COLLEGE OF SCIENCE
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Analysis of Bacteriocin Expression in Urinary Lactobacilli

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Introduction and Objective

An alternative treatment is needed to counter the increase in antibiotic resistant pathogens as they evolve to resist different antibiotics. Bacteriocins are toxic peptides and proteins often produced by lactic acid forming bacteria such as lactobacilli. Recent research revealed that genomes of urinary isolates of *Lactobacillus gasseri* S1 and *Lactobacillus delbrueckii* S9 bacteria encode for several putative bacteriocins. We also discovered that these lactobacilli inhibit the growth of major uropathogens. The goal of this project is to test whether identified bacteriocin genes are expressed. Primers were designed and tested that allowed us to access transcription of the bacteriocin genes via reverse transcription PCR. If our analysis finds bacteriocins capable of inhibiting the uropathogens, these bacteriocins can serve as a possible alternative treatment for complicated or drug-resistant urinary tract infections.

Materials and Methods

- **Complementary DNA (cDNA) was prepared by reverse transcription reaction from the total RNA isolated from lactobacilli strains *Lactobacilli gasseri* S1 & *Lactobacillus delbrueckii* S9.**
- **Genomic DNA (gDNA) of S1 and S9 strains were used for testing the newly designed primers in polymerase chain reaction (PCR) (Table 1, Fig. 1) using Q5 Polymerase MasterMix**
- **PCR program:**

  98°C - 60 s
  98°C - 40 s
  55-6°C - 15 s } **25 cycles**
  72°C - 5 s
  72°C - 120 s

- **cDNA was used to test for expression of bacteriocin genes while gDNA and RNA were used as positive and negative controls respectively (Fig. 7)**
- **PCR products were analyzed on 2% agarose gel with ethidium bromide staining (Fig. 2-3).**

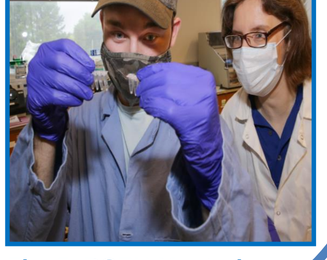

Fig. 1. PCR Machine

Fig. 2. Gel Box

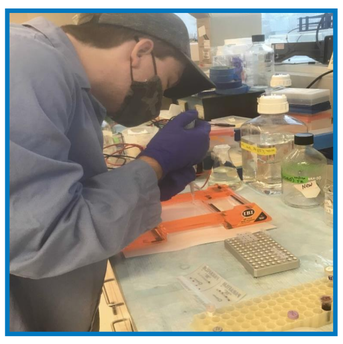

Fig. 3. Gel Analysis

Fig. 4. Lab Team

Fig. 5. RCEU Mentoring

Results

- **Four pairs of primers were successful in amplifying the predicted genes of Enterolysin A and Helveticin J genes (oSL 39 – oSL 48, Table 1) and these primers were used for initial tests of bacteriocin expression.**

Table 1. Primers to amplify predicted bacteriocin genes

Lactobacillus Strain	Predicted Bacteriocin	Primer ID	Sequence	Temperature Predicted	Temperature Used	Expected Product Size
L. gasseri S1	Helveticin J	oSL 39	CGTAGCAGTTTCTGGATATC	61°C	59°C	203 bp
		oSL 40	GCCCTGTTATGGTTGATGT			
L. delbrueckii S9	Enterolysin A	oSL 43	CTGTTTTGGCTATTCACCC	62°C	59°C	173 bp
		oSL 44	CTTGTCGCCTAGCTTGAC			
	Helveticin J1	oSL 45	GCAAACTTGGGAATATGCTG	62°C	59°C	172 bp
		oSL 46	GTTGAGGTAAGCCAAACG			
	Helveticin J2	oSL 47	GTGTCGATATCAAAGCACAG	62°C	59°C	194 bp
		oSL 48	GATTGTAAAGTAGCCGTTGC			

- **PCR from cDNA of S1 strain revealed the expected PCR product; however, so did the negative control of RNA template as well. This is an indication of gDNA contamination in the RNA and cDNA preparations.**
- **Neither of the three predicted bacteriocin genes in S9 strain were amplified from cDNA template suggesting no expression of these genes under tested condition.**

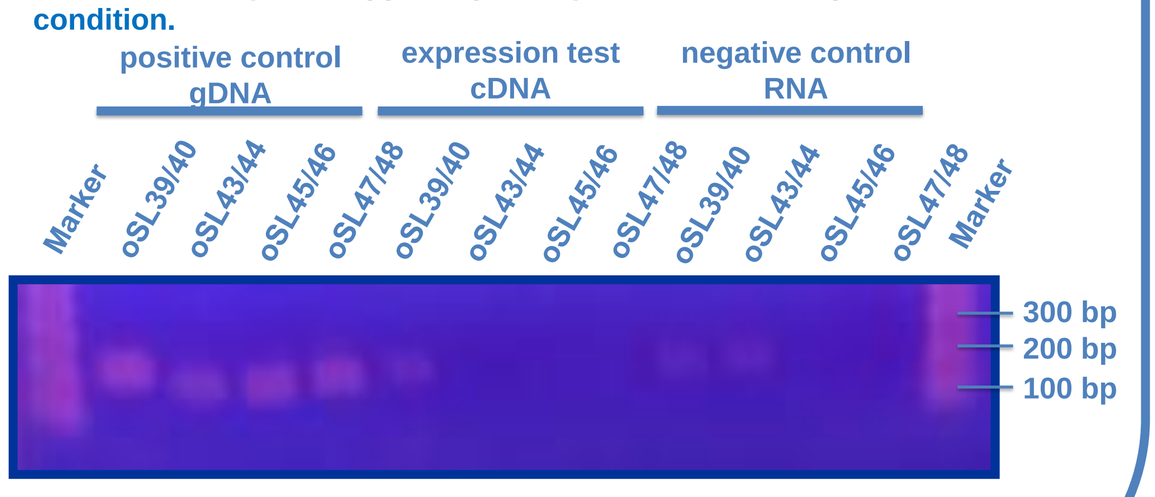

Fig. 7. Analysis of PCR products using Lactobacilli S1 & S9 samples

Conclusions

Our results confirmed the presence of bacteriocin genes in lactobacillus strains S1 and S9, but did not show strong expression of these genes. Future study will involve continued PCR testing using DNA samples from different growth stages of lactobacilli to discover the stages in which bacteriocins are produced.

Acknowledgements

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