

Characterization of Red Pigment Producing Bacteria: An Honors Project

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Abstract

This is a continuation of a project submitted as an Honors Proposal in April of 2019. The project focuses on five bacterial strains that are capable of producing both a red pigment and a green sheen on Marine Agar. The project was intended to further classify these organisms, through several experiments that originally included a carbohydrate utilization test, an Analytical Profile Index (API), determination of fatty acid composition, examining morphology, multi-locus sequence comparison, and/or comparing the absorption spectra. Since then, the project has switched gears and become more focused on genomic comparison. The five protein sequences studied were Filamenting temperature-sensitive mutant Z (FtsZ), Glyceraldehyde-3-phosphate A (GapA), RecA, RNA Polymerase A (RpoA), and Topoisomerase A (TopA). An attempt was made to isolate the sequences of each of the five genes in the following organisms: MI3, JD-17, JD-18, Renegade, and Little Penny. Unfortunately, the project was cut short due to lab closures in response to the COVID-19 Pandemic, and the only isolates sequenced were RecA, RpoA, TopA and 16s isolated from MI3. These sequences were compared to the known sequences of the genes from two strains of *Zooshikella ganghwensis* (15267 and JC2044) and one strain of *Hahella chejuensis*.

Keywords: *Zooshikella ganghwensis*, *Hahella chejuensis*, RecA, RpoA, TopA

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The red pigment in our isolates is believed to be prodigiosin. According to researchers at the Sokoto State University, the genus *Serratia* can produce this and is “known to have an antibiotic and antimalarial effect and immunosuppressing activity” (Usman HM, Abdulkadir N, Gani M, et al. 2017). Antibiotic properties in bacteria are becoming an increasingly important tool for scientists. The invention and widespread use of antibiotics such as Penicillin have caused resistant strains of pathogens to develop such as MRSA. To keep up with rapidly changing pathogens, researchers are working on finding other microbes that are evolving defenses to these new threats at just as rapid a rate. The identification of bacteria that produces a new antibiotic compound that can be harnessed, processed and mass produced can have a huge impact on the world by saving the lives of millions of people from infections that would otherwise be fatal and untreatable.

The green sheen is most often linked to the growth and metabolism of *Escherichia coli* (*E. coli*) on Eosin Methylene Blue (EMB) agar. It indicates “rapid fermentation of lactose and formation of strong acids” (Tankeshwar, 2013). Characteristics such as these are used to select for and differentiate between many different types of bacterial strains. This is important for identifying and classifying isolates which is necessary to advance scientific knowledge. This is applicable to situations such as culturing mucus samples to determine which medications will be effective for a suffering patient.

It is quite uncommon for bacteria to produce both a red pigment and green sheen on marine agar. The first report of this unique combination was from research in Korea where the then novel species *Zooshikella ganghwensis* was isolated, identified, and characterized ((Yi, Chang, Oh, Bae, & Chun, 2003). Another similar organism, *Zooshikella marina* was isolated

from a sample collected from Shivrajpur–Kachigad beach, Gujarat, India (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). This species differs from *Zooshikella ganghwensis* because of its production of cycloprodigiosin and eight other prodigiosin pigment analogues (Ramaprasad, Bharti, Sasikala, & Ramana, 2015).

“16s rRNA analysis is a very important step toward identifying a bacterial strain because it is the primary method used to define and distinguish bacteria at the genus level. Since a variation of this sequence is found in virtually all bacteria, it is a convenient way to compare isolates. This analysis begins with the extraction of DNA from the sample using a detergent, which breaks open the cell membrane and the nucleus, releasing the contents of the cell. The DNA can be isolated through a series of filtrations. In this case, the isolated DNA is sequenced by an outside lab.

The small amount of DNA present in a sample from a bacterial colony can be best sequenced if it is first amplified with a process called Polymerase Chain Reaction (PCR). PCR consists of a series of steps that separate the two strands of DNA using high heat and allow new nucleotides to replicate each strand, doubling the amount of DNA each cycle. This reaction requires primers, which mark the segment of the DNA to be replicated, or amplified. Additionally, free nucleotides must be present along with an enzyme that can assemble new strands of DNA at high temperatures, usually taq-polymerase. The amplified DNA can then be used to sequence the 16s rRNA” (Mauzy, 2019).

If these isolates are found to be one of the *Zooshikella* species already discovered, that would imply that bacteria can travel a great distance since all other isolates have been from the other side of the world. If the isolates are not a match, this would imply that a novel species has been identified which would open up many doors for exploration.

Materials and Methods

The first step was to design primers for isolating each of the protein sequences. <https://www.patricbrc.org/> was used to collect data on the known sequence of each gene for the two strains of *Zooshikella ganghwensis* and a closely-related strain, *Hahella chejuensis*. Next, these sequences were aligned and compared using ClustalOmega. Then, the sequences were trimmed and sent to an outside lab for primer development. Once the primers were developed, master mixes containing the forward and reverse primer for each gene were created. These were used to isolate each gene from each bacterial strain. PCR and gel electrophoresis were used to analyze the success of the isolation. Successful isolates were sent out for sequencing at an outside lab. Unfortunately, attempts to clean up samples from within the gel were largely unsuccessful.

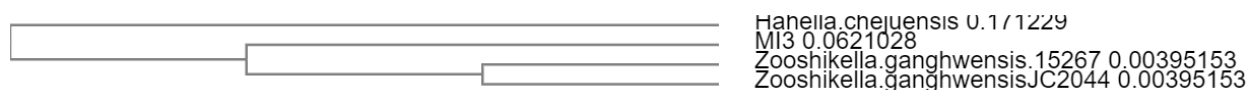
The MI3 sequences for RecA, RpoA, and TopA were able to be successfully sequenced. Once the sequences were returned, they were put into ClustalOmega and compared with the two *Zooshikella ganghwensis* sequences and the *Hahella chejuensis* sequence. At first, comparisons were made between each gene separately. For example, the sequence for the RecA gene from the known sequences was compared with the RecA sequence for MI3 that came back from the lab. This was done so that each gene could be truncated in a way that the most similar sections of each gene could be compared. These segments were merged to form a single sequence of the same length consisting of RecA, RpoA, and TopA in that order. These merged sequences were then compared with ClustalOmega.

Results

The sequences obtained are shown in appendices A-D. The results of the 16s data indicate that the isolate is very likely to be *Zooshikella Marina*. Below is a cladogram that was created with ClustalOmega, which uses sequence alignment and similarity to determine relationships between samples. No data was available for the other gene sequences in *Zooshikella marina*, so comparisons could not be made with regard to RecA, RpoA, or TopA. Furthermore, no satisfactory isolates of FtsZ or GapA were able to be sequenced from any of the samples available.



The data appears to indicate that the sequences collected from MI3 were partial sequences, because they were all shorter than the known gene sequences and produced only partial matches. The full sequences and the later truncated sequences can be seen in appendices H-P. In each of these scenarios, our isolate, MI3, was more similar to *Zooshikella ganghwensis* than it was to *Hahella chejuensis*. However, the two *Zooshikella ganghwensis* strains were more similar to each other than they were to MI3. This indicates that our isolate is likely to be closely related to *Zooshikella ganghwensis* but is not the same. This supports the earlier conclusion that our isolate is likely to be *Zooshikella marina*. The cladogram below depicts the relationships determined by ClustalOmega.



Discussion

Knowing that we have isolated a strain of *Zooshikella marina*, we can make several assumptions about this isolate that could later be tested. Phylogenetic analysis would likely show a 99.24 % sequence similarity to *Zooshikella ganghwensis*, and no other similarity greater than 91.94% (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). It is also likely to be a member of Gamma-proteobacteria (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). Uubiquinone-9 is likely to be its major respiratory quinone (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). Additionally, the lipids diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine are likely to be contained in the bacterial membrane. (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). Furthermore, C16:1 ω 7c/C16:1 ω 6c, C16:0, C18:1 ω 7c and C12:0 fatty acids and the pigment prodigiosin are likely to be present as well. (Ramaprasad, Bharti, Sasikala, & Ramana, 2015). Lastly, unlike *Zooshikella ganghwensis*, the isolate is likely to have cycloprodigiosin and eight other prodigiosin pigment analogues and differences in physiology (Ramaprasad, Bharti, Sasikala, & Ramana, 2015).

Future research would include sequencing more genes of *Zooshikella marina*, so that more comparisons could be made between isolates. Another step in the right direction would be creating a 16s primer for isolation of this gene from the five cultures with similar properties. Further research would include sequencing the other 5 common genes. Also, it would be good to verify the identification of MI3 through a carbohydrate utilization test, an Analytical Profile Index (API), determination of fatty acid composition, examining morphology, Multi-locus sequences, and/or comparing the absorption spectra.

References

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Appendix A- MI3 RecA Sequence

nnnnnnnnnnnnnnntgacagancattccagccattcaacaggctcattaggttagatattgctctgggtattggtggttacaaaa
ggacgtatcgttgaaatttatggaccagagtcttcaggtaaaacaacactcacattgcaagtcattgctgaagcgcaaaaacaaggggtac
gtgtgcctttgtcgacgtgagcatgcgttgaccctgaatatgcacgtaagctaggggtaagcgttgataacctttatgtatctcagcctgata
ctggagagcaagcgcttgaaatcacggacatgttggttcgttcgggtgcgggtgatgtcattattgttgactctgttgccgcgttaacgcctaag
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gtcgttaaaaataaggttcacctccttccgtcaggctgagtttcagatcctttatggtaaaggtatttatcatatgggtgaggttatagatcttgg
nnaaaaaaaaannnnnnnaaaaa

Appendix B- MI3 RpoA Sequence

nnnnnnnnnnnnngnctgggtgttgtaactgcagcagatattcagctagatcatgatatcgaagtggtaatcctgatcatgtgattgctcat
ttgagcgatgcgggttcgttaaataatgaagcttaagggtccgtcgtggccgcggctatgaacctgctgatgcacgaatcagtgatgatgatgaa
agtcgctctattggtagacttcagttgatgccacctatagccccgttttcgagtgctctacgttgctgaaagcgctcgtgttgagcaacgtac
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gaccttgagctaacagttcgcagtgcacaaactgcctgaaagctgaaaacatttactacataggtgacttgatccagcgctacggaagttgagctg
ttgaaaaccctaacttgggtaaaaaatcgctcacagaaatcaaagacgtactgnnncnnnnngnngtttnnnnaaan

Appendix C-MI3 TopA Sequence

nnnnnnnnnnnccctgggtagnannatatcgtaaagtcagtggtgggtcatattcgtgacttaccgtaagtggtagtaataaagcggtaga
ccctaaggcacgcgcaaaagcggcggcagaaacacgtaaactgacgcctgaacaacgcgctatccataaggagcgtaggctaaagag
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GGGATTATCCGCTGGGCGGTACAGTCAGTGGCTGTTGATTAAATGNNNNNNGGGGAGCGA

nnnnnnnnnancnngctcttgctgacgagcggcggaagggtgagtaacgcataaggaaatctgcccatgggtgggggatagcccggagaa
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nnnnngnngannannnnnnnnnnnnnggggggnnnnnnnnnntcanntcnnnnnnnnnnnntnngnncanggnnnnnnnncnnnnn
nnnnnnnnnnnggnnnnnnnnnnnnnnn

Appendix E- Zooshikella Marina 16s Sequence

agctaccatgcagtcgggcggtaacaggagagcttgctctcgtgacgagcggcgacgggtgagtaatgcataggaatctgcccatgg
gtgggggatagcccgagaaatccggattaataccgcataatgccctgaggggtaaagcaggggatcttaggaccttgcccgatggatga
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gtggggactctaaggagactgccggtgacaaaccggaggaagggtggggacgacgtcaagtcacatggcccttacggccagggtaca
cacgtgtacaatggggcgaacagagggtgccaagctgcgaagtggagctaatctctaaaacgtctcgtagtccggattggagtctgca
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aagtaccaag

Appendix F- *Zooshikella ganghwensis* 16s Sequence

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Appendix G- *Zooshikella ganghwensis* Strain JC2044 16s Sequence

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ggttcgatatttatgtaaaaaaggcataccgcattaa

Appendix H: *Zooshikella ganghwensis* DSM 15267 RecA Full vs. Truncated (highlighted)

atggatgacaacaagaaaaagccttagccgctgcagtttctcaaattgaacgacagtttgggaaaggtgccatcatgcggatgggtgatca
tgaacaagaagctatccctgccatttcaacaggctcattgggttagatattgctctgggtattggtggcttacaaaaggacgtatcgttgaag
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attgcttgggaaatcgagaaaaaatacgtcagaagcatgacacactgccagcgaggattcacctcaagaagccattgaacccgcgagc
aaagataagaaaggaaaatcaaaggcaaccacggattaa

Appendix I: *Zooshikella ganghwensis* Strain JC2044 RecA Full vs. Truncated (highlighted)

atggatgacaacaagaaaaagccttagccgctgcagtttctcaaattgaacgacagtttgggaaaggtgccatcatgcggatgggtgatca
tgaacaagaagctatccctgccatttcaacaggctcattgggttagatattgctctgggtattggtggcttacaaaaggacgtatcgttgaat
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tgcttgggaaatcgagaaaaatacgtcagaagcatggcacactgccagcgaggatgcacccaagaagccattgaacccgcaagca
aagataagaaggaaaatcaaaggcaaccacggattaa

Appendix J: *Hahella chejuensis* RecA Full vs. Truncated (highlighted)

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ccagttgctcctgttgaggctgaataa

Appendix K: *Hahella chejuensis* RpoA Full vs. Truncated (highlighted)

atgcagcgttcattaaacgaatttcttacacctcgactatcgaggtgaatcaaatcaatgcgactcacgcaaagtgaccctggagccgctc
gaacgcgggttcgggcatactttaggaagtgcattacgccgcattttgctgtcttcaatgccgggtgcgctgctcgaagttgagatcgacg
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aaatccagatatggttatttgcaaccttaactcaaatggcgacgttaagatgaaactgaaagtggctcgtgggcgtggttatgagcctgcgga
tcaacggaactcagctgatgatgagacaagagcaattggaagattgcagcttgactcctcttccagcccagtctccgtgttgcttataacgtt
gaaagcgctcgggttgagcagcgtaccgatttgacaagttggttatcgacttgaaacaaacggaacgattgaccctgaggaagcaatcc
gtagggcggcgacaattctgcaacagcagcttgctgtctttgtagattttgatcagcaaaatgagccagaaaaagtgaagagcaagaagag
atcgatccgattcttctgcggccgggttgatgatttagaactgactgtgcgttcagcgaactgcttaaaagctgagaacatctactatatcggtga
tctcattcaacgcactgaggtggagcttctgaagactcctaacctgggaaagaaatcattaaccgaaattaaagacgtgcttgcttcacgcgg
ctgtcttttaggtatgcgtcttgaaaactggcctcccgcagcattcggggcgatgatcgggtattagcgcgctaa

Appendix L: *Zooshikella ganghwensis* DSM 15267 RpoA Full vs. Truncated (highlighted)

Atgcatggctgatgaagcagttctgagcctatctaagcaaggcttaggtgtgtaactgcagcagatattcagctagatcatgatatcgaa
atcgtaatcctgatcatgtgattgctcacttgagtgatgcaggctcattaaatatgaaactgaaggctcgtcgtggccgtggttacgagcctgc
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gaaagcgctcgtgttgagcaacgtacagacttagataagttggtacttgatttagaaactgacggtactctggatccagaggaagccatccgt
cgggctgcaacaattcttcaacaacaattggctgtctttgttgatttagaaggcgacaaagaacctgagccagaagaaattgaagaagaaatc
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cttggcatgcgtctcgataactggcctcccgtagcttaaaaaatgacgataaggtttctgcctag

Appendix M: *Zooshikella ganghwensis* Strain JC2044 RpoA Full vs. Truncated (highlighted)

atgcagagttcggtaacagactttctcacaccacgtcacatagacgttcaggaaaacagcgcaactcgtgcaaaggttactctggagccgct
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gggcgttttacacgagtatagtgccatcgaaggtgtacaggaggacgttattgaaatcctgttaaaccctaaagggtgtgctattaagatgcatg
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cctgatcatgtgattgctcacttgagtgatgcaggctcattaaatatgaaactgaaggtccgtcgtggccgtggttacgagcctgctgatgcac
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gcgtctcgataactggcctcccgtagcttaaaaaatgacgataaggtttctgcctag

Appendix N: *Hahella chejuensis* TopA Full vs. Truncated (highlighted)

atgggtaaatacgtggaatacgtggagtcacctgcaaaggcaaaaccataaacaataatcttggttcggaataatgtagttaaatccagtgtg
gggcatattcgtgatttcccagcagtgaggtaaggaaacatcatctccgaaagagcgtgcggcggaagcggcgaaaaatgcgtaagct
gcctgctgaagaacgcgaaagcatcaagaaacgtaaagccagggatcaattgatctctgtatgggcgttgatccagaaaatgactgggc
ggccccgtatgaagtccttcaggtaagaaaaggcgttgaagagctgaggcgtctggcgaaaagcgtgaccagatctatctcgcaac
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tcgatcgtgtggtcggtacatggtgtcgcccttggtgtggtccaagatagcgcgtggtttgtctcgggggcgggtgcaatcggttgcaatgc
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gcaccggttagggttagcgtcaagaagaccatggtgtggcgacgcgtcttatgaggcgggttacatcacctatatgagaacggactcca
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gagtaagagcgggcgaggaagcgcacgaggcgatacgtccttctgatatcaacgtggcgctaatcagatttccggtctcgaaaagga
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Appendix O: *Zooshikella ganghwensis* DSM 15267 TopA (topo I)

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ggcgtatttttaggtgttctggctatgcactgccacaaaagagcgttgcaaatcaaccatcaatctgggtgccgggtgaagaagcgggtgcg
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Appendix P: *Zooshikella ganghwensis* Strain JC2044 TopA (topo I)

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gccggaacagcgcgtatccataaggagcgtgaaggccaaagaacaactcattggcgtatgggggtgatccggaaaatgactggaaag
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