Grading Rubric

Student Name: _____ Unknown Number: _____

Proposed Unknown: _____

_____/12 Correct identification of Unknown

_____/15 Quality of Lab Journal Entries

- Complete Sentences, complete biochemical explanation of results, adherence to format….

_____/13 Report/Summary of Findings

- Quality of research, proper use of references….

_____/40 Total

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Microbiology

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Unknown Project

There are several types of bacteria that are unique in their structure, color, function, metabolic properties, and more. Additionally, there are a multitude of tests that a microbiologist may conduct to deduce what kind of bacteria they are observing. These tests all happen within a sterile environment where many unique techniques are performed that will eventually fulfill all conditions to determine an unknown bacteria labeled 35a7.

In order to properly isolate the given unknown bacteria 35a7, a *T-Streak* technique was performed which would be used for all subsequent tests. 35a7 was given as a broth culture and then stained with a sterile loop across four quadrants of a tryptic soy agar (TSA) plate. After the TSA plate had been incubated invertedly for over 48 hours, colonies of the unknown bacteria had grown that appeared white and opaque. 35a7 had now been successfully isolated, but should not solely remain on a TSA plate for future retrieval of samples. A steady stock of 35a7 should be made more readily available for future tests, so the next step was to conduct an *Inoculate Slant*.

After having colonies form on the TSA plate using the T-Streak technique, a TSA slant tube was obtained next by using a sterile loop and swabbing the new tube in a zig-zag motion. After incubation at an optimal temperature for bacterial growth, another colony was visible on the surface of the slant that would be used for the next following tests to find what bacteria 35a7 is.

The following technique used was the *Gram Stain* in order to rule out a large amount of bacteria based on their easily distinguishable appearance after being cultured. The Gram Stain technique involves staining both known gram-positive and gram-negative dishes as the controls

as well as the unknown bacteria 35a7. After performing this test, the results revealed that 35a7 contained chained rod-like cells that appeared purple due to cells having a thicker peptidoglycan wall. This result matched the control and determined that 35a7 was gram-positive. In contrast, a gram-negative result would be presented as pink. Being gram-positive and specifically having a more rod-like appearance rather than being circular leaves a few bacteria to choose from such as *Bacillus lazuli*, *Bacillus Thuringiensis* [Bt], *Bacillus Subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Mycobacterium smegmatis*, and *Streptomyces grisieus*. Knowing that the unknown sample is a part of the Bacillus bacterial group, a common metabolic test to determine specific growth character at such an early stage of testing could be searching for *Motility*.

The next test determined whether or not the bacteria was *Motile*, or having the ability to move using hair-like structures called flagella. The motility test involved using a sterile inoculating needle and "stabbing" the 35a7 tube straight down leaving a line of separation. The goal of this test was to observe any bacterial growth moving away from the stab line. The tube showed red and cloudy growths that were indeed forming away from the stab line thus resulting in positive motility. The group of Bacillus bacteria (remaining: *Bacillus cereus*, *Bacillus lazuli*, *Bacillus Thuringiensis* [Bt], and *Bacillus subtilis*) are characterized by being motile and containing flagella, so more tests must be conducted to identify 35a7.

The Mannitol Salt Agar test (MSA) was conducted next to determine if 35a7 could grow in an environment of high salt concentration and be able to ferment mannitol as a byproduct. A sample of 35a7 was streaked in a zig-zag pattern along the surface of a slanted MSA tube that presented red/pink in color. After incubation of 48 hours, the MSA tube would reveal any growth found on the slant meaning it would be able to withstand high salt levels. That growth could also have displayed yellow coloration, or not, which would be indicative of mannitol fermentation.

Results showed that the MSA tube indeed had some growth on top of the slanted agar surface, but did not show any signs of yellow coloration and remained red/pink. Many other bacillus and cocci bacteria may actually be salt-tolerant as well, but Bt resulted as the only bacillus bacteria that did not ferment mannitol while in a high salt concentration environment. Following this test, another differentiating test must be performed to especially distinguish between the two bacillus bacteria remaining: Bt and B. subtilis. These two are both gram positive, motile, and salt-tolerant and two more tests remain to confirm the identity of 35a7.

The *Citrate* test was conducted as an indicator of 35a7's ability to use citrate as its sole carbon source and thus prompting a potential color change within a Simmons citrate agar slant tube. If 35a7 did cause bacterial growth, then a blue shade would begin to emerge resulting in a positive test. After streaking the agar slant with an inoculating loop in a zig-zag motion and allowing time for incubation, there ultimately was no color change thus indicating a negative result. 35a7 may finally be concluded as Bt, but one last test was conducted to further confirm this result.

Lastly, another test that could differentiate between the two bacillus bacteria was the *Methyl Red and Voges-Proskauer* (MR/VP) test. The purpose of this test is to determine if 35a7 would ferment glucose in either one of two pathways: glucose to stable acids (MR positive) or glucose to acetoin and butanediol (VP positive). A MR-VP broth tube was inoculated with 35a7 and left to incubate over 48 hours. After incubation, 15 drops of methyl red were added to the MR-VP broth tube and left for observation of any change in coloration. The color change to red in the MR-VP tube indicated that 35a7 was positive for stable acid fermentation deriving from glucose. 35a7 did not cause any color change in the VP tube suggesting that it was negative for

acetoin and butanediol fermentation. After this final test, it is determined that 35a7 can be categorized as *Bacillus Thuringiensis* [Bt].

After several metabolic tests were conducted, the unknown bacteria displayed specific characteristics in appearance, function, and growth within stable and controlled environments that best aligns with Bt. This bacteria is presented as gram-positive which is highly distinguishable due to its purple rod-like chains along with evidence of containing flagella as a means of motility. Bt was found to have the ability to grow in a high salt-content environment and also was able to metabolize citrate or glucose to create certain byproducts unique to this bacteria. After concluding these tests, it is important to discuss Bt and its prevalence in the real world.

Bt could commonly be found in soil as an agent for pest control. This bacteria mainly produces “crystalline proteins that show high insecticidal activity” (Rajadurai et al., 2023). This means that Bt creates toxins that are pest-specific so that spray-pesticides kill invasive insects allowing for successful crop/plant production. Bt causes “negligible harm towards non-target organisms, animals, and avian species” (Rajadurai et al., 2023). This implies that Bt may not be harmful towards human and animal ingestion, but another study suggests that “Bt may adversely affect human health upon constant exposure to pesticides and preceding immunity weakening” (Belousova, et al. 2021). Studies have been conducted in patients suffering from gastroenteritis outbreaks, but have been inconclusive or not associated with Bt as other other cytotoxins were found in stool samples or causation of other diseases such as Norovirus were observed. In fact, Bt spores are not completely digested in vertebral organisms such as large animals or humans, thus it does not negatively affect any blood, tissues, or organs. Conversely, Bt may be confused or misattributed with Bacillus Cereus which is another gram-positive bacterium that is also found

in soil and pesticide-affected foods. One case in particular observed by Butcher et al. in 2022 describes an autopsy of a late five year-old who ultimately passed due to leukemia, hepatomegaly, multi-organ hemorrhaging, among more malignant findings. Two Bacillus species, Cereus and Thuringiensis, were present in several organs, but no inflammatory response was ever observed in order for these bacterial groups to claim causation of the child's death. Overall, it is inconclusive to incriminate Bt as the main factor of disease when most, if not all, cases of patients are immunocompromised or already possess several comorbidities and this bacteria would need further long-term and controlled testing to deem its toxicity to living organisms.

It was necessary for 35a7, among other bacteria groups, to have gone through all these metabolic tests so that a microbiologist or even a student may observe how a bacteria could survive and be found in certain environments. One could learn the metabolic processes and how it could react in an organism's body leading to virality. Bacteria exists everywhere in every area of life and could affect a human's health in several ways. To understand its production and further processes may help in fighting against any negative afflictions that bacteria may cause.

References

Rajadurai, G., Anandakumar, S., & Raghu, R. (2023). *Bacillus thuringiensis in pest*

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Butcher, M., Puiu, D., Romagnoli, M., Carroll, K. C., Salzberg, S. L., & Nauen, D. W. (2021). *Rapidly fatal infection with Bacillus cereus/thuringiensis: Genome assembly of the responsible pathogen and consideration of possibly contributing toxins*. Diagnostic Microbiology and Infectious Disease, 101(4), Article 115534. https://doi.org/10.1016/j.diagmicrobio.2021.115534