E. miricola's β-lactamase Genes, A antibiotic resistant bacterium from space?

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ABSTRACT

In this experiment, the bacteria Elizabethkingia miricola was cloned into E. coli cells using PCR and heat shock treatment to test for the specific β-lactamase gene E.mir_ATCC33958_bla4255|putative SBL. The nitrocefin colorimetric assay confirmed that this gene did survive and multiply after PCR and heat shock, as well as being suspended in media containing Kantamyacin. The assay also concluded that the gene is in fact a β-lactamase, and is therefore resistant to certain antibiotics. The next step in this line of experimenting would be to test the gene for the specific antibiotics to which it is resistant.

INTRODUCTION

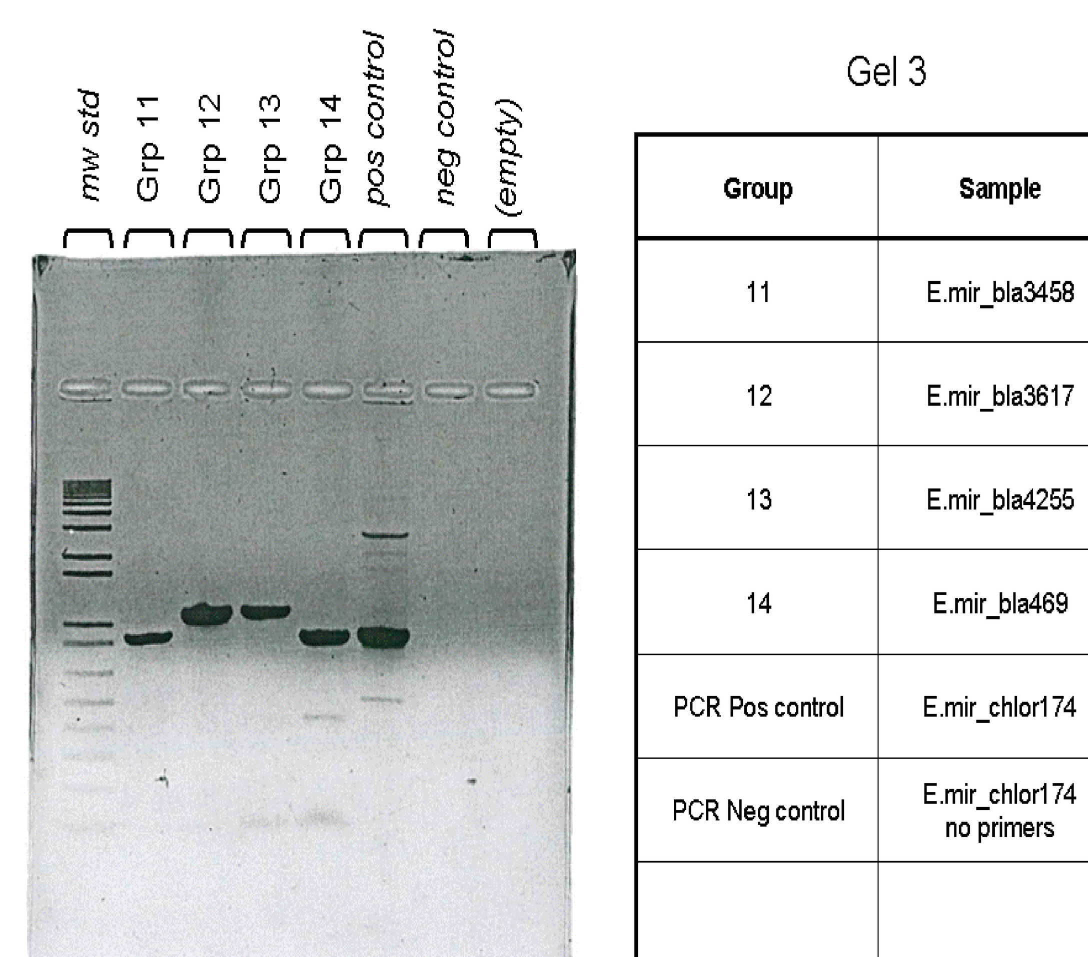
The bacteria tested was E. miricola, which was first isolated from the Mir space station, from which it derives its name, when scientists took a random sample of the condensation around the space station. E. miricola is one of the three Elizabethkingia bacterium, the others being E. meningoseptica and E. anopheles (Kämpfer 2011). E. miricola has recently been thought to contain several antibiotic resistant β-lactam genes, none of which had been previously researched and sequenced. This group, along with our colleagues in 1990 biochemistry freshman research, cloned some of these likely β-lactam genes into E. coli bacterium in order to discover whether or not they are antibiotic resistant.

MATERIALS AND METHODS

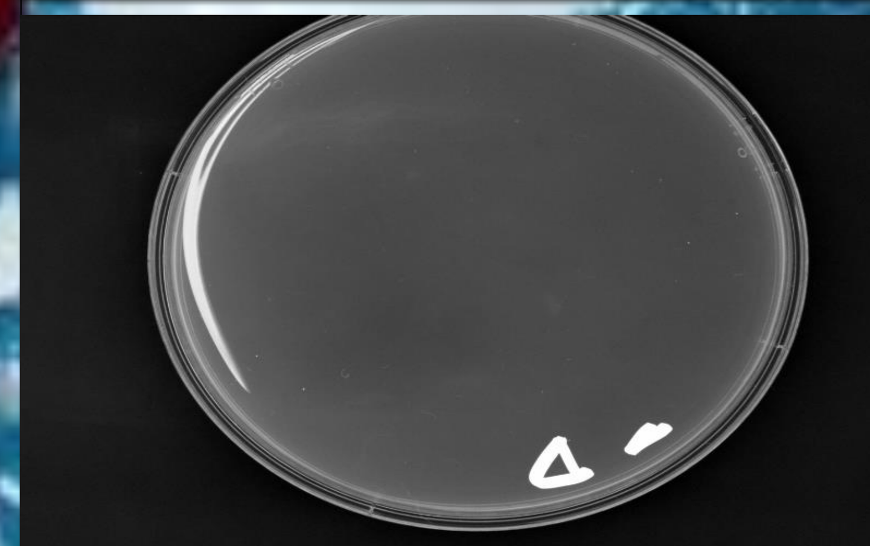
Polymerase Chain Reaction (PCR) cloning was the first step of this experiment and was checked by agarose gel. Preparing for the PCR included ordering the primers. PCR is cloning a part of the DNA sequence and duplicating it with heat treatment, denaturing the DNA, which allows the primers to attach to the specific DNA sequence wanted to produce an exponentially greater amount of clones. Then Electrophoreses by agarose gel was used to check if the PCR was successful. The next step was DNA ligation, then E. coli heat shock. After the PCR and agarose gel check was conducted, the ligation, i.e. gluing the strand of DNA containing the suspected beta-lactamase gene to an E.coli bacteria. The vector DNA used was pSKB3. This was to test if the E. coli would start producing beta-lactamase. During the process of ligation the E.coli was heated and cooled down repeatedly. The transformation plates was later checked for transfomants, with the assistance of Dr. Matts recombinant clones were identified by screening the colonies for the cloned DNA fragment. By qualitative colorometric assay using nitrocefin saturated disks the clone was compared to a positive and two negative controls. This assay determined if the gene carried by the clones was a beta-lactamase.

RESULTS

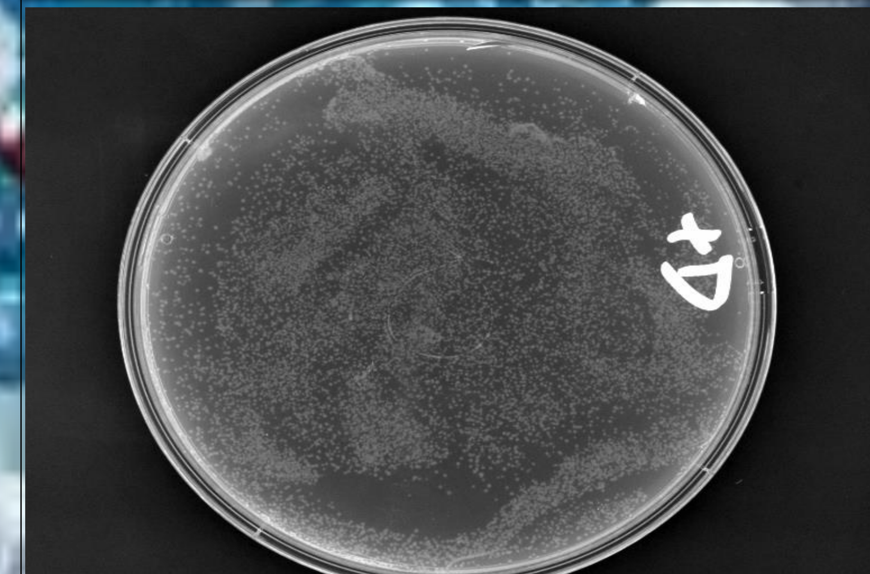
Our results are promising and show that the tested gene is most likely a beta-lactamase producing strand. The electrophoreses check showed that our PCR was successful this is illustrated to the right



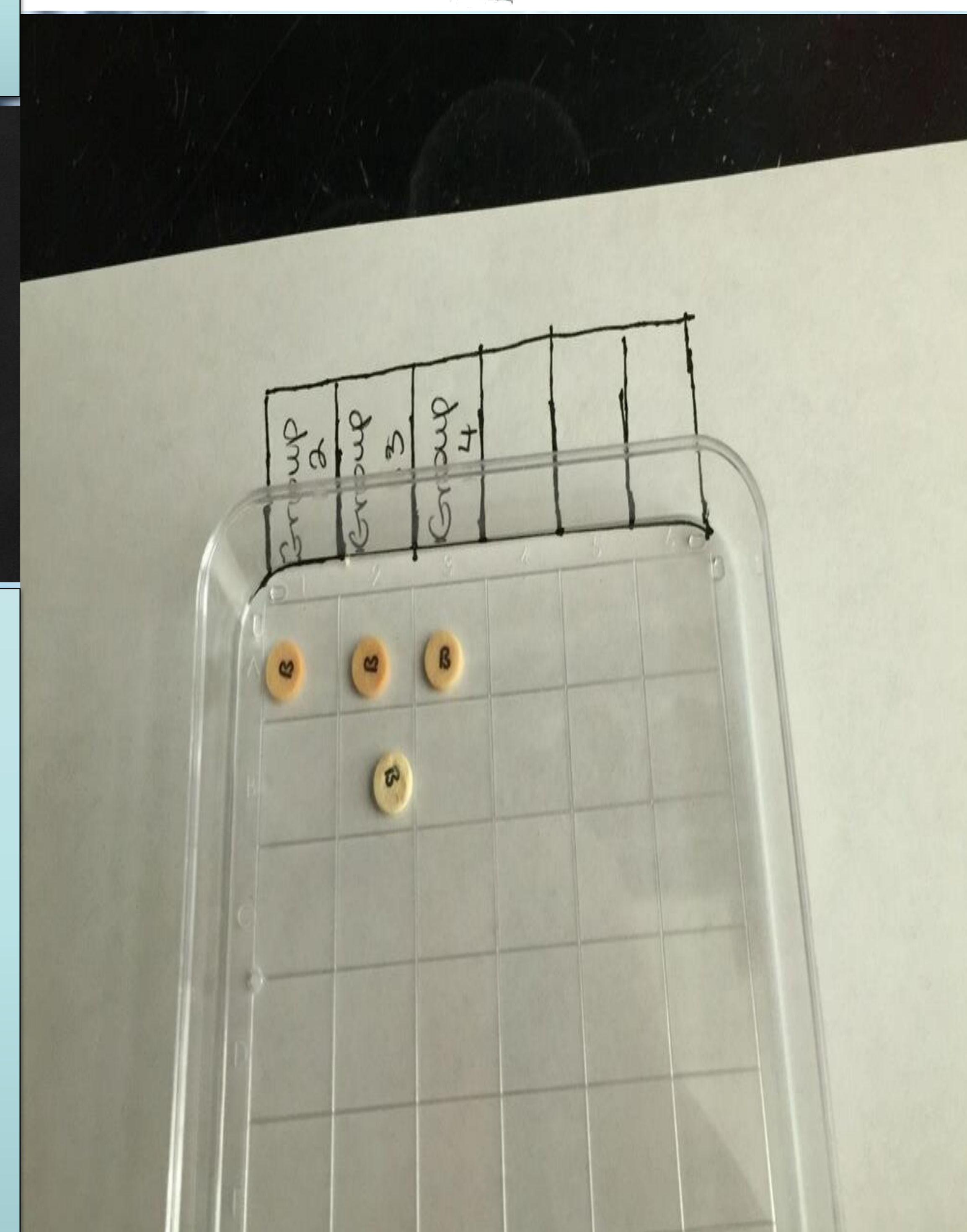
Group	Sample
11	E.mir_bla3458
12	E.mir_bla3617
13	E.mir_bla4255
14	E.mir_bla469
PCR Pos control	E.mir_chlor174
PCR Neg control	E.mir_chlor174 no primers



A picture of our bacteria colonies was not available but the negative and positive control plates are represented by picture seen below, (positive below, negative above).



The qualitative colorometric assay using nitrocefin saturated disks are shown in the illustrations below. Our clones are labelled as group 13 and as seen in the picture the disk has gotten a reddish colour signalling that the nitrocefin has been hydrolysate by the beta- lactamase produced by the E.coli combined with the suspected beta-lactamase gene. The second saturated disk in the "group 13" row is a control to show the difference between a disk where nitrocefin has been hydrolysed (turned red) and one that has not reacted (stayed yellow)



DISCUSSION

As stated before the results of this studie indicate that the gene E.mir_ATCC33958_bla4255|putative SBL in E. miricola is a beta-lactamase gene. The PCR cloning was shown to have worked in the electrophoreses of the agarose gel. The nitrocefin colorometric assay showed that a beta-lactamase gene was present in the clones, when vectored into E. coli cells. The controls of the experiment demonstrated that the cloning procedure was effective in transferring the desired phenotype into E. coli cells. The ability of this gene to block certain antibiotics can possibly be used to determine which anitbiotics would be effective against genes of this type, should they be present in more dangerous bacteria. This would require more testing of specific antibiotics, rather than the domain-wide test we performed on the gene in this experiment. There are several other potential beta-lactamase genes that also need to be tested, in addition to the ones tested in this class experiment. These experiments are easily reproducible. The experiments performed in the class may yield interesting results when put together and analyzed to see how many of the putative beta-lactamase genes were hydrolysed by the nitrocefin assay.

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