What is the optimal way to purify and express a new BYK protein? Different methods from the DNA level to protein expression and purification to achieve an extremely pure protein for X-ray crystallography

Students: Juliana Martes Sternlicht, Tahlia Aviva Altgold

Mentor: Michal Mayer, MSc candidate Assistant Prof. Meytal Landau Faculty of Biology, Technion

INTRODUCTION

Antibiotics, drugs used to treat bacterial illnesses, are quickly becoming ineffective due to the rise of antibiotic resistant bacteria[1]. In the process of killing bacteria, these drugs put life or death pressure on the organisms, which most likely causes them to divide rapidly and mutate in an effort to survive, causing resistant strains that are harder to combat. The new goal is to make drugs that do not put the same pressure on the cells by harming their virulence without killing them. Thus, BY-kinases emerge as a potential target. BY-Kinases are a group of proteins that participate in many different functions, most notably in biofilm production by contributing exopolysaccharides[2]. Within bacteria, biofilm (an extracellular matrix) acts as a defence mechanism against drugs that may harm it[3]. The working hypothesis is that by inhibiting the expression of BY-Kinases and thus the contribution of exopolysaccharides, the biofilm is severely compromised, which may allow antibiotics to be effective in resistant bacteria. Therefore, the study of BY-kinase structure could be used to develop a new group of antibiotics that would aim to inhibit BY-kinase synthesis. The goal of this research is to compare methods of purifying and expressing BY-Kinase proteins in order to study their structure for further research as each protein is unique and requires optimization to the expression and purification protocol.

MATERIALS AND METHODS

PHASE #1 – SUBCLONING OF *wzc* AND *bceF* INTO pET11d

- **Digestion via restriction enzymes**: CIP
- **Ligation into pET11d**: T4 DNA Ligase
- **Transformation**: *E. Coli* strain XL-1

Figure 1: Flowchart of methods of Phase #1 – Subcloning of *bceF* and *wzc* into pET11d

PHASE #2 – OPTIMIZATION OF THE EXPRESSION

- **Transformation to BL-21**: *wzc* in pET9d | *wzc* in pET11d
- **Protein expression**: pET9d 37°C | pET11d 37°C 1mM IPTG | pET11d 16°C 0.1mM IPTG
- **Cell membrane lysis**: Sonicator
- **Protein gel**

Figure 2: Flowchart of methods of Phase #2 – Optimization of the Expression

PHASE #3 – PURIFICATION OF Wzc

- **Wzc expressed from pET11d vector induced with 1mM IPTG at 37°C**
- **Cell membrane lysis**: French press
- **Affinity column**: His-Trap column
- **Size exclusion column**

Figure 3: Flowchart of methods of Phase #3 – Purification of Wzc

PHASE #4 – CRYSTALLIZATION

RESULTS

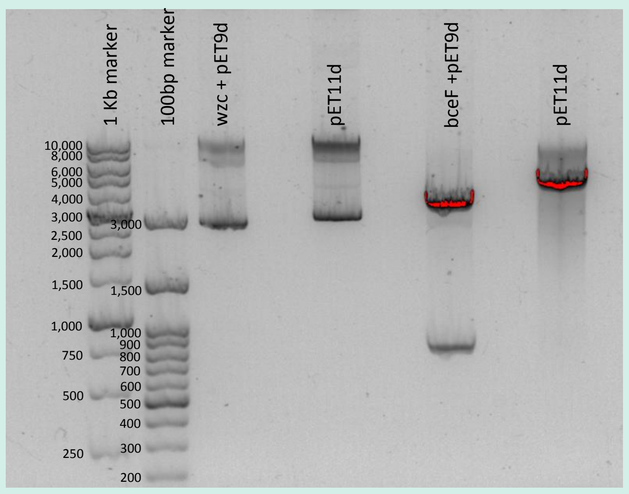

Figure 4: Gel results of the vector exchange of bceF and wzc from pET9d into pET11d. All samples were cut with BamHI-HF and NcoI-HF and ran in 1% agarose gel.

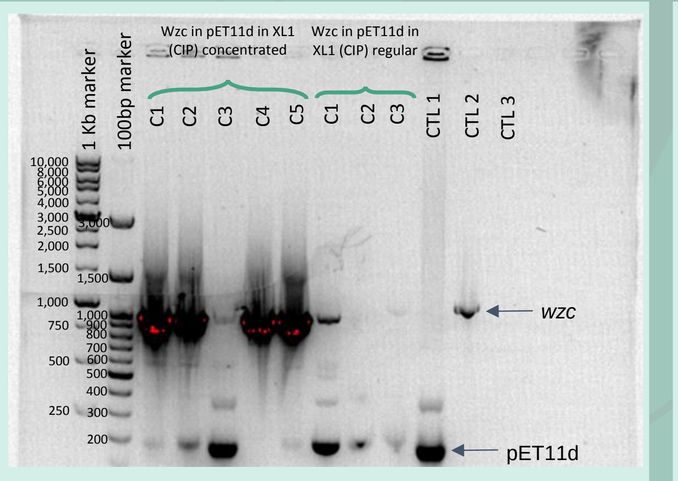

Figure 5: Colony PCR gel results of the ligation of wzc into pET11d. C1 refers to colony 1 etc. CTL 1, 2, and 3 stand for, respectively, pET11d in XL1, *wzc* in pET9d, and no bacteria cells. All samples ran in 1% agarose gel.

XL-1 colonies

SAMPLE	N° OF COLONIES
WZC in pET11d in XL-1 (CIP) (regular concentration)	10
WZC in pET11d in XL-1 (CIP) (concentrated)	46
WZC in pET11d in XL-1 (CIP) (diluted)	1
Control 1 - WZC + pET11d in XL-1 (without ligase) (CIP)	62
Control 2 - WZC + pET11d in XL-1 (without ligase and insert) (CIP)	46

Table 1: Number of colonies in different concentrations of wzc an bceF in pET11d transformed in XL-1.

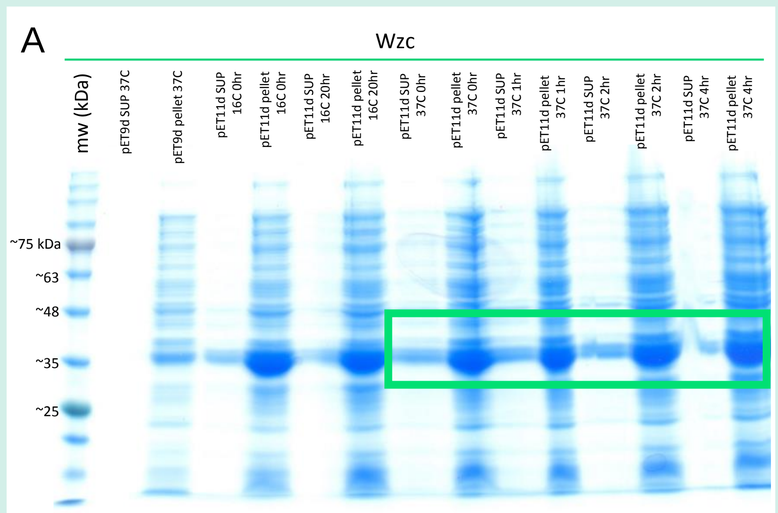

Figure 6: Gel results of Wzc in pET9d and pET11d with coomassie stain (A) on SDS-PAGE 12% acrylamide and Western-blot results (B) of same gel with antibody anti-His-HRP (Thermo) 1:1000

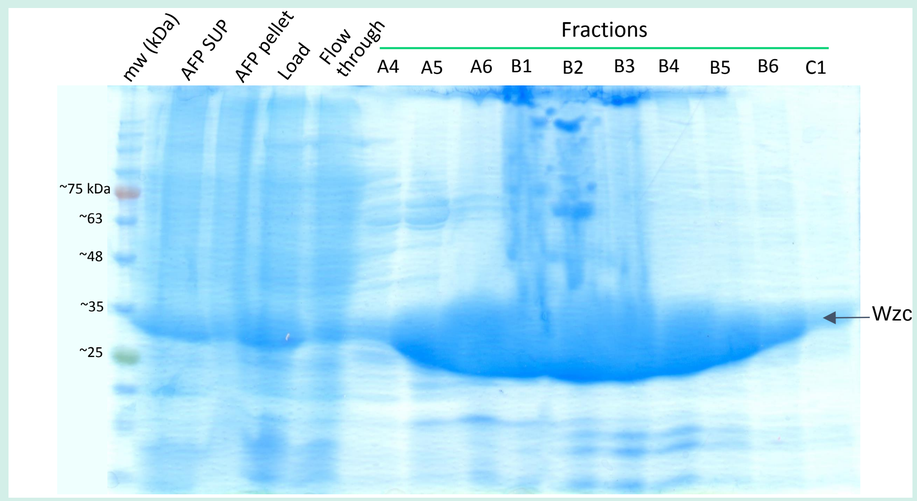

Figure 7: Affinity column gel results of Wzc with coomassie stain on SDS-PAGE 12% acrylamide

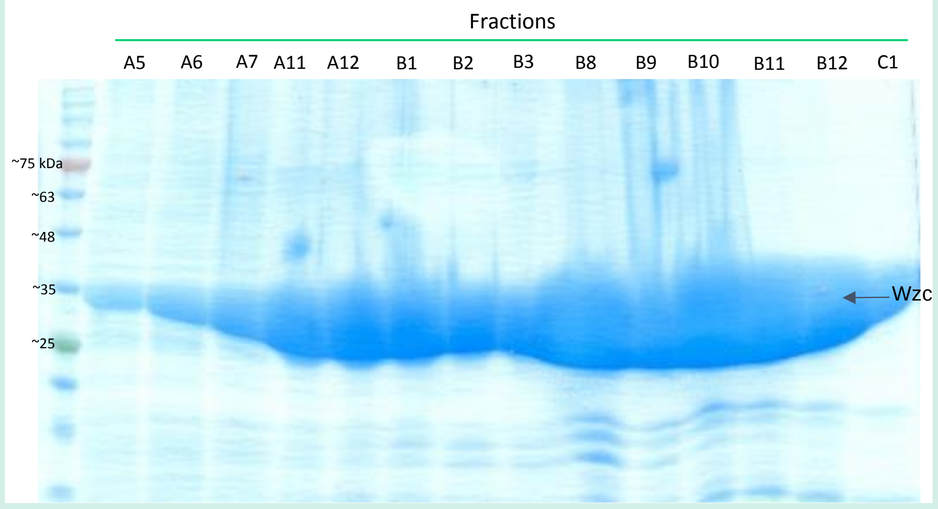

Figure 8: Size column gel results of Wzc with coomassie stain on SDS-PAGE 12% acrylamide

CONCLUSIONS

During phase 1 (Figure 1), we digested *wzc* in pET9d, *bceF* in pET9d, and pET11d using restriction enzymes. As shown in Figure 4, the digestion was not completely successful for *wzc* and pET9d as well as pET11d. Although *bceF* was successfully digested, it was not successfully ligated unlike *wzc,* as seen in Figure 5 and Table 1. In phase 2 (Figure 2) the optimal expression conditions were found to be inducible expression (pET11d) at 37°C as seen in Figure 6. In the purification phase (Figure 3) we used a His-Trap affinity column (Figure 7) and a Size Exclusion Column (Figure 8) and achieved a large amount of protein, yet not sufficiently pure (there is no single band of Wzc, bands of smaller proteins appear as well). From Figure 8 that Wzc has a tendency to aggregate which affects its ability to crystallize.

REFERENCES

[1] GRANGEASSE, Christophe; TERREUX, Raphaël; NESSLER, Sylvie. Bacterial tyrosine-kinases: Structure–function analysis and therapeutic potential. Biochimica Et Biophysica Acta (bba): Proteins and Proteomics, [s.l.], v. 1804, n. 3, p.628-634, mar. 2010.

[2] MARCUZZO, Alberto Vito et al. Hyaluronate effect on bacterial biofilm in ENT district infections: a review. Apmis, [s.l.], 24 jul. 2017. Wiley-Blackwell.

[3] HØIBY, Niels et al. Antibiotic resistance of bacterial biofilms. International Journal Of Antimicrobial Agents, [s.l.], v. 35, n. 4, p.322-332, abr. 2010.

ACKNOWLEDGEMENTS

We would like to thank Michal Mayer, MSc candidate, and Assistant Prof. Meytal Landau for hosting and guiding us through our research in her laboratory.
We would also like to thank the TCSB for allowing us to use their facilities.
Finally, we would like to thank the foundations and donors for their generous support of the SciTech Program.