The use of Principle Component Analysis and MALDI-TOF MS for the differentiation of mineral forming *Virgibacillus* and *Bacillus* species isolated from Sabkhas

Rim Abdel Samad[1], Zulfa Al Disi[1], Mohammad Yousaf Mohammad Ashfaq[1], Sara Mohiddin Wahib[1] and Nabil Zouari[1]
[1]Qatar University, Biological and Environmental Sciences

Graduate Students
Energy, Environment & Resource Sustainability

ABSTRACT

Occurrence of mineral forming and other bacteria in mats is well demonstrated. However, their high diversity shown by ribotyping was not explained, although could explain the diversity of formed minerals. In this study, combination of MALDI-TOF MS with PCA was shown a powerful tool to categorize 35 mineral forming bacterial strains isolated from Dohat Fshaikh sabkha, at northwest of Qatar (23 mineral forming *Virgibacillus* from decaying mats and 12 from living ones). Combination of isolation of bacteria on selective mineral forming media, their MALDI TOF MS protein profiling and PCA analysis established their relationship in a phyloproteomic based on protein biomarkers including m/z 4905, 3265, 5240, 6430, 7765, and 9815. PCA analysis clustered the studied strains into 3 major clusters, showing strong correspondence to the 3 phyloproteiomic groups that were established by the dendrogram, evidently demonstrating a relationship between known *Virgibacillus* strains and other related bacteria based on profiling of their synthesized proteins.

OBJECTIVES

-To study the biodiversity of microorganisms by isolating, identifying and differentiating the types of bacteria found in living and decaying mats.

-To differentiate isolates belonging to the same species using cluster and principal component analysis (PCA) for further characterization and of Qatari *Virgibacillus* and other bacterial strains.

Introduction

Gulf Countries, including Qatar, belong to the most arid coastal ecosystems of the world. Dohat Faishakh Sabkha is a flat supratidal expanse that has been recognized as one the few places on Earth where dolomite forms at ambient temperature. Numerous studies have evidenced the role of microorganisms in biomineralization process leading to the formation of these carbonate rocks. In Dohat Faishakh sabkha, it was shown that bacteria, especially those belonging to the *Virgibacillus* genus were able to mediate mineralization process. The compared amplified 16s rRNA sequences was insufficient to explain the diversity of *Virgibacillus* in biomineralization potential. However, the relationship between the possible diversity of function and diverse capacities to form minerals in terms of magnesium incorporation into the carbonate minerals is not well elucidated. This diversity of *Virgibacillus* isolates might be established through clustering these isolates based on their respective protein profiles, if appropriate cultural conditions are used for examining the biomineralization potentials. The matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) technique is suitable for the purpose. It is utilized for identification and differentiation of microorganisms, relying on protein proteins to identify strains of specific genera, species and subspecies.

METHODOLOGY

Bacterial Isolates from decaying mats, used as reference

Twenty-three bacterial isolates were used as reference strains were previously isolated from decaying mats sampled from Dohat Faishakh sabkha, northwest of Qatar. 7 strains of *Virgibacillus marismortui*, 3 strains of *Virgibacillus salarius,* and 13 *Virgibacillus sp.*

Identification of bacterial isolates by MALDI-TOF

The identification was carried out by the Bruker Biotyper software, where a log scale from 0 to 3 defines the identification matching level with the database

Data processing

Principle component analysis (PCA) was performed to decrease dimensionality of the data set and maintain the original information present.

Dendrogram clustering was also carried out.

RESULTS and DISCUSSION

MALDI TOF identification and Analysis

***Table 1**: Identification of the 35 bacterial strains isolated from decaying and living mats of Dohat Faishakh sabkha (Qatar), using MALDI-TOF MS.*

Strain Code	Identification by Ribotyping	MALDI TOF Score	Identification by MALDI TOF	Code for PCA analysis
DF112	*Virgibacillus marismortui*	< 1.7	NR	31
DF221	*Virgibacillus sp.*	< 1.7	NR	19
DF231	*Virgibacillus marismortui*	< 1.7	NR	1
DF241	*Virgibacillus sp.*	< 1.7	NR	18
DF252	*Virgibacillus sp.*	< 1.7	NR	27
DF281	*Virgibacillus salarius*	< 1.7	NR	21
DF282	*Virgibacillus sp.*	< 1.7	NR	3
DF291	*Virgibacillus sp.*	< 1.7	NR	4
DF322	*Virgibacillus marismortui*	< 1.7	NR	7
DF341	*Virgibacillus marismortui*	< 1.7	NR	14
DF351	*Virgibacillus salarius*	< 1.7	NR	8
DF411	*Virgibacillus marismortui*	< 1.7	NR	11
DF431	*Virgibacillus sp.*	< 1.7	NR	30
DF451	*Virgibacillus sp.*	< 1.7	NR	5
DF461	*Virgibacillus salaries*	< 1.7	NR	2
DF472	*Virgibacillus marismortui*	< 1.7	NR	15
DF491	*Virgibacillus marismortui*	< 1.7	NR	20
DF2102	*Virgibacillus sp.*	< 1.7	NR	22
DF2121	*Virgibacillus sp.*	< 1.7	NR	12
DF2131	*Virgibacillus sp.*	< 1.7	NR	13
DF2141	*Virgibacillus sp.*	< 1.7	NR	28
DF2161	*Virgibacillus sp.*	< 1.7	NR	29
DF2172	*Virgibacillus sp.*	< 1.7	NR	10
K011	ND	1.81	*Bacillus licheniformis*	26
K1031A	ND	2.25	*Bacillus cereus*	33
K103B	ND	1.87	*Bacillus cereus*	6
K9-3-1	ND	1.76	*Bacillus circulans*	24
K012A	ND	< 1.7	NR	35
K012B	ND	< 1.7	NR	9
K9-1-1	ND	< 1.7	NR	16
K9-1-2	ND	< 1.7	NR	25
K9-1-4	ND	< 1.7	NR	34
K915A	ND	< 1.7	NR	32
K9-2-1	ND	< 1.7	NR	17
K9-2-2	ND	< 1.7	NR	23

ND: Not determined. NR: Not reliable

Relationships and clustering of isolates using MALDI-TOF MS and PCA

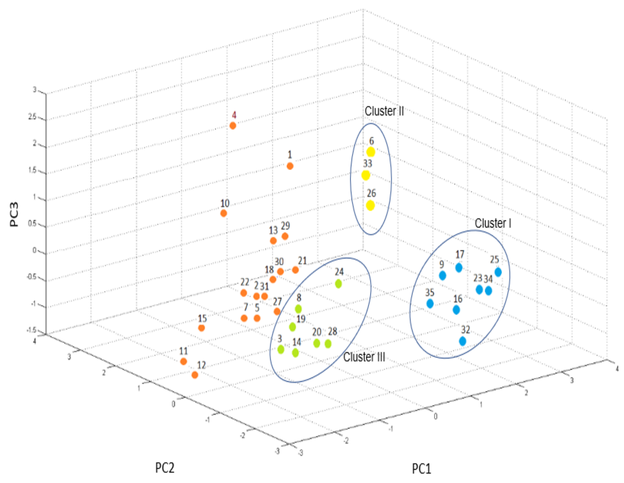

Figure 1: Principal component analysis plot of the strains under study.

Clearly the strains exhibit large biodiversity at the protein level. The three principle components i.e. PC1 (32%), PC2 (21.5%) and PC3 (12.5%) combined, revealed three clusters. The distance between the clusters shows the variation at groups level, while the distance between the strains (within the cluster) shows the differences in protein profiles at strain level. Cluster 1, which has positive correlation to PC1 and negative correlation to PC2 and PC3, include unidentified strains (K012B, K911, K921, K922, K914, K912, K915A, and K012A). Whereas, cluster 2 has positive correlation to all three components and is mainly comprised of *B. cereus* and *B. licheniformis* (K1031B, K011 and K1031A) demonstrating variation in their protein profiles in comparison to other studied strains.

Phylloproteiomic tree

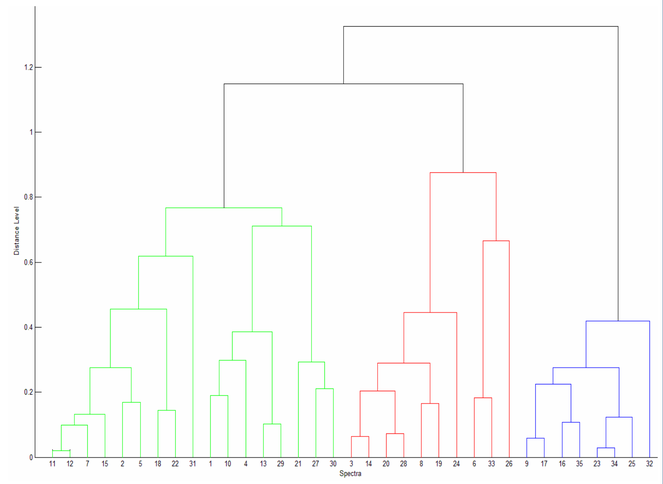

***Figure 2:** Phylloproteiomic tree illustrating the relationship among the strains used in this study using similarity coefficient and single linkage.*

The 23 *Virgibacillus* which are used as reference, are divided into two separate groups (A and B) in the phylloproteiomic tree. The isolates *V. marismortui* (DF112, DF231, DF322, DF411 and DF472), *Virgibacillus sp*. (DF 241, DF252, DF291, DF451, DF2121, DF2102, DF2131, DF2161 and DF2171), *V. salarius* (DF281, DF461 and DF431) were grouped together (group A). The reference strains *Virgibacillus sp*. (DF221, DF282 and DF2141), *V. marismortui* (DF341 and DF491) and *V. salarius* 351 were categorized separately into another group (group B) along with four newly isolated strains that have been identified by MALDI-TOF, *Bacillus cereus* (K1031A and K1031B), *Bacillus licheniformis* (K011), and *Bacillus circulans* (K931). The eight remaining strains were categorized in one group (group C).

CONCLUSION

The combination of MALDI-TOF MS with PCA is shown to be a powerful tool for rapid identification and categorization of strains isolated from the same niche or comparable ones. Here, combination of isolation of bacteria from Sabkhas mats by enrichment cultures in a medium that allows formation of many types of minerals, to MALDI TOF MS protein profiling and PCA analysis guides on their rapid identification as a first step. Then, larger number of bacterial isolates can be easily screened for their potential to exhibit certain activities, which is of ecological, environmental and biotechnological significance. These findings demonstrate the high occurrence and diversity of *Virgibacillus* strains in the same mat and their high similarity with others from other mats.

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