Eva Star

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Microbiology M01

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Identification of an Environmental Unknown

The purpose of this environmental unknown project was to understand that microbes are ubiquitous. They can be found on living and inanimate things. This project also provided the opportunity to apply all the knowledge and techniques acquired throughout the course for the identification of the unknown bacterium. For this project each student obtained a specimen by swabbing the location place of their choice.

The source of the swab for this particular report was a computer keyboard. A swab was taken with a sterile cotton swab and transported in a sterile saline solution to the lab. In the lab, the bacteria on the cotton swab were transferred in a zigzag pattern onto a nutrient agar plate and incubated for 24 hours to achieve growth of the collected bacteria. The transferred bacteria grew into 4 colonies. Each colony was approximately 2 mm in diameter, opaque, and had a cream color.

One of these colonies was taken aseptically and transferred to a streak plate to isolate one colony forming unit and produce a pure culture. After the 24 hour incubation the streak plate had opaque cream colored, dull and dry growth and the margins were smooth. Present on the streak plate were a few colony forming units. One unit was chosen to inoculate aseptically in the two nutrient agar slants (one work slant and one stock slant), the deep stab and the nutrient agar broth. The growth on the slants was dense, opaque and had smooth edges. The nutrient broth had a precipitate with almost no turbidity, which indicated that this bacteria was a facultative anaerobe. This was confirmed by the deep agar stab which showed growth along the whole stab

line, confirming the previous result that the unknown bacteria was indeed a facultative anaerobe.

The next step was to perform a gram stain. Bacteria were transported aseptically from the working slant to a microscope slide and viewed with an oil immersion lens at 1000X magnification. A review of the slide found purple cocci in arrangement of pairs, tetrad, and clusters which differentiate the bacteria to be a gram positive cocci, and as indicated by the agar stab and nutrient broth, was facultative anaerobic. In the *Bergey's Manual of Determinative Bacteriology* facultative anaerobic gram positive cocci comprise Group 17.

This now limited the findings to on group, however there were 13 genera in Group 17, which were *Aerococcus, Enterococcu, Gemella, Lactocoocus, Leuconostoc, Melissococcus, Pediococcus, Saccarococcus,* Staphylococcus, Stomatococcus, Streptococcus, Trichococcus, and Vagococcus. To further narrow down the search, a motility stab was preformed. This test result was negative for motility. After becoming increasingly familiar with *Bergey's Manual* the realization occurred that none of Group 17 bacteria were motile, therefore the test that was performed did not eliminate bacteria from the original 13 genera.

The next test that was performed was on a 6.5% NaCl agar slant. The result showed excellent growth with smooth margins, and a translucent, shiny, and wet appearance. This bounded the results to 3 genera of Group 17: *Aerococcus, Enterococcus* and *Staphylococcus*. Now as suggested by *Bergey's Manual* the next logical test to be performed was the Catalase test. This test result was positive for catalase which was indicated by bubbles. With this test both genera of *Aerococcus, Enterococcus* could be excluded. The genius of *Staphylococcus* was the only genius positive for catalase reaction.

The next step was to get familiar with the species and subspecies of the genus *Staphylococcus* which are the following: *S. arlettae, S. aureus supsp.anaerobius, S. aureu supsp. aureus, S. auricularis, S. capitis supsp. capitis, S. capitis supsp ureolyticus, S. caprae, S. carnosus, S. caseolyticus, S. cohnii/cohnii, S. chromogenes, S. cohni supsp ureolyticus,*

S. delphini, S. epidermidis, S. equorum, S. felis, S. gallinarum, S. haemolyticus, S. hominis, S. hyicus, S. intermedius, S. kioosii, S. lentus, S. lugdunensis, S. saprophyticus, S. saccharolyticus, S. sciuri, S. schleifer supsp coagulans, S. schleiferi supsp. schleiferi, S. simulans, S. warneri, S. xylosus. The next test was a urease test based on the indicators found in the *Bergey's Manual* tables of *Staphylococcus* characteristics. The test result was positive for strong urease production; the medium turned pink, therefore the species left were the following *Staphylococcus* species and subspecies: *S. aureu supsp. aureus, S. capitis supsp ureolyticus, S. caprae S. cohni supsp ureolyticus, S. delphini, S. epidermidis, S. equorum, S. felis, S. gallinarum, S. hominis, S. intermedius, S. saprophyticus, S. schleifer supsp coagulans, S. simulans, S. warneri, S. xylosus.*

To further eliminate the species and subspecies of *Staphylococcus* a coagulase test was performed. The coagulase test also came back positive for coagulation showing light coagulation of the medium. Through this result ¾ of *Staphylococcus* species and subspecies could be eliminated and only 4 choices were left, which were *S. aureu supsp. aureus, S. delphini, S. intermedius* and *S. schleifer supsp coagulans.* The final defining test was the blood agar test which showed only white growth on the surface of the agar and no hemolysis. This negative result eliminated all but *Staphylococcus intermedius,* making this the environmental unknown.

In review of the project there were no special problems encountered and the tests performed were important to obtaining the correct outcome. Moreover, searching the internet confirmed that *Staphylococcus intermedius* was a reasonable result. The *Journal of Clinical Microbiology* states this bacteria is a common organism found on the skin of healthy dogs and causes nosocomial infections in humans. It would not be unreasonable to have found it on the location of the swab.

Test	Observation	Results
NA plate	2 mm, opaque, smooth edges	Bacteria was present
Streak plate	Single colonies isolated	CFU formed
2 Agar slant	Flat, dry, opaque, smooth edges	Bacteria multiplied
NA broth	Precipitate, little turbidity	Facultative anaerobe
Deep stab	Growth along the stab line	Facultative anaerobe
Gram stain	Purple Cocci	Gram positive cocci
Motility stab	Red growth only on stab line	Organism is not mobile
6.5% NaCl agar slant	Translucent growth	Positive growth on high NaCl
Catalase test	Bubbles formed	Catalase is present
Urease Test	All pink	Strong urease production
Coagulase test	Medium slightly thickened	Plasma has been coagultated
Blood Agar	White growth but no change in the medium	No hemolysis

Works Cited

Holt, John G. at al. *Bergey's Manual of Determinative Bacteriology*. Ninth Edition. Ed. William R. Hensyl. Philadelphia: Liffincott, Williams, & Wilkins. 2000.

Pottumarthy, Sudha. at al. December 2004. Clinical Isolates of *Staphylococcus intermedius* Masquerading as Methicillin-Resistant *Staphylococcus aureus*, Journal of Clinical Microbiology. 14 July 2008. http://jcm.asm.org

Environmental Unknown