Isolation of Halophilic Bacterium from Makai Deep Pure Sea Salt

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INTRODUCTION

Edible salts have long been used in the food industry for not only the flavoring of foods but also in food preservation. While food storage advancements like refrigeration and vacuum sealing of products have decreased the need for salt preservation, it is still utilized today. Salt is known for its ability to reduce the growth of pathogens and other microorganisms that can result in spoilage of food products or illness. It does this by limiting the amount of unbound water available to be used by microbes and their chemical reactions (4). However, salt can also be the source of living microorganisms called halophiles that may affect human health, especially the microbiome.

OBJECTIVES

- Isolate halophilic microorganisms, especially bacteria from edible salt solution.
- Purify isolated bacterium for further testing.
- Identify bacterium using biochemical tests as well as 16S ribosomal RNA sequencing.

METHODS

Makai Deep Pure Sea Salt (Figure 1) was obtained from the local food store and 1g of this salt was placed into a 15mL screw cap centrifuge tube. 12mL of a resuscitation buffer was added to the salt and gently mixed until salt was dissolved. The tube was then filled with more resuscitation buffer. This was placed on ice for 2 hours. 0.5mL of this solution was spread plated onto the resuscitation media. Makai samples were then incubated for 10 days before first growth was noted. The colonies noted on the original plates were further isolated using quadrant streaking techniques (Figure 2) on prepared modified R2A media, this process was repeated a total of 4 times. The isolated colonies were Gram stained (Figure 3) and examined under a light microscope to check for purity as well as Gram reactivity. Biochemical tests were carried out (Table 1) and the sample was sent for 16S ribosomal RNA sequencing.

RESULTS

Table 1: Classification and general features of *Gracilibacillus* sps according to the MIGS recommendations (3)

MIGS ID	Property classification	Term	Evidence code[a]
		Domain: Bacteria	TAS (7)
		Phylum: Firmicutes	TAS (5)
		Class: Bacilli	TAS (7)
		Order: Bacillales	TAS (7)
		Family: Bacillaceae	TAS (7)
		Genus: *Gracilibacillus*	TAS (6)
	Gram strain	Positive	IDA
	Cell shape	Rods	IDA
	Motility	Motile	IDA
	Sporulation	Sporulating	IDA
	Temperature (°C)	Mesophile	IDA
	Optimum temperature	41°C	IDA
	pH range: optimum	6.0–8.0	IDA
	Carbohydrate utilization	Glucose and Mannose	IDA
MIGS-6	Habitat	Salt environment	IDA
MIGS-6.3	NaCl range: optimum	12-16%	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
MIGS-15	Biotic relationship	Free-living	IDA
MIGS-14	Pathogenicity	Unknown	IDA

[a]Evidence codes – IDA, inferred from direct assay; TAS, traceable author statement (i.e. a direct report exists in the literature). These evidence codes are from the Gene Ontology project (1).

Figure 1: Makai Deep Pure Sea Salt

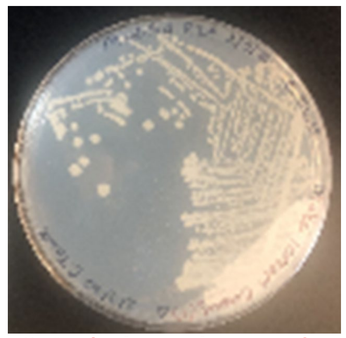

Figure 2: Isolation using quadrant Streaking technique

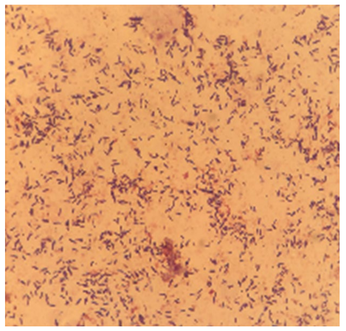

Figure 3: Gram staining of the Isolated colony obtained from Makai Deep Sea Salt

CONCLUSION

In this study, we performed culturomics and genomics-based analyses to survey the microbial content of commercially obtained edible salt. The isolated bacterium was identified to be of the genus ***Gracilibacillus***. These bacteria originating from an edible salt means that they are normally ingested when used on food. There are many surfaces in the oral cavity that microorganisms can reside and colonize within, including newly introduced bacteria. Due to the normal alkalinity of saliva (pH 6.5-7) and the average oral temperature being 37º C this would help provide an optimum growth environment for the identified bacterium (2). The oral microbiome houses a wide variety of different microorganisms all of which play a crucial role in maintaining and protecting the oral cavity. Introduction of a new bacterium has the potential to disrupt this homeostatic environment.

FUTURE PLANS

- Identify isolated bacterium to the species level.
- Further investigate role of halophiles isolated from edible salts in human health.

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