Analysis of Antibiotics (bacteriocins) from Antibiotic-Producing Bacterial Isolates

Microbiology

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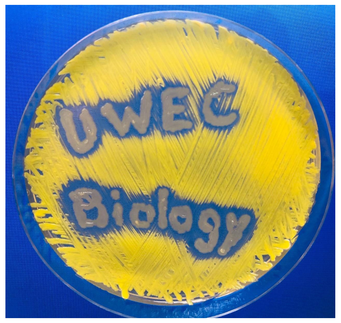
Introduction

Some bacteria, specifically ones that are present within the environment, have been found to have antibiotic-producing abilities in order to have a higher advantage when competing for food and nutrients with other microorganisms. This concept has been an up and coming topic for microbial research. The antibiotics/bacteriocins produced from these bacteria, if isolated, could potentially be used as a way for food preservation or as an antibiotic against pathogenic bacteria.



Strain M20G showing inhibition properties against *Micrococcus luteus*
Artist – Dr. Sasha Showsh

Abstract

The purpose of our research was to potentially find new strains of bacteria with antibiotic-producing abilities that could be effective against pathogenic microorganisms. A variety of soil samples were taken from multiple environments around Wisconsin. Each sample was screened for their ability to produce antibiotics against Escherichia coli and Staphylococcus aureus through an assay technique. A total of 12 microbial strains showed consistent inhibition properties and underwent further testing. Of the 12 strains, one of them exhibited inhibition of E. coli only, five demonstrated inhibition of S. aureus only, and six strains demonstrated inhibition against both. We are currently identifying the biochemical and genetic properties of the strains, as well as attempting to purify the antibiotics in cell-free extracts for further chemical analysis. We have already been attempting to assay purified cell-free extracts from several of the strains but have not gotten consistent results yet.

Methods

Sampling

A variety of environmental samples were taken from all over Wisconsin including Eau Claire, Menomonee Falls, Arkdale, and Kewaskum. Several samples were placed in separate test tubes from each location and labeled. All samples were documented with details about what kind of environment they were taken from and then kept refrigerated unit screening could take place.

Screening

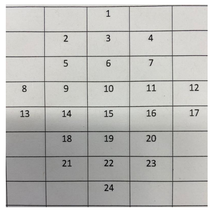
Each sample was mixed with an appropriate amount of distilled water and then mixed thoroughly to suspend the soil. A serial dilution was made for each sample and then spread plated on a variety of media including CBA (Columbia Blood Agar) and R2A (Reasoner's 2A), along with others.

After incubation of the plates, they were all carefully examined for colonies displaying possible inhibition properties against other bacteria present on the plate. Those colonies were then separated and purified through streaking.

Assaying

Purified colonies were assayed against pathogenic bacteria – *Staphylococcus aureus* (Sa) and *Escherichia coli* (Ec). To do this, Sa and Ec were spread plated on CBA plates. Using the grid formation (pictured), colonies from the purified samples were aseptically picked up with toothpicks and placed on the spread plates one by one.

After overnight incubation of the plates, they were examined for inhibition properties. Inhibition properties were exhibited when a zone of clearing is present around the newly formed sample colony on the plate.



Grid used for assay

Cell-free extract collection

Colonies from strains that showed strong inhibition properties against Sa and Ec were then taken to make cell-free extracts. Colonies were aseptically transferred and grown in THB (Todd Hewitt Broth). The newly grown cells suspended in the broth were then spun in a centrifuge to separate the cells from the cell-free extracts.

After being spun, there was a formation of a pellet (collection of cells at the bottom of the tube) and a supernate (the cell-free extract). In order to separate the two, the supernate was removed by syringe and then placed in a different tube. After this process was done for all strains being tested, the cell-free extracts were re-assayed against Sa and Ec using the same technique described above.

Results

Table 1: Environmental Samples Inhibition Properties Against Sa and Ec

#	Name	Sa	Ec
1	V03	-	+
2	V05	+	-
3	T3A	+	-
4	T3B	+	-
5	T6	+	+
6	M20G	+	+
7	M20W	+	-
8	A1	+	-
9	A7	+	+
10	M21A	+	+
11	M21B	+	+
12	C1	+	+

(+) indicates the strain showed inhibition
(-) indicates the strain did not show inhibition

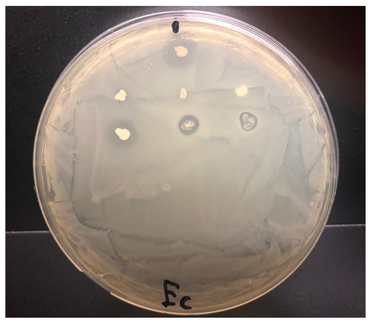
- Table 1 shows 13 strains of microbes that were found and then kept for further testing due to consistent results and demonstration of inhibition against Sa and Ec
- V03 showed inhibition against E. coli only
- V05, T3A, T3B, M20W, and A1 showed inhibition against S. aureus only
- T6, M20G, A7, M21A, M21B, and C1 showed inhibition against both E. coli and S. aureus

Table 2: Samples and Inhibition Properties Against Sa and Ec

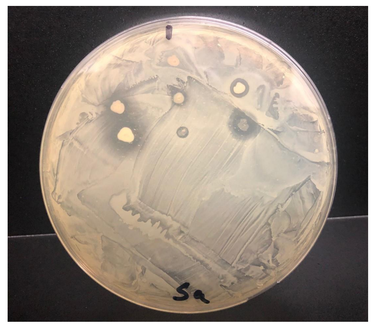
#	Name	*Sa*	*Ec*
1	VO3	-	+
2	VO5	+	-
3	M20G	+	-
4	M20W	+	-
5	M21A	+	+
6	M21B	+	+
7	C1	+	+

(+) indicates the strain showed inhibition
(-) indicates the strain did not show inhibition

- Table 2 shows the results of another assay attempt
- Only 7 of the 13 emphasized strains are shown. This is due to possible pursuit of isolating their cell-free extracts to then further concentrate and identify their antibiotic producing agents
- Pictures of these results are shown below



Assay with microbial strains showing inhibition against *Escherichia coli*



Assay with microbial strains showing inhibition against *Staphylococcus. aureus*

- Some of the strongest performing colonies – M21A, M21B, M20G, M20W - were then put through the cell-free extract collection process (described in the methods section)
- The original results of cell-free extracts from the colonies of the chosen strains showed inhibition in an assay, but only on diluted plates of Sa and Ec.
- The cell-free extracts were then put through a variety of processes to concentrate them. However, none of the attempts were able to show consistent inhibition properties.

Discussion

- The results show that several strains obtained from the environment have possible antibiotic producing agents
- Cell- free extracts from a few of the strains have showed some inhibition properties but are inconsistent at this time.
- A more precise concentration process of the cell-free extracts needs to be perfected
- Further research would include analyzing the samples physical properties and understanding the chemical make up of the antibiotic agents being secreted from them

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