti-Fatimah-Zahra and Rahmat,O.
  TITLE    Direct Submission
  JOURNAL  Submitted (20-APR-2011) Molecular Medicine, University of Malaya,
            Jalan Pantai, Kuala Lumpur, WP 50603, Malaysia
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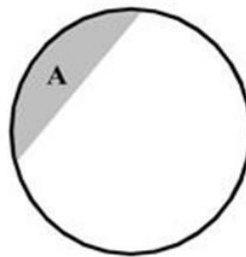
## Appendix 2A: Procedure for the streak plate technique

(Adapted from Practical 9 of Lab Manual Microbiology ©Salmah Ismail 2011/2012)

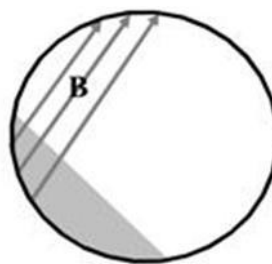
### Methods

Prepare a streak plate bacterial culture from the broth cultures:

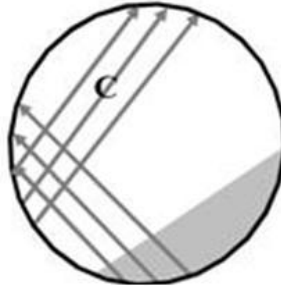
1. Loosen the top of the bottle containing the inoculum.
2. Hold the loop in the right hand and flame the loop to allow it to cool.
3. Lift the bottle/test tube containing the inoculum with the left hand and remove the lid/cotton wool plug/cap of the bottle/test tube with the little finger of the left hand.
4. Flame the neck of the bottle/test tube and insert the loop into the culture broth and withdraw.
5. Flame neck of the bottle/test tube and replace the lid/cotton wool plug/cap on the bottle/test tube using the little finger. Place bottle/test tube on bench.
6. Partially lift the lid of the Petri dish containing the solid medium.
7. Hold the charged loop parallel with the surface of the agar; smear the inoculums backwards and forwards across a small area of the medium (see diagram 1 streaked area =A). Remove the loop and close the Petri dish.



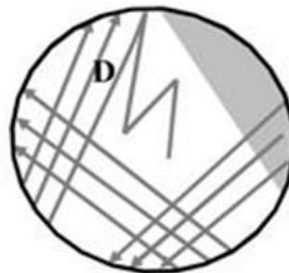
8. Flame the loop to allow it to cool and turn the dish through 90° anticlockwise. With the cooled loop streak the plate from area A across the surface of the agar in three parallel lines to B. Make sure that a small amount of culture is carried over. Remove the loop and close the Petri dish.



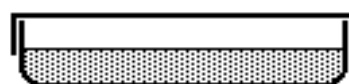
9. Flame the loop and allow to cool. Turn the dish through 90° anticlockwise again and streak from B across the surface of the agar in three parallel lines to C. Remove the loop and close the Petri dish.



10. Flame the loop and allow to cool. Turn the dish through 90° anticlockwise and streak loop across the surface of the agar from C into the centre of the plate to D. Remove the loop and close the Petri dish. Flame the loop.



11. Label the plate with your name and the name of the bacteria. You must carefully label your plates and tubes. There will be a lot of plates and test tubes incubating together making clear labelling crucial to avoid confusion. Always label the **BOTTOM** of the plate so that, even if lids get accidentally switched or broken, we will know what was in each plate. Likewise, do not label tubes on the cap. The label should include your name, the date, and what was put into the plate.
12. Seal with parafilm, invert and place plates in the incubator. Incubate overnight at 37°C.



Cover on top.  
"Right side up."



Cover on bottom.  
"Upside down."

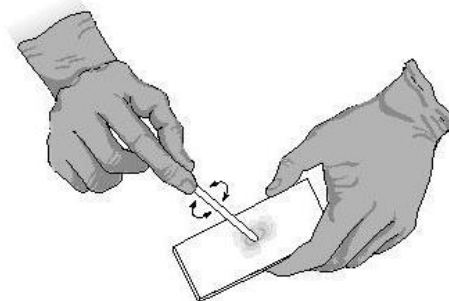
13. The disposal/unwanted plastic Petri dishes are placed in the red disposal bin and are destroyed by autoclaving.
14. Record your observations.

## Appendix 2B: Staining and viewing microbes

(Adapted from *Practical 4 of Lab Manual Microbiology* ©Salmah Ismail 2011/2012)

### A. Preparing/making the smears

1. Put on your gloves. Remember to use aseptic technique and flame the loop before and after each use.
2. Clean a plain microscope slide thoroughly using lens tissue.
3. Label a microscope slide with a marker pen to record the culture being used, date and initials; this is also a useful reminder of which side of the slide is being used. (It is important to label the slides accordingly; be sure you know which side is up.)
4. Flame a wire loop to ensure that no culture accidentally remains from a previous operation.
5. Transfer one or two loopfuls of tap water on to the centre of the slide.
6. Flame loop and allow it to cool.
7. Using aseptic technique, transfer a very small part of a single colony from a plate or slope of agar medium into the tap water.
8. Make a suspension of the culture in the tap water on the slide and thoroughly but gently spread it evenly over an oval area of up to 2 cm length:

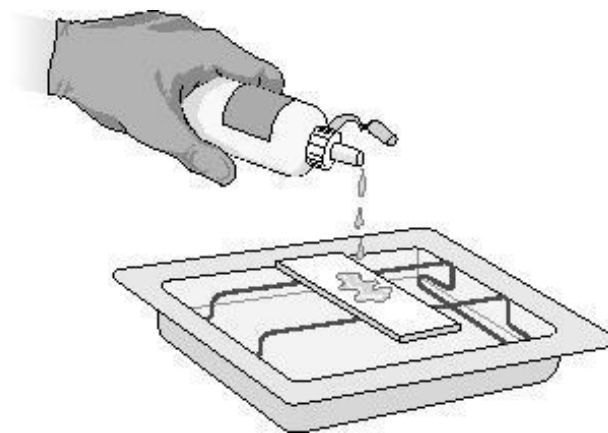


*Preparation of a smear*

9. Flame the loop. If it is necessary to use a liquid culture or sample, the use of tap water to prepare the smear will probably be unnecessary and may result in a smear with too few cells.
10. Dry the suspension by warming gently over a Bunsen burner flame and then “fix” it by quickly passing it through the flame a few times.
11. This is called a **heat-fixed smear**; it should be visible to the naked eye as a whitish area. Fixing is necessary to ensure that cells adhere to the slide and to minimize any changes before staining.

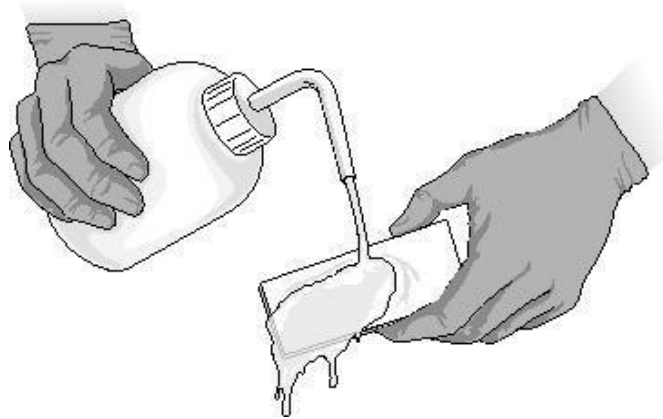
**B. Staining the smears – A differential stain: Gram's staining method**

1. Put the slide with the fixed smear uppermost on a staining rack over a sink or staining tray.
2. Thoroughly cover the smear with crystal violet solution and leave for 1 minute.



*Adding stain to a slide*

3. Hold the slide with forceps (optional but avoids stained fingers), at a 45° angle over the sink.
4. Pour off the stain, wash off any that remains (and any on the back of the slide) with iodine solution.
5. Put the slide back on staining rack.
6. Cover the smear with iodine solution and leave for 1 minute. Iodine solution acts as a “mordant” (a component of a staining procedure that helps the stain to adhere to the specimen), a crystal violet-iodine complex is formed and the smear looks black.
7. Hold the slide with forceps at a 45° angle over the sink wash off the iodine solution with 95% (v/v) ethanol (not water); continue treating with alcohol until the washings are pale violet.
8. Rinse immediately and put the slide back on staining rack.



*Rinsing off a slide*

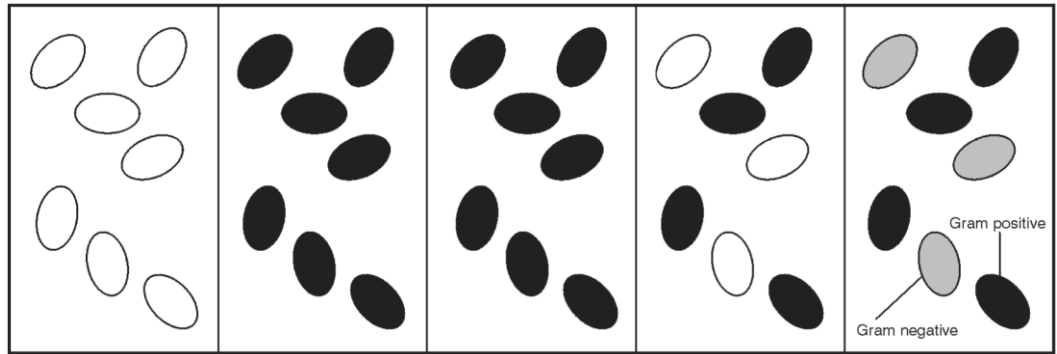
9. Cover the smear with the counterstain, e.g. safranin solution, 0.5% w/v, for 30 seconds and rinse off the stain with tap water.
10. Blot-dry the smear with filter/fibre free blotting paper using firm pressure but not sideways movements that might remove the smear:



*Drying a slide*

### **C. Observing and evaluating the smears**

1. Examine the slides under oil immersion. Observe the bacterial cells to determine their Gram reaction:
  - ⇒ These colors are hard to differentiate at first. The purple or violet coloring is Gram positive, the pink coloring is Gram negative.
  - ⇒ The *Staphylococcus epidermidis* and the *Bacillus* sp. should stain Gram positive.
2. Record your observations concerning the Gram reaction, cell shape and arrangement.



(a) before staining all bacteria are colourless

(b) after basic stain (*crystal violet*) all bacteria are stained violet

(c) after mordant (*Lugol's iodine*) stain is fixed more firmly into the cell

(d) after decolouriser (alcohol) some bacteria are colourless (Gram negative) while others are still violet (Gram positive)

(e) after counterstain (safranin) colourless bacteria (Gram negative) have taken up stain and appear red: Gram positive bacteria remain violet

### The most common bacterial shapes

