OSU Center for Health Sciences, Department of Biochemistry and Microbiology

Culturomics Approach to Identify Halophiles in Edible Salts

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INTRODUCTION

The phrase "you are what you eat" comes up in our everyday conversations, however does this phrase hold its integrity when discussing our microbiome? The oral and gut microbiome play an important role in the health status of individuals, thus making the food which we consume an important factor in maintaining healthy living. While the chemical ingredients of the food we ingest are known, the microorganisms present in the food may be unknown.

Advances in genomic technologies have enabled us to identify halophilic microorganisms present in edible salts, which are often obtained from salt mines (figure 1). Some halophiles have been shown to be pathogenic and/or have the potential to be pathogenic. However, the capacity that these halophiles have to our improve health has not yet been determined. The undiscovered relationship between halophiles and health thus becomes relevant due to our heavy use of salt in preservation or therapy. So, in this project we used a culturomics approach to isolate and purify halophilic archaea in store bought edible salt. We used biochemical tests and 16s rRNA sequencing to identify the organism as *Haloferax alexandrinus*.

OBJECTIVES

The long-term objective of this project is to analyze a halophilic archaeal strain isolated from edible salt for factors that dictate colonization and interaction with the gut microbiome and the host.

METHODS

Store-bought edible salts were obtained and dissolved in specially designed resuscitation buffer to promote growth of halophiles. Dissolved salts were inoculated onto salt enriched media to obtain isolated colonies which were then subject for further identification. One store-bought edible sample showed presence of microbial growth. Phenotypical characterization using biochemical tests(Table 1 and Table 2) helped to identify the halophilic organism. For genotypic identification, DNA was isolated from the pure culture, extracted, and purified using a DNA extraction kit. 16s ribosomal RNA sequencing of the purified DNA was used to identify the halophilic organism growing on the edible salt.

Figure 1: Salt mine

The Weather Channel. Retrieved January 25, 2022, from https://weather.com/science/nature/news/yekaterinburg-russia-salt-mines-mikhail-mishainik-20140614

Biochemical tests

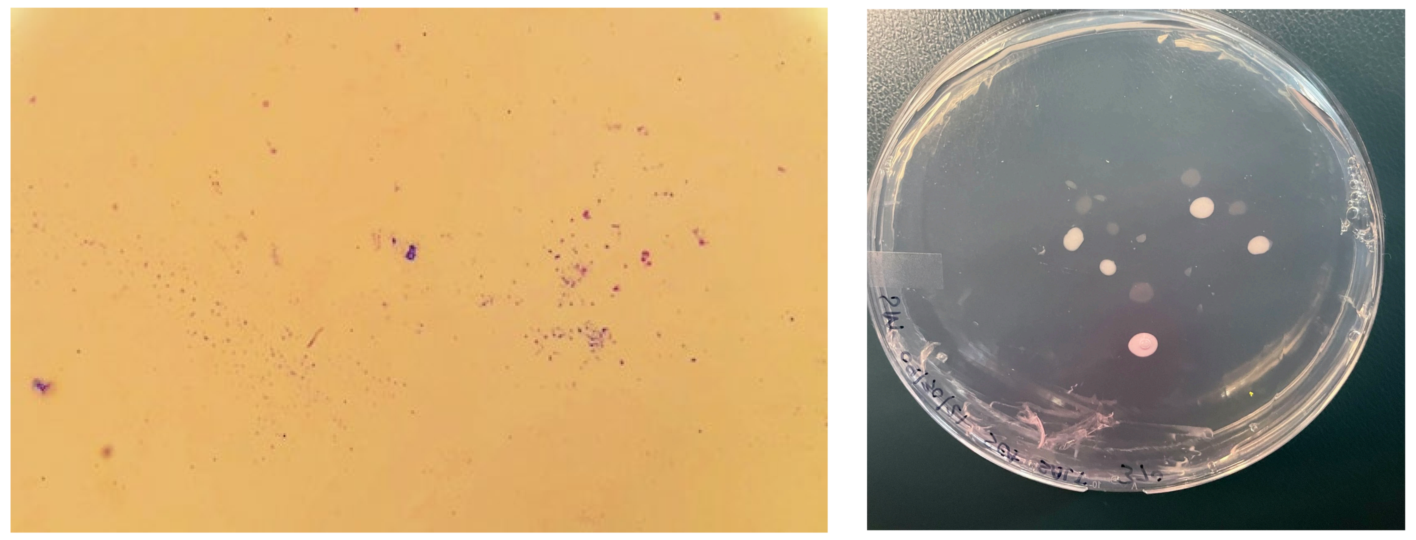
Table 1: Enzyme and fermentation tests

API 20 NE			
Test	Active Ingredients	24h	48h
NO3	Potassium nitrate	-	na
TRP	L-tryptophane	-	na
GLU	D-glucose	+	+
ADH	L-arginine	-	-
URE	Urea	+	+
ESC	Esculin ferric citrate	+	+
GEL	Gelatin (bovine orgin)	-	-
PNPG	4-nitrophenyl-BD-galactopyranoside	-	-
GLU	D-glucose	-	-
ARA	L-arabinose	-	-
MNE	D-mannose	-	-
MAN	D-mannitol	-	-
NAG	N-acytl-glucosamine	-	-
MAL	D-maltose	-	-
GNT	Potassium gluconate	-	-
CAP	Capric acid	+	+
ADI	Adipic acid	-	-
MLT	Malic acid	-	-
CIT	Trisodium citrate	-	-
PAC	Phenylacetic acid	-	-
OX	Oxidase	na	na

Table 2: Carbohydrate utilization tests

API 50 CH					
Test	24h	48h	Test	24h	48h
CONTROL	-	-	Esculin ferric citrate	-	-
Glycerol	-	-	Salicin	-	-
Erythritol	-	-	D-cellobiose	-	-
D-arabinose.	-	-	D-maltose	-	-
L-arabinose	-	-	D-lactose (bovine origin)	-	-
D-ribose	-	-	D-melibiose	-	-
D-xylose	+	+	D-saccharose (sucrose)	-	-
L-xylose	-	-	D-trehalose	-	-
D-xylose	+	+	Inulin	-	-
Methyl-beta-D-xylopyranoside	-	-	D-melezitose	-	-
D-galactose	+	+	D-raffinose	-	-
D-glucose	+	+	Amidon (starch)	-	-
D-fructose	+	+	Glycogen	-	-
D-mannose	+	+	Xylitol	-	-
L-sorbose	-	-	Gentiobiose	-	-
L-rhamnose	-	-	D-turanose	-	-
Dulcitol	-	-	D-lyxose	-	-
Inositol	-	-	D-tagatose	-	-
D-mannitol	-	-	D-fucose	-	-
D-sorbitol	-	-	L-fucose	-	-
Methyl-alpha-D-mannopyranoside	-	-	D-arabitol	-	-
Methyl-alpha-D-glucopyranoside	-	-	L-arabitol	-	-
N-acetylglucosamine	-	-	Potassium gluconate	-	-
Amygdalin	-	-	Potassium 2-ketogluconate	-	-
Arbutin	-	-	Potassium 5-ketogluconate	-	-

Figure 2 : Gram stain and colony morphology



RESULTS

Phenotypical and genomic identification identifies the organism as *Haloferax alexandrinus*

CONCLUSION

Based on the phenotypic results obtained through biochemical testing and genotypic 16s ribosomal RNA sequencing the presence of *Haloferax alexandrinus* was detected in the store-bought edible salt.

FUTURE DIRECTIONS

These results provide basis for performing future studies such as the ability to form biofilms, colonize the gut and be part of its microbiome, and how their presence potentially affects heath conditions.

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