Discovery of Glycocins from Pathway Refactoring

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1. Introduction

Motivation and Significance

- There has been an increasing amount of multi-drug resistant bacterial strains, which requires more research in the discovery of new antibiotics to counter the multi-drug resistant bacterial strains
- Glycocins, known as glycosylated bacteriocins, are bacterial toxins that constitutes a subset of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products.
- Glycocins contain cationic N- terminal regions that bind to negatively charged cell membranes. This allows hydrophobic C-terminal regions to form pores which kills the target cells..

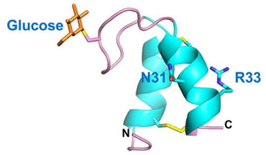

Fig. 1.1 Structure of Sublancin, an antimicrobial peptide that belongs to the glycocin family of natural products

Heterologous Expression

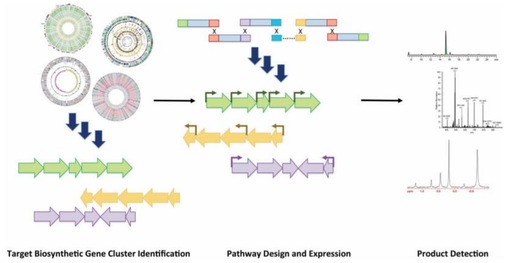

Fig. 1.2 Overview of the heterologous expression strategy for natural product discovery

Novel Contributions

- Heterologous expression of the biosynthetic gene clusters is an effective strategy used for natural product discovery as it can activate gene clusters that are typically silenced in native hosts.
- Synthetic biology tools such as Golden Gate cloning technology is an effective and efficient way for rapid reconstruction of gene clusters.
- Escherichia coli is very cost effective have been engineered to optimize and balance natural product biosynthesis
- The discovery of newly found glycocins can later be used for drug-therapeutic uses.

2.1 Materials and Methods

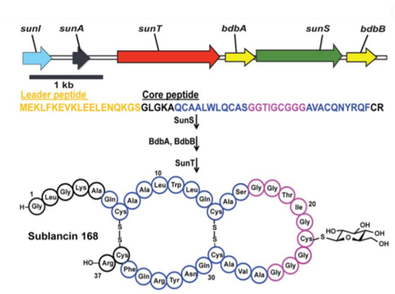

Fig. 2.1 Biosynthesis of Sublancin in *Bacillus subtilis* 168. Leader peptide is indicated in gold. Leader peptide is cleaved off after the S in "GSG" sequence.

- The glycocin from *Geobacillus* will be biosynthetically reconstructed from a gene cluster made up of 5 genes homologs of Sublancin
- Glycocin plasmid is cloned using *Escherichia coli* DH5α cells
- T7 promoters and terminators are used which makes them functional in BL21(DE3) *E.coli* strain for expression

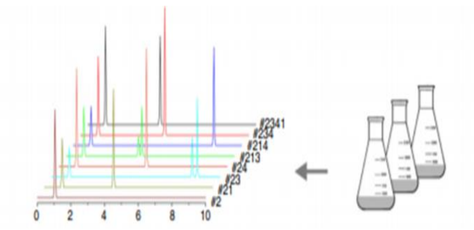

Figure 2.2 Fermentation is followed by product characterization using MALDI

- Restriction enzyme digest using XmnI and EcoRV are used to check whether the glycocin pathway is assembled correctly
- Fermentation followed by matrix-assisted laser desorption ionization as mass spectrometry (MALDI MS) will be used to characterize the glycocin.
- A bioassay with the indicator strain *Bacillus cereus* atcc 14579 is used to check the bioactivity of the glycocin of interest.

2.2 Experimental Work-flow

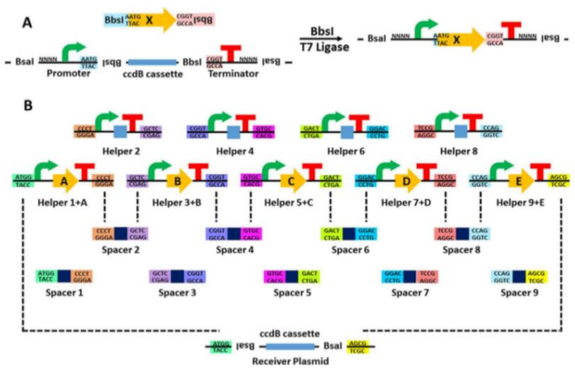

Fig. 2.2 Scheme of the plug-and-play pathway refactoring workflow

- (A) The first tier Golden Gate reaction. Gene is synthesized of amplify via PCR and cloned into the helper plasmid using BbsI to catalyze the reaction
- Gene is synthesized of amplify via PCR and cloned into the helper plasmid using BbsI to catalyze the reaction
- (B) The second tier Golden Gate reaction. All the inserts are assembled into the final construct using BsaI

3. Results

- **Heterogenous expression** is a quick and effective technique to engineer the pathway of the glycocin. This allows for fermentation, fractionation and purification of the product

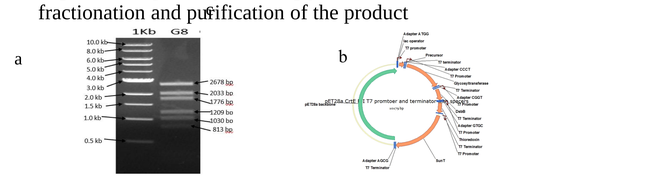

Fig 3.1 a) Restriction Enzyme digest of glycocin from Geobacillus using EcoRV and XmnI. b) pET28a plasmid with all the Sublancin gene homologs added to form the glycocin of interest.

- During the expression of the glycocin pathway, the leader peptide is cleaved off and core peptide is expressed. MALDI is used to detect the glycocin following fractionation.

Fig. 3.2 a) Amino acid sequence of *Geobacillus* in comparison with SunA. Leader peptide is cleaved after the S of the "GSG" sequence. *Geobacillus* has a modification at hexose.

b) MALDI result after desalting supernatant using Ziptip. The peak highlighted in yellow shows where our product is. Expected weight of our glycocin is 4222.7238 Da.

- Glycocin is purified using HPLC and detected using MALDI. Bioassay is used to test for the bioactivity of the glycocin.

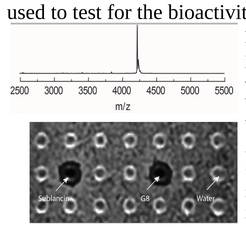

Fig. 3.3 a) MALDI results following ammonium precipitation of the glycocin. A strong peak around 4220 which is approximately the same molecular weight as the glycocin of interest

b) Bioassay with the indicator strain Bacillus cereus atcc 14579 growing in the LB agar. Sublancin and Geobacillus are added to the bio assay and shows zone of inhibition. The water spot indicates no zone of inhibition.

Conclusion

- With synthetic biology techniques available to us, we can identify the characteristics of the natural product of interest
- The data generated by this method showed that the glycocin isolated from *Geobacillus* is correctly constructed and no bioactivity was lost during the purification process.
- In the end, one more glycocin was discovered from a thermophilic strain, something that has never been done before, which can be potentially used for therapeutic drug use.

Acknowledgments

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