Purpose

The purpose of this experiment is to determine the strain of bacterium isolated from soil samples from Lake Forest beach, IL. The bacterium is known to be antibiotically resistant as it will be taken from a McConkey agar plate with 3 µg/ml of Tetracycline concentration. We will use amplifcation of gene 13S ribosomal RNA which is common across prokaryotes to aid us in its identifcation. Sequenced data will be searched through gene databases. Techniques used include bacterium restreaking, genomic DNA isolation, Polymerase Chain Reaction (PCR), and gene sequence analysis using APE plasmid editor. We expect to fnd a close or identical match to a bacterium strain with known antibiotic resistance (Delventhal, 2022).

Procedures

1. Bacterial Restreak

A soil sample from Lake Forest beach IL was taken and bacteria from this sample was grown on a MacConkey agar with diferent concentrations of Tetracycline antibiotic (Urgacova, 2022). The bacterium that was restreaked came from a plate Tet3 that contained 3 µg/ml of Tetracycline and 1/10 dilution of the original soil sample (Figure 3). The single bacterium colony was restreaked on sterile agar media using a toothpick. The agar plate was then incubated for 72 hours at 28 °C. Afterwards the petri dish was sealedadde The column containing the gDNA sample was then transferred into a new collection tube, 500 µl of gDNA Wash Bufer was added, and the sample was centrifuged for 1 minute at maximum speed. After discarding the fowthrough, the column was reinserted into the collection tube and the wash procedure was repeated. Afterwards, the collection tube with fowthrough was discarded (Delventhal, 2022)

The column was then put into a DNase-free 1.5 ml microcentrifuge and 50 µl of preheated gDNA Elution Bufer was added, allowing it to incubate at room temperature for 1 minute. Afterward, the sample was centrifuged at the maximum speed for 1 minute. Lastly, the gDNA amount was quantifed using a Biotek spectrometer (Delventhal, 2022).

3. Polymerase Chain Reaction (PCR)

To identify the bacteria strain, the sequence for 16S ribosomal RNA (rRNA) was selected for and amplifed using PCR. This was done

5'-AGG GTT CGA TTC TGG CTC AG-3'

Figure 2. Mix of 27F primers used from 18S rRNA PCR amplifcation (Frank et al., 2008)

For the experimental condition, 7 µl of dH2O was put into the PCR reaction tube followed by 1 µl of 1492R primer (10 µM), 1 µl of 27F primer mix (10 µlM), 1 µl of isolated genomic DNA (217.6 ng/µl) and 10 µl Phusion Master Mix (MM). For the positive control, 7 µl of dH2O was put into the PCR reaction tube followed by 1 µl of 1492R primer (10 µlM), 1 µl of 27F £ 1 µl Ŗ ș ž Mµl µ] ˛ я l kM

a microcentrifuge tube. Afterwards the sample was placed in a thermocycler and incubated at 37 °C for 15 minutes and then at 80 °C for 15 minutes. The samples were then sent to University of Chicago Sequencing Facility (Delventhal, 2022).

6.

may cause a problem to rainbow trout fsheries.

Our question, however, is how did a Citrobacter tructae get to a soil sample from Lake Forest beach. Antibiotic resistant bacteria are more commonly found around livestock because of increased antibiotic use with livestock (Nogrado et al., 2021). It is possible that the bacteria entered into Lake Michigan through water waste, or that it may have originated in Lake Michigan itself. Additionally, there are Rainbow Trout living in Lake Michigan so it is possible that they may be carrying that antibiotic resistant bacteria (Rainbow Trout, n.d.). Either way this illustrates the danger of antibiotic overuse and how quickly novel mutated bacteria can spread into the environment. A strain that may have originated in a fshery that uses a lot of antibiotics can now be found in the lakes surrounding soil. Looking at Figure 3, the bacterium was one of the largest on the 3 µg/ml of Tetracycline McConkey agar plate, which also suggests higher antibiotic resistance.

During the procedure there were a few limitations. For example, looking at the restreaked plate in Figure 4, we can see both horizontal and vertical growth which is unusual. This may have been due to two diferent bacteria being accidentally restreaked on one plate. Additionally, during gDNA extraction, the column was not centrifuged at maximum speed which may have lowered overall purity of the sample. Even though our sample was within an acceptable range, in the future this can be a way to improve the results. Finally, another source of error could have included improper pipetting as the amounts were very small.

For future studies, there are a variety of ways forward. As the paper

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