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Environmental Unknown

Genus *Staphylococcus*

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The environmental unknown was isolated from the palm of my left hand after I washed it once with SoftCIDE soap. I believe it to be of the genus *Staphylococcus*, a Gram (+) coccus. Surprisingly, only one colony developed on the nutrient agar plate, and from this colony, I did two quadrant streaks to obtain a pure culture. One colony from this second streak plate was used to make two continuous streaks, and one of these was used to do our three required tests: a Gram stain, Gas Pak anaerobic test, and a motility test.

Our lab book explained that Eubacteria belong in one of two groups depending on their cell wall structure (Vilgalys, 2000). The Gram stain differentiates between these two groups. After Gram-staining my unknown using both the lab book and alternate version of the Gram stain to compare results, I obtained purple-violet cocci from both methods. This result indicated Gram (+) bacteria (Vilgalys, 2000). A 3% KOH test was done to confirm the Gram stain results. Upon swirling a loopful of bacteria in a drop of KOH, the bacteria dissolved, and there was no sign of stringiness. The KOH test confirmed that I had Gram (+) bacteria.

Next was the Gas Pak anaerobic test. Two plates were made for this test—one for testing, and one for a control. The aerobic control plate was used as a standard for comparing the test plate to. This way, I could see how well the bacteria grew under anaerobic conditions, as compared to aerobic conditions. After 24 hours of incubation with no oxygen, the plates were removed, and the test plate showed roughly half as much growth as the control plate. The Gas Pak test had shown that my Gram (+) cocci were facultatively anaerobic (Vilgalys, 2000). To confirm these results, I made two agar stabs, and after 48 hours of incubation, I noticed growth all along the tube. This result confirmed that I had facultatively anaerobic bacteria, since they could grow in both aerobic and anaerobic conditions (Vilgalys, 2000).

Next was the motility test. The motility plate contained four strips. Two were for my given and environmental unknowns, and the other two were control strips. The strips were allowed to soak up moisture for 15 minutes. After that, the edge of one control strip was inoculated with motile bacteria, and the other was inoculated with non-motile bacteria. After incubating for 48 hours, the non-motile bacteria showed movement. This meant that the test was inconclusive and had to be redone, since I could not trust my test results if the control results were wrong (Vilgalys, 2000).

I ended up repeating the motility test four times. Motility Test #2 produced the same results as Test #1—the non-motile bacteria moved, so I had to do the test again. For the third try, I made several changes to my procedure. First, I used the hood to reduce my chances of airborne contamination. Second, I made sure to obtain freshly-sterilized motility strips and to be one of the first people in line to use them. Lastly, I allowed the motility strips to soak up moisture overnight in a freezer before inoculating the plate with bacteria. This last step was to eliminate any carrying over of bacteria while moisture was diffusing throughout the strips, since wicking of moisture could explain why the non-motile bacteria kept moving across the strip. These changes to the procedure were ultimately successful. Motility Test #3 yielded correct control results, and my environmental unknown was non-motile. I did the test once more since other students had been experiencing identical problems with their motility tests, and I wanted to make sure I had reliable results. Motility Test #4 gave the same results as Motility Test #3. I felt comfortable about recording that my environmental unknown was non-motile.

While waiting for the second motility test, I had proceeded to do other tests. Up to now, I knew I had a Gram (+) facultatively anaerobic coccus. Doing a catalase test would be a quick way to eliminate a large number of facultatively anaerobic Gram (+) cocci from further consideration (Table 2). To do the catalase test, I added a drop of hydrogen peroxide to a loopful of my unknown. Bubbles were immediately produced, which indicated a positive test for catalase (Vilgalys, 2000).

After the catalase test, I narrowed my list down to four genii, all of which were Gram (+), facultatively anaerobic, catalase (+) cocci. These four genii were *Melissococcus*, *Saccharococcus*, *Staphylococcus*, and *Trichococcus*. After comparing colony properties and cell shape descriptions given in the Bergey's Manual, I was finally left with one genus, *Staphylococcus*. Other features also helped me exclude the other three genii from consideration. For example, *Saccharococcus* did not grow well at 37°C since it was thermophilic, having an optimal temperature of 60-68°C. Also, *Trichococcus* formed chain colonies on solid media, whereas the environmental unknown formed small, white individual colonies on solid media.

Of the two unknowns I worked with, this environmental unknown was the easiest to identify. There was no ambiguity in my test results, except for the motility test; it was not until after my third and fourth trials that I began to get consistent results for this test.

I feel quite certain that I have a species of Staphylococcus. Although I did not use a hood to avoid airborne contamination until I started my third motility plate, contamination was kept to a minimum. I observed that the streak appearance of the unknown bacteria never changed, and I never saw anomalous colonies growing in the unknown streaks. However, there were a few cases where I did observe one or two colonies outside of the streak. This was indicative of airborne contamination, which may have changed a once-pure culture into a mixture of bacterial cells. However, although I cannot be absolutely certain I have a species of Staphyloccus, there is a reason why it might be likely. I had isolated this environmental unknown from the palm of my hand, and Staphylococcus is one of the common bacteria on the skin (Vilgalys, 2000). As far as tests were concerned, the most problematic test was the motility test, but after four trials, I finally found worthwhile results. An important lesson from this lab was the importance of aseptic technique, especially when it comes to setting up good motility plates. Also, I developed a greater appreciation for trying to obtain clear test results on whatever test one is doing. This will help greatly, since experimenters will not want to miss the correct identification of the unknown because of an incorrect or uncertain test result.

Table 1:
Summary of Tests Done, in Order

1. Gram stain—narrowed down to Gram (+) cocci
2. KOH test—confirmed Gram (+)
3. Gas Pak test w/ stabs—narrowed down to facultative anaerobes
4. Motility test, #1—non-motile bacteria moved, so test was inconclusive
5. Catalase test—narrowed down to catalase (+) bacteria
6. Motility test, #2—non-motile bacteria moved, so test was inconclusive
7. Motility test, #3—controls worked, and unknown was non-motile
8. Motility test, #4—to confirm results in test #3: controls worked, and unknown was non-motile—narrowed down to non-motile bacteria
9. Consulted Bergey's Manual

Table 2:
Process of Eliminating Gram (+) Cocci by Three Tests
From Group 17 of Bergey's Manual, p. 527

| | Gas Pak test: | Catalase test: | Colony/Cell Shape: |
|---|---|---|---|
| Results of Test: | Facultative Anaerobes | Strong (+) Result | Small round white colonies; forms clusters on slide |
| *Aerococcus* | | | |
| *Coprococcus* | | | |
| *Deinobacter* | | | |
| *Deinococcus* | | | |
| *Enterococcus* | *Enterococcus* | | |
| *Gemella* | *Gemella* | | |
| *Lactococcus* | *Lactococcus* | | |
| *Leuconostoc* | *Leuconostoc* | | |
| *Marinococcus* | | | |
| *Melissococcus* | *Melissococcus* | *Melissococcus* | |
| *Micrococcus* | | | |
| *Pediococcus* | *Pediococcus* | | |
| *Peptococcus* | | | |
| *Peptostreptococcus* | | | |
| *Planococcus* | | | |
| *Ruminococcus* | | | |
| *Saccharococcus* | *Saccharococcus* | *Saccharococcus* | |
| *Salinicococcus* | | | |
| *Sarcina* | | | |
| *Staphylococcus* | *Staphylococcus* | *Staphylococcus* | *Staphylococcus* |
| *Stomatococcus* | *Stomatococcus* | | |
| *Streptococcus* | *Streptococcus* | | |
| *Trichococcus* | *Trichococcus* | *Trichococcus* | |
| *Vagococcus* | *Vagococcus* | | |

## References

Bergey's Manual of Determinative Bacteriology. 9th ed. Williams & Wilkins, Baltimore, 1994.

Bergey's Manual of Systematic Bacteriology. 1st ed. Williams & Wilkins, Baltimore, 1984.

Vilgalys, Rytas. Laboratory Exercises for BIO 103, General Microbiology. Fall 2000.