Transformation of the methicillin resistance gene (mecA) from methicillin resistant Staphylococcus aureus (MRSA) into Escherichia coli

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram positive, cocci-shaped bacterium that is genetically different from other strains of *Staphylococcus aureus* (*S. aureus*) and is responsible for several difficult to treat infections in humans. It is of great concern as a human pathogen because of its ability to cause a diverse array of life-threatening infections and its ability to adapt to different environmental conditions. MRSA is one of the leading overall causes of healthcare associated infections globally (Pournajaf et. al, 2013[1]; Gill et. al, 2005[2]). There are many anti-staphylococcal drugs including penicillins, chloramphenicol, macrolides, and tetracycline, but they quickly become ineffective due to the ability of the bacterium to develop mechanisms to confront these antibiotics. Over 60% of *S. aureus* isolates are resistant to methicillin, which is a type of penicillin (Gill et. al, 2005[2]). Currently, the only effective treatment for MRSA infections is with vancomycin, however resistance to this antibiotic has been seen in ten MRSA cases in the United States since 2002 (Tortura, Funke, and Case, 2016[3]).

MRSA is a carrier of the mecA gene, a 533 base pair sequence found in bacterial cells which allow the bacterium to be resistant to the antibiotic methicillin (Pournajaf et. al, 2013[1]). In nature, some bacteria, after cell lysis, release their DNA into the environment. Other cells encounter the DNA and depending on the particular species and growth conditions, take up fragments of DNA and integrate them into their chromosomes (Fig. 1). Transformation occurs naturally among very few genera of bacterium including *Bacillus, Streptococcus,* and *Staphylococcus*. *Escherichia coli* (*E. coli*) is a gram negative bacterium that is easily transformed in the laboratory environment. The purpose of this study was to take the mecA gene from a MRSA strain and transform it into *E. coli* cells.

Methods

DNA was extracted from MRSA cells (ATCC: 43300, Fig. 2) using the protocol and supplies provided by an Invitrogen© DNA extraction kit. Four replicate extractions were done and the mecA gene was amplified from extracted DNA using polymerase chain reaction (PCR). The mecA specific primers used for amplification were forward 5'-AAAATCGATGGTAAAGGTTGGC-3' and reverse 5'-AGTTCTGGAGTACCGGATTTGC-3' (Pournajaf et al., 2013[1]). The amplified products were run and visualized by electrophoresis on a 1.5% agarose gel (Fig. 3). PCR product was then purified using the protocol and supplies provided by an Invitrogen© PCR purification kit.

Transformation of competent *E.coli* cells (ATCC: 15597) was done using the purified PCR product following a variation of the calcium chloride heat shock method described by Chang et. al, 2017[4]. Four replicate transformations were performed and transformed cells were then plated on Eosin methylene blue (EMB) agar (Fig 4). The plates were incubated at 37^{0}C for 24 hours. DNA was extracted from the transformed *E. coli* cells, following the same protocol as above, and a PCR was done to amplify the mecA gene. Final PCR products were run and visualized by electrophoresis on a 1.5% agarose gel (Fig. 5).

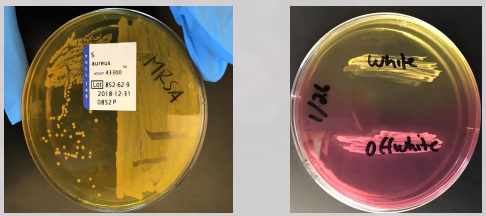

Fig. 2: Left: MRSA colonies on a mannitol salt agar (MSA) plate. **Right:** MSA is a selective media for *Staphylococcus* species and is normally red in color. The yellow coloration is characteristic of *S. aureus* as it has the ability to ferment mannitol sugar differentiating it from other *Staphylococcus* species.

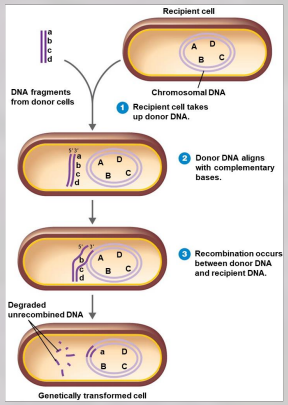

Fig. 1: The mechanism of transformation of free DNA into a bacterial cell (Tortura, Funke, and Case, 2016[3])

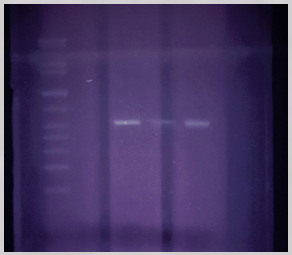

Fig. 3: Agarose gel electrophoresis of amplified mecA genes from four replicate MRSA extractions. The size of the PCR product is in between the 500bp and 600bp ladder fragments. Lane 1 is a 1kb ladder containing 100 bp fragments. Lanes 2 through 5 contain sample from each of the four replicate extractions.

Fig. 4: Growth of transformed *E. coli* on Eosin methylene blue (EMB) agar. The metallic green sheen is characteristic of *E. coli* as it undergoes acidic lactose fermentation that interacts with the methylene blue dyes contained in the media.

Fig. 5: Agarose gel electrophoresis of amplified mecA genes from four replicates of transformed *E. coli* cells. The size of the PCR product in lane 2 is in between the 500bp and 600bp ladder fragments. Lane 1 is a 1kb ladder containing 100 bp fragments. Lanes 2 through 5 contain sample from each of the four replicate extractions. Lane 6 is a negative control.

Results

The results of the gel electrophoresis pictured in Fig. 3 show that the MRSA DNA extraction and subsequent PCR of the mecA gene was successful for 3 of the 4 replicates (lanes 3 – 5). The bands present in those lanes are between the 500bp and 600bp marks of the ladder in lane 1 indicating that the PCR product is most likely at the 533bp size of the mecA gene. The lack of a band in lane 2 indicates that there may have been a problem in either the DNA extraction or PCR procedures.

Growth on the EMB agar plate (Fig. 4) indicates that the four replicates of transformed *E. coli* did in fact survive the transformation procedure. The metallic green sheen is characteristic of *E. coli* as *E. coli* ferments the lactose contained in EMB media creating acid products that interact with the methylene blue dye.

The results of the gel electrophoresis pictured in Fig. 5 show presence of the 533bp mecA gene in only 1 of the 4 replicates (lane 2). This result most likely indicates that only 1 of the 4 *E. coli* transformants were able to successfully incorporate the mecA gene inside of their cells.

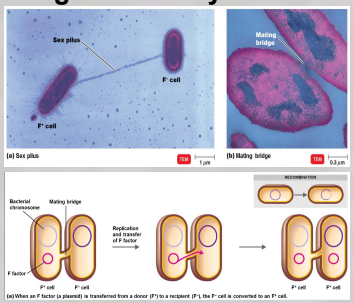

Fig. 6: Top: Electron micrograph showing conjugation in gram negative (a) and gram positive (b) bacteria. **Bottom:** Mechanism of plasmid conjugation between bacteria. (Tortura, Funke, and Case, 2016[3]).

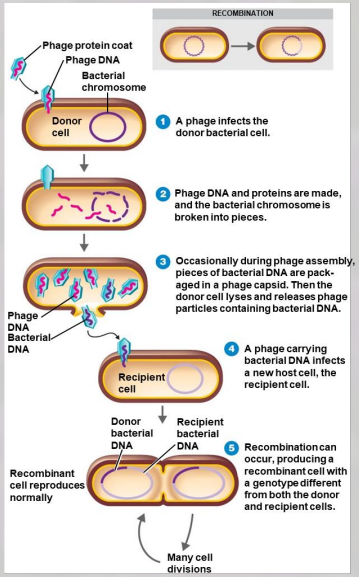

Fig. 7: The mechanism of phage transduction in bacteria (Tortura, Funke, and Case, 2016[3]).

Discussion

Antibiotic resistance is a growing concern in the medical field as it gives rise to many hard to treat infections in humans. While MRSA is one example, there are many other emerging pathogens such as antibiotic resistant strains of *Staphylococcus epidermidis* (Shoenfelder et. al, 2010[5]), *Neisseria gonorrhea*, and vancomycin resistant *Enterococcus* (Tortura, Funke, and Case, 2016[3]). Overuse of prescription antibiotics puts selective pressures on bacteria that leads to the evolution of resistance to those antibiotics (Ohlsen et. al, 2003[6]). In the case of MRSA, the only effective treatment remaining is with the antibiotic vancomycin. However, the use of this antibiotic has led to strains of vancomycin resistant *S. aureus* which will eventually render that antibiotic no longer effective for treatment of a MRSA infection. Another side effect of vancomycin use is the emergence of vancomycin resistant *Enterococcus* which can be a digestive tract pathogen (Tortura, Funke, and Case, 2016[3]).

Resistance factors are often associated with mobile genetic elements such as conjugative plasmids (Fig. 6), bacteriophage transduction (Fig. 7), or as free DNA in the environment (Fig. 1). The results of this study do in fact show that the methicillin resistance gene, mecA, was easily transferable from MRSA cells to *E. coli* cells. With the ever increasing emergence of antibiotic resistance in bacteria, and the ease at which these resistance factors can be transferred from one bacteria to another, new methods will need to be developed to treat these emerging pathogens.

Future Research

While the results of this experiment show that the mecA gene was successfully transferred from MRSA into *E. coli*, there is no evidence to support expression of methicillin resistance in *E. coli*. *E. coli* is a gram negative bacteria which has a natural resistance to penicillin and other penicillin antibiotic derivatives, such as methicillin. In order to provide evidence that the transformation leads to expression of the methicillin resistance gene, a gram positive bacteria needs to be used as the transformation donor. Preferably this should be done with another strain of *Staphylococcus* that does not already have the mecA gene, but the process of *Staphylococcus* transformation involves electroporation which requires expensive equipment (Kraemer and Iandolo, 1990[7]). Without electroporation there are natural restriction barriers involved with *Staphylococcus* that greatly reduce laboratory transformation success (Monk and Foster, 2012[8]). Another gram positive cell that could be used is *Bacillus subtilis* which has been shown to be successfully transformed with genes from a *Staphylococcus* bacterium (Ehrlich, 1977[9]) and *Bacillus* can easily be made competent in the laboratory setting (Saito, Taguchi, and Akamatsu, 2006[10]).

References

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