Overexpression and Purification of MRSA Associated Proteins BlaR1 and MecR1

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Introduction

The Methicillin Resistant Staphylococcus Aureus (MRSA) pathogen is a global concern due to its prevalence and ability to resist β-lactam antibiotics [2,3]. β-Lactam antibiotics (BLAs) are one of the largest antibiotic classes and are characterized by the presence of the β-lactam ring in their structure[4]. BLAs function by interfering with bacterial cell wall biosynthesis [3,4]. Specifically BLAs target the peptidoglycan transpeptidase PBP which prevents effective cross linking of the cell wall[4].

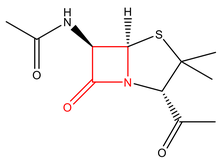


Figure 1: The backbone of the penicillin family of β-lactam antibiotics. The β-lactam ring is highlighted in red.

MRSA has two main mechanisms of resistance to BLAs [1,3,4]. The first mechanism involves the production of the β-lactamase *bla*Z which renders BLAs useless by hydrolyzing the β-lactam ring[3]. The *bla*Z production pathway has two main components, a sensor protein *Bla*R1 and a repressor blaI[1,2].The *mec*A mechanism involves the production of *mecA* which is an alternative transpeptidase that has a lower binding affinity for BLAs [3,4]

Figure 1: The C-terminal sensor domain of BlaR1

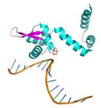


Figure 2: blaI protein bound to DNA

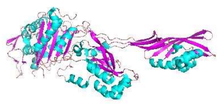
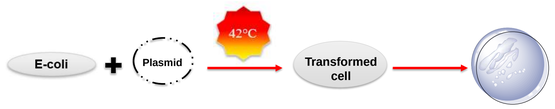


Figure 3: The structure of the transpeptidase MecA

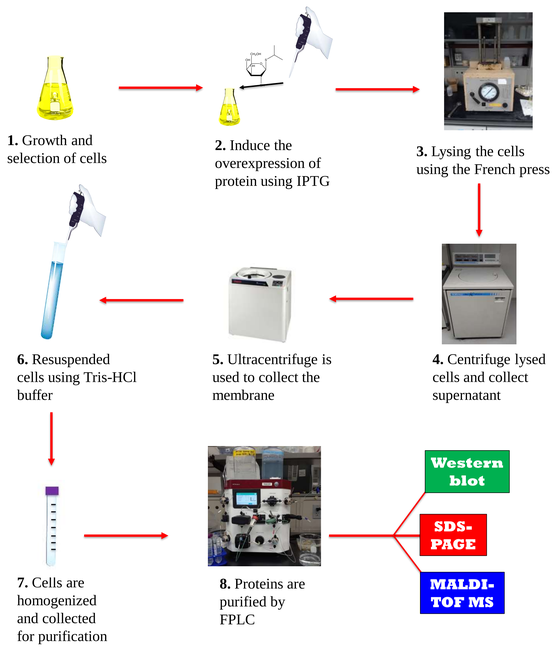
Our investigation has two distinct goals. Our first goal is to design a procedure that will allow us to purify BlaR1 minus the C-terminal domain. Our second goal is to further elucidate the *mec*A mechanism of resistance.

Transformation of BL21 (DE3) competent cells with Plasmid containing protein sequence



Transformation of BL21 (DE3) strain of E-coli using heat shock. The transformed cells are then cultured for future procedures

Purification of BlaR1 & MecR1



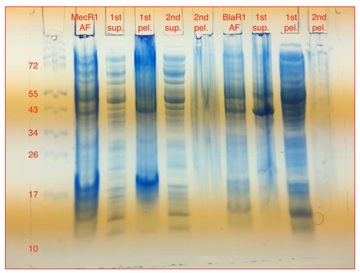


Figure 4: SDS-page gel of MecR1 and BlaR1 containing samples of both proteins throughout the experiment.

Sample	01	02	03	04	05	06	07	BlaR1
m/z	832.276	855.026	1233.602	855.099	833.155	1233.719	832.434	720.4654
	930.398	1060.004	1326.658	871.031	855.129	1326.793	1233.719	870.3662
	1008.444	1233.538	1598.756	907.994	908.088	1567.944	1326.782	916.5138
	1233.566	1249.483	1617.704	1060.099	1060.140	1598.908	1598.921	953.4430
	1249.496	1631.667	1631.727	1168.377	1252.745	1631.876	1629.925	961.5465
	1326.626	1763.663	1780.830	1249.587	1265.704	1713.906	1631.900	1032.5320
	1567.787	1781.732	1795.816	1280.675	1299.713	1717.925	1781.016	1116.5796
	1598.738	1794.735	1803.789	1409.685	1361.800	1764.004	1794.981	1237.6059
	1631.725	1803.729	1847.757	1654.836	1394.798	1779.992	1803.963	1255.5816
	1713.724	1847.701	1961.942	1746.686	1742.969	1781.988	1962.108	1310.6335
	1763.808	1964.793	1964.865	1763.685	1757.936	1794.974	1965.051	1343.7609
	1780.808	2601.170	2004.938	1847.868	2447.144	1845.984	2117.278	1357.7263
	1794.793	2769.167	2117.097	2601.386	2522.411	1965.047	2304.334	1391.7317
	1803.816		2304.095	2751.363	2847.717	1968.033		1418.7373
	1845.851		2601.232	2768.450		2304.309		1561.7605
	1847.786		2769.220	2769.399		2371.237		1640.8318
	1964.870					2653.505		1957.1924
						2851.638		2075.0154
						2856.680		2234.9618
								2435.3997
								2646.4875
								2673.3379
								2835.5694
								2940.6419
								3101.2849
								3812.7768

Figure 5: The peaks yielded by the MALDI-TOF MS analysis of BlaR1. The numbers highlighted in red the peaks that were predicted by trypsin digesting BlaR1

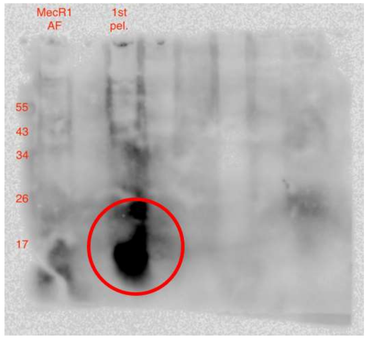
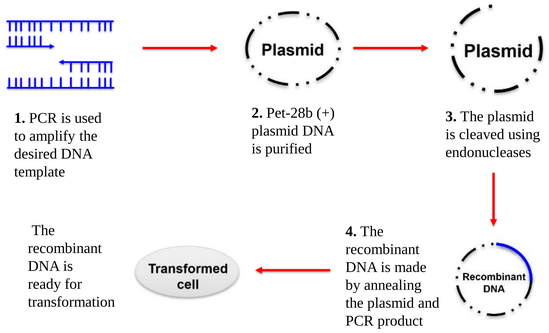


Figure 6: Analysis by western blot yielded a band for a low weight protein which could be the C-terminal domain of MecR1 that was self cleaved

Design of recombinant DNA with sequence for truncated BlaR1



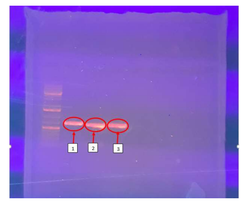


Figure 7: Agarose gel showing the products of the PCR reaction. The 3 circled bands verify that the desired DNA was amplified.

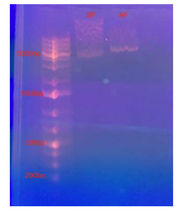


Figure 8: An agarose gel was run in order to verify if the endonucleases cleaved the Plasmid.

Conclusions

- Different conditions need to be explored for the overexpression and purification of both BlaR1 and MecR1.
- Future work includes using Urea to resuspend the pellets of both proteins after the initial centrifugation. The reasoning behind this is that SDS-page analysis of MecR1 shows expression of a protein around the 17 kilodalton region.
- Additional follow up work also includes using MALDI-TOF mass spectrometry to identify the previously mentioned protein.

Acknowledgements

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