Jen Johnson BIOL 310 Unknown 15

I identified my unknown as Gram negative rods, so I narrowed my search down to Groups 2-6 in Bergey’s Manual of Systematic Bacteriology. It grew on a plate, in a broth on the wheel, and on a plate in a bag with a GasPak in the warm room. I excluded the “strict” characteristic of the oxygen profile. Growth occurred all over a thioglycolate broth. I concluded it was a facultative anaerobe. Therefore, I focused on Group 5.

Next, I used Table 5.1 to eliminate some genera/families. I decided that motility, oxidase, catalase, and nitrate reduction were the most reliable tests. I confirmed the motility using the methods of a hanging drop, motility deep, and SIM deep. Therefore, the nonmotile nature of my unknown is reliable. My unknown was oxidase negative and catalase positive. I confirmed both of these tests multiple times along with control organisms so I also have high confidence in their results.

I eliminated most families based on these characteristics (see dichotomous key for details). This left the genera Calymmatobacterium, the family Enterbacteriaceae, and the family Pasteurellaceae. I eliminated Calymmatobacterium because there was only species in that group, so it did not seem likely that this would be used in an assessment of biochemical identifications. I eliminated the family Pasterurellaceae because the manual stated that differentiation of this group is being reevaluated and identification should not be attempted. Therefore, I continued with the family Enterbacteriaceae.

I used Table 5.2 to eliminate all species who did not fit characteristics named above. When a result was missing from the table or labelled as “d” for depends, I kept the species. The next most reliable test was the glucose fermentation test. I decided that the color change was easy to observe, but the gas bubble was a result that could have been misinterpreted. Therefore, I eliminated glucose fermenters that were negative for both acid and gas production, and kept those that were only negative for gas production.

Next, I filtered based on tests that were positive and had been confirmed multiple times. These included lipid hydrolysis, indole production, phenylalanine deaminase presence, nitrate reduction, and the methyl red test. I scored the remaining species out of 5 for these tests, where a point was added if the species is positive for that test and therefore shares the characteristic with the unknown.

None of the species shared all 5 characteristics, but *Rahnella aquatilis, Serratia plymuthica*, *Providencia heimbachae*, and *Escherichia coli* (inactive) all shared 3 properties. I eliminated all other species that scored less than or equal to 2/5.

Next, I filtered based on negative tests. These included sucrose fermentation, casein hydrolysis, DNA hydrolysis, Voges-Proskauer, gelatin hydrolysis, citrate utilization, beta galactosidase presence, and urea hydrolysis. I scored the remaining 4 species based on differences in characteristics, subtracting a point out of 7 for differences. See the last row of the table on the dichotomous key for differences between the species and the unknown.

To make this decision, I considered the importance and reliability of the disparities between the unknown and the species (See the table on the dichotomous key for the ranking/importance of different factors). The literature said *R. aquatilis* was rare and there were too many negative tests that should have turned out positive, so I eliminated it. I considered the unambiguous results I obtained from the lipid hydrolysis test. I observed the diameter of the colonies on a plate from the cold room: ~1mm. These 2 features suggested *S. plymuthica*. I repeated the SIM/indole test and compared the shade of red in my result to online literature. I decided that the unknown’s shade of pink-orange could have been a weak positive or even negative result. I had been noticing a strange smell coming from my unknown, even from day 1, so I attributed that to the musty smell. Therefore, I concluded that my unknown was *Serratia plymuthica*. I ruled out *E. coli* (inactive) because the colony morphology did not match and the lipid hydrolysis test was clearly positive. I also ruled out *P. heimbachae* because of the lipid test.

I did not include Lysine and Ornithine decarboxylase, litmus milk, and lactose fermentation because the positive reactions were weak, meaning the results were ambiguous (Photos attached in lab notebook pg 72-73). Furthermore, after the filtering down to the last 4 species, all were the same for these characteristics, so I decided against testing again. I also had realized from redoing other tests that the initial tests performed were correct. I saw ambiguous results for hydrogen sulfide production (SIM test), so I repeated the test a third time to confirm a negative result. This did not contradict any of the final candidates.

Disparities and Macrocharacteristics of the final candidates. The higher the row, the more important the test.

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| Species | *R. aquatilis* | *S. plymuthica* | *P. heimbachaea* | *E. coli* (inactive) |
| Pre-filtering |  | Glucose gas - | Glucose gas - | Glucose gas - |
| 5 Should-be Positive | Lipase – Indole – | Indole –  Phenylalanine deaminase – | Lipase –  Indole – | Lipase –  Phenalanine deaminase – |
| Other: | rare. | 1 mm colonies  cream-white, opaque, shiny, smooth colonies.  musty smell. | 4 mm colonies  grey-white, opaque, shiny, smooth, convex colonies. | 3-6 mm colonies  /Users/jen/Desktop/escherichia-coli-pseudomonas-aeruginosa-tsa.jpg  characteristic odor. |
| 7 Should-be Negative | sucrose +  VP +  citrate +  ONPG + | sucrose +  VP +  DNAse + |  |  |