Int.J.Curr.Microbiol.App.Sci (2024) *13*(12): 284-291

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 13 Number 12 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1312.030

Heterotrophic Bacteria from an Aztec Archaeological Zone

Olvera-Ramírez Roxana, Rodríguez-Dorantes Angélica María and Medina-Jaritz Nora Beatriz*

Department of Botany. National School of Biological Sciences. National Polytechnic Institute. prol. De Carpio s / n, Casco de Santo Tomas. Mexico City. C.P. 11430. Mexico

**Corresponding author*

Keywords

Biodeterioration, biofilm, heterotrophic bacteria

Article Info

Received:
28 October 2024
Accepted:
30 November 2024
Available Online:
10 December 2024

A B S T R A C T

The archaeological zones suffer alterations because they are exposed to diverse physicochemical and biological processes such as changes in temperature and humidity, the dissolution or crystallization of salts (carbonates, sulphates and silicates), the development of microbial colonies producing pigments, organic acids, siderophores, enzymes, etc. The incidence of these processes affects the stone monuments structurally and aesthetically. Ten strains isolated from the archaeological zone of Malinalco were tested for the production of siderophores with Luria Bertani Cromazurol S (LB-CAS) media and for the production of organic acids with Bromocresol Green Foster agar (presumptive test) and Foster agar with calcium carbonate (confirmatory test). The halos produced were measured at 24 and 48 hours. Subsequently, routine biochemical tests were performed to determine a presumptive genus and were determined taxonomically by analysis of 16S RNA sequences. The results show that the heterotrophic bacteria studied are related to the production of different metabolites such as organic acids and siderophores that contribute to the deterioration of archaeological monuments, among the genera found are *Bacillus*, *Arthrobacter*, *Pantoea* and *Proteus*.

Introduction

The deterioration of the archaeological zones depends as much on its mineral composition as on the environmental conditions (climate and human activities) (Warscheid and Braams, 2000), reason why the decay of the stony material is consequence of physical, chemical and biological deterioration processes. The knowledge of such processes is crucial to define strategies for the restoration and conservation of historical monuments (Berdoulay and Salvado, 2009; Scheerer *et al.,* 2009). Among the microorganisms commonly found on the surfaces of buildings are autotrophic and heterotrophic bacteria, fungi, algae and lichens (Gorbushina, 2007), this heterogeneous consortium of microbial species forms a biofilm whose internal cohesion and adhesion to the Support surface are secured by extracellular polymeric substances (Echevarría-García, 2013).

The main deteriogenic effects of microbial biofilms are Berdoulay and Salvado (2009): changes in the color of the stone surface due to the presence of photosynthetic or protective pigments; physical alteration of the structure of the material due to the bacterial or fungal penetration and by the differential mechanical pressure imposed by the changes of hydration of the biofilm; chemical

modification of the support mineral by acidolytic processes or by oxidation-reduction corrosion generated by products of microbial metabolism (Warscheid and Braams, 2000).

The presence of diverse groups of microorganisms in the biofilms of some archaeological zones of both Mexico and the rest of Latin America has been reported: Proteobacteria, Bacteroidetes and Cyanobacteria on Mayan ruins in Uxmal, Yucatán (Ortega-Morales *et al.,* 2004); cyanobacteria and algae in historic buildings in Latin America (Crispim *et al.,* 2003); Epiliticα-proteobacteria and epilithic and endolytic actinobacteria at the archaeological site of Ek-Balam, Yucatán (McNamara *et al.,* 1972). The objective of this study was to identify heterotrophic bacteria in biofilms growing in Malinalco archaeological monuments.

Collection site

Malinalco is in the mountainous area that separates the states of Mexico and Morelos, in the center of the country; at 1770 m.s.n.m. and at 18 ° 58 07 "LN and 99 ° 30 06" LW. The climate is subhumid semi-warm with summer rains and an average annual temperature of 20 ºC and a maximum of 34.5 ºC (Galván, 1984; White and Zepeda, 2005).

The archaeological site of Malinalco was built by Aztec stonemasons who carved five buildings, among which stands out the monolithic pyramid known as Cuahcalli, which means Casa del Águila, and is the only temple carved into a single rock found in Mexico (figure 1) (White and Zepeda, 2005), is a truncated pyramid carved directly into the volcanic tuff with veins of tepetate, facing south (Noguez, 2006); the other buildings are the monument II, a truncated pyramid that although it is consolidated shows some deterioration; monument III, which is a structure sculpted in the rock; the monument IV, in which half of the building is carved in the rock, while the other half, the front facade, is made with carved stones; monument V, another masonry circular building in poor state of preservation and monument VI, which was under construction at the time of the Spanish conquest, so that it was never finished (García, 1958). The feldspathic minerals, of igneous origin, are abundant on the surface of the soil and constitute one of the main components of the structure of the buildings I and VI (Torres *et al.,* 1989), besides that they erode easily, they can also deteriorate by the action of some metabolites of microbial origin (Sugumaran and Janarthanam, 2007).

Materials and Methods

Sampling and bacterial strains isolation

Samples were taken aseptically with segments of filter paper moistened in Ringer's solution that were placed on the surface of the monument for three minutes; a total of 18 samples were collected (three from each temple), each one was stored in sterile microtubes and kept at 4 ºC. Samples from each monument were mixed and diluted in Ringer's solution (10-1, 10-2, 10-3 and 10-4) and inoculated on Luria Bertani (LB) media plates (Miller, 1972), subsequently incubated at 28 ° C for 24 and 48 hours.

Characterization of bacterial strains

The isolates were characterized considering the colonial morphology (color, shape, size, surface, margin, elevation, appearance and consistency) as well as the microscopic morphology and the Gram tintorial affinity. Tests were also performed on TSI (triple sugar iron agar), SIM (mobility, indole and sulfhydric acid production), Simmons citrate agar, urea oxidase and catalase base agar (McFaddin, 1990).

The production of siderophores was also determined with the LB-Cas Azurol medium (Pandey *et al.,* 1994) and organic acids with Foster-green agar of bromine cresol (presumptive) and Foster-calcium carbonate agar (confirmatory) (Foster and Davis, 1949). The halos produced in each medium were measured at 24 and 48 hours of incubation.

Isolated microorganisms were identified by the similarity and phylogenetic analysis of the 16S rRNA gene partial sequence. The amplification of 16S rRNA genes was performed with a PCR System 9700 (Applied Biosystems, Foster City, CA).

Reaction mixtures contained 5 ng of template DNA, 1x reaction buffer, 25 mM MgCl2, 0.25 mM of each dNTP, 10 pM of each primer, and 1 U of Taq Polymerase, adjusted to 25µL.

Ribosomal 16S rRNA genes were amplified using the universal bacterial primer 8 (5′-GCG GAT CCG CGG CCG CTG CAG AGT TTG ATC CTG GCT CAG-3′) forward and 1492 (5′-GGC TCG AGC GGC CGC CCG GGT TAC CTT GTT ACG ACT T-3′) reverse (Relman, 1993). The PCR conditions used were: an initial

denaturation step at 94 ºC for 5 min; 35 cycles of 45 s at 94 ºC, 45 s at 55 ºC, and 90 s at 72 ºC; and a final extension step at 72 ºC for 10 min. PCR products corresponding to the 16S rRNA genes were purified using the QIA quick PCR purification kit according to the manufacturer's instructions (Qiagen Inc., Valencia, CA) and sequenced using the Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ) and an ABI PRISM 310 Genetic Analyzer (Perkin- Elmer Applied Biosystem, Inc., Boston, MA).

16S rRNA gene sequences were subjected to BLAST (Altschul *et al.,* 1990) and Analysis Tools of Ribosomal Database Project-II Release 10 (http://rdp.cme.msu.edu) were used. A collection of taxonomically related sequences obtained from the National Center for Biotechnology Information (NCBI) Taxonomy Homepage and Ribosomal Database Project-II Release 10 were included in the multiple alignment analyses with CLUSTAL X (Thompson *et al.,* 1997) that were edited using Bioedit and MEGA 5.2 software to build the phylogenetic tree and to achieve the species identification.

Results and Discussion

A total of thirty-one strains were isolated, of which ten were selected based on their morphological and metabolic characteristics (Table 1, figure 2).

Average diameter of halos produced by each strain in the LC-Cas azurol media and Foster agar shown in the figure 3.

All sequences were identified to genus level, and 8 to species level (Table 2).

Three isolates were members of the alpha-Proteobacteria and identified as *Azospirillum lipoferum* and *Bradyrhizobium japonicum*; the other members of the *Phylum firmicutes* corresponded to *Paenibacillus* spp. Figure 4 shows the phylogenetic tree obtained.

The biggest solubilization halos were observed in the Foster medium with carbonates and in the minimal medium with potassium salts after 48 hours of incubation, the strains that produce the greatest amount of organic acids (those that do it in the three media) belong to the genera *Proteus* and *Serratia*.

The production of organic acids by the isolated strains is important because they promote the dissolution of different types of minerals (Sugumaran and Janarthanam, 2007).

The production of organic acids can be related to the leaching of the rock mainly from feldspar and pyroxenes. Pyroxenes contain calcium, which once exposed to organic acid they begin to wash off the surface of the monuments causing erosion (Sugumaran and Janarthanam, 2007).

The other physiological test carried out was the production of siderophores, extracellular chelating compounds that sequester and solubilize iron (Ahmed and Holmström, 2014). In the archaeological zone of Malinalco, iron is found as oxidized forms of ferromagnetic minerals: pyroxene and biotite (Torres *et al.,* 1989), that causes surface bacteria on archaeological monuments to produce the chelating molecules.

To determine the production of siderophores, the Luria-Bertani medium added with chromium azurol S was used. Chromium azurol is a ferri-chromogen complex wich color changes when it loses ferric ions. It is worth mentioning that siderophores have a greater affinity for ferric than chromogen, so they can capture and assimilate iron faster, causing a change in color from blue to yellow.

Furthermore, in this study it was observed, that strains 1 (*Proteus* sp.), 8 and 27 (*Serratia nematodiphila*), 21 and 25 (*Serratia ureilytica*), 26 (*Providencia* sp.) and 30 (*Proteus penneri*) produce siderophores. This means that they do not have the iron available for their metabolism, since the siderophores will only be secreted when there are deficiencies of reduced iron.

Dybas *et al.,* (1995), mention that these molecules are produced mostly by Gram-negative bacteria which can be corroborated in Figure 4, where we observed that isolates 9 and 22, are related to *Exiguobacterium*, Gram positive bacteria that do not produce siderophores.

Additionally, there are bacteria capable of using not only the siderophores that they synthesize, but also those produced by other bacterial species that may be the case of *Exiguobacterium*, which would not result in a halo of siderophore production in the CAS-LB medium or be observed with a small diameter (Morrissey *et al.,* 2000).

Table.1 Characterization of the isolated bacterial strains.

Strain		1A	8A	9B	20C	21D	22B	25C	26C	27A	30D
Colonial morphology	Size	2	2	3	4	4	3	4	3	4	3
	Form	c	c	c	c	c	c	c	c	c	am
	Elevation	cvx	cvx	cvx	cvx	cvx	cvx	cvx	cvx	cvx	fl
	Border	who	who	who	who	who	who	who	who	erod	who
	Appearance	s, br	s, br	s, br	s, br	s, br	s, br	s, br	s, br	s, br	s, br
	Consistency	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
	Opacity	op	op	op	op	op	op	op	op	op	op
	Color	w	w	or	pch	w	or	w	w	w	w
	Pigmentation	-	-	-	-	+	-	-	-	-	-
Microscopic morphology	Forma y tamaño	bc	bc	bc	bc	bc	bc	bc	bc	bc	bc
	Gram	-	-	+	-	-	+	-	-	-	-
TSI	P/F	K/A	K/A	K/A	K/A	A/A	K/A	K/A	K/A	A/A	K/A
	G	-	-	-	-	-	-	-	-	-	-
	Ac	-	-	-	-	-	-	-	-	-	-
CI		+	+	-	+	+	-	+	+	+	+
SIM	M	+	+	-	-	-	-	-	+	-	+
	I	+	-	-	+	+	-	+	+	+	-
	S	-	-	-	-	-	-	-	-	-	-
U		-	-	-	-	-	-	+	+	-	+
O		+	-	-	+	-	-	-	-	-	-
C		+	+	+	+	+	+	+	+	+	+

am=ameboid, w=white, br=bright, c=circular, cvx=convex, who=whole, erd=eroded, pch=peach, wt=wet, or=orange, op=opaque, fl=flat, s=soft

Table.2 Molecular identification of isolated strains.

Strain	Taxon
1	*Proteus* sp.
8	*Serratia nematodiphila*
9	*Exiguobacterium acetylicum*
20	*Aeromonas hidrophila*
21	*Serratia ureilytica*
22	*Exiguobacterium acetylicum*
25	*Serratia ureilytica*
26	*Providencia* sp.
27	*Serratia nematodiphila*
30	*Proteus penneri*

Figure.1 The monolithic pyramid known as Cuahcalli (Eagle's House).

Figure.2 Produced halos by isolated strains on the selected media.

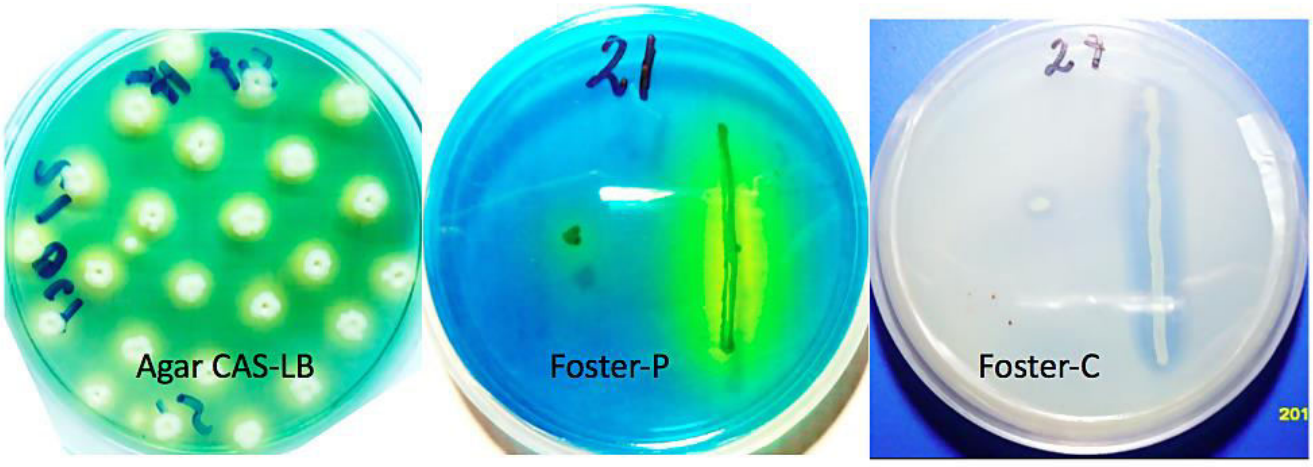


Figure.3 Inhibition halos.

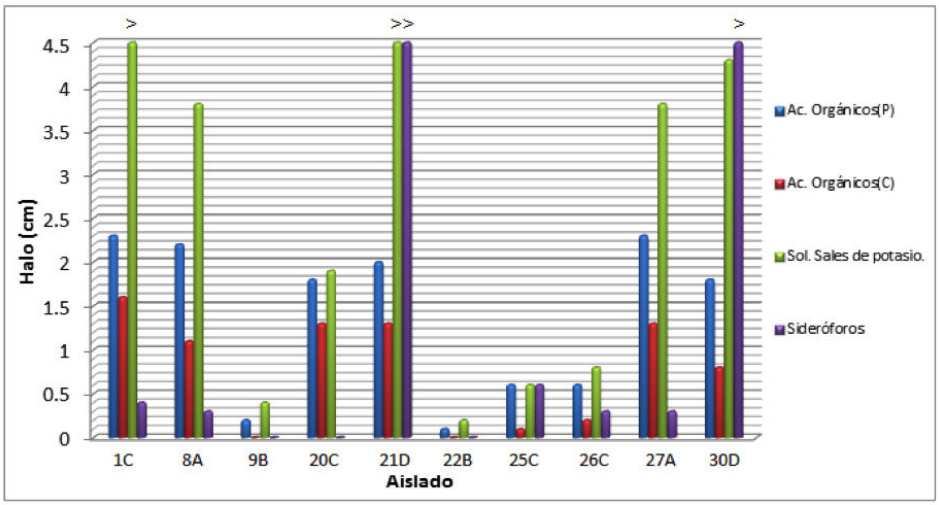
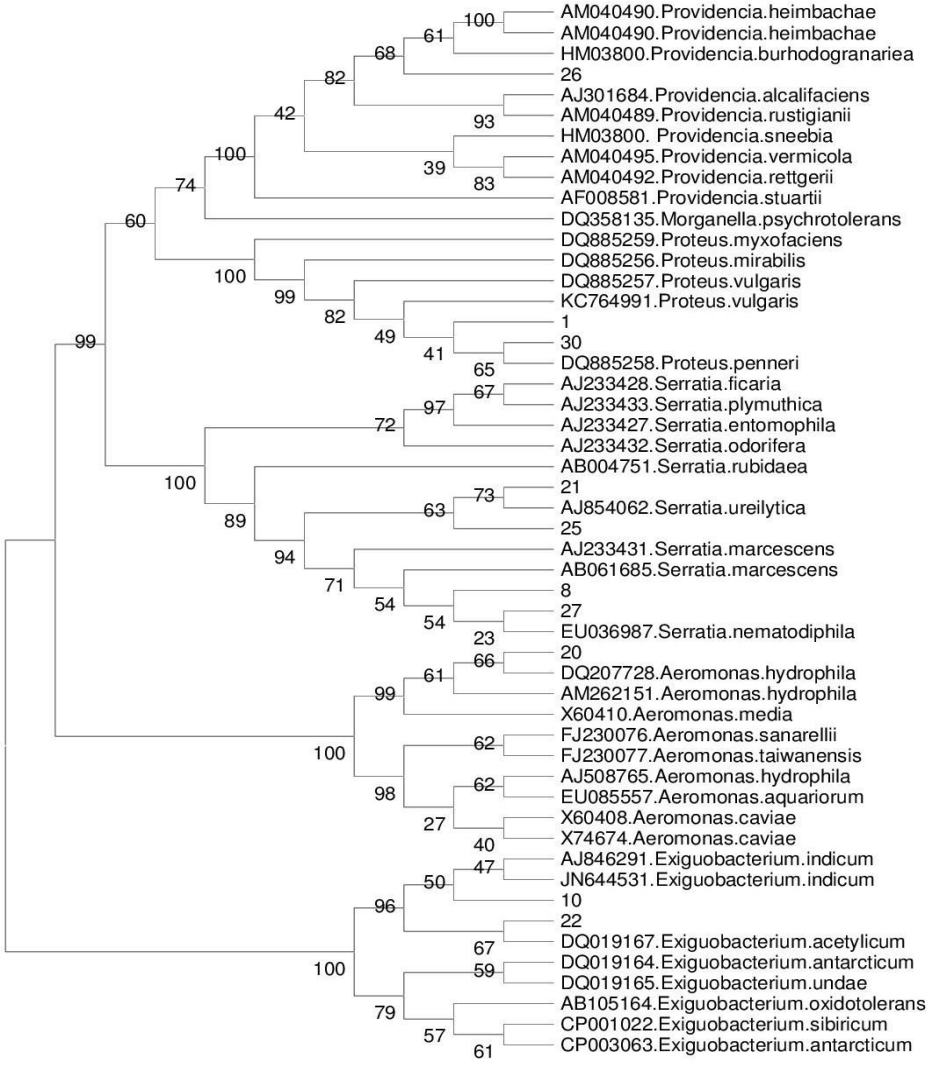


Figure.4 Phylogenetic tree obtained from sequences.



There are heterotrophic bacteria present on the surface of the archaeological zone that can produce as organic acids and siderophores that contribute to the deterioration of archaeological monuments. These strains include 1 (*Proteus* sp.), 8 and 27 (*Serratia nematodiphila*), 21 (*Serratia ureilytica*), and 30 (*Proteus penneri*) as the best producers of siderophores and producers of organic acids. Finally, *Exiguobacterium* was the strain that presented the slowest metabolism, this bacterium is a yellow pigment producer, it is a short Gram-positive bacillus, which has a low production of organic acids and siderophores. The afore mentioned does not make it less important because the production of the pigment affects the archaeological zone in an aesthetic way, besides it is reported to be a psychrophilic bacterium which says that it supports temperatures in which other microorganisms do not grow well (Morrissey *et al.,* 2000).

We can conclude that from the samples collected in the archaeological zone of Malinalco it was possible to isolate heterotrophic bacteria, which contribute to the deterioration of the archaeological zone by the production of secondary metabolites such as organic acids and siderophores. The bacteria that most produce organic acids are related to the Enterobactericeae family and are the ones with the highest production of siderophores.

The genera related to the degradation of the archaeological zone were *Proteus*, *Serratia*, *Aeromonas*, *Providencia* and *Exiguobacterium*. The isolates identified as *Exiguobacterium acetylicum* affect the archaeological zone in an aesthetic way due to the production of a yellow pigment, as well as being a psychrophilic bacterium.

Acknowledgements

This work was supported by Comisión de Operación y Fomento de Actividades Académicas (COFAA), Programa de Estímulos al Desempeño Docente (EDD) and Programa de Estímulo al Desempeño de Investigadores (EDI), from Instituto Politécnico Nacional (IPN).

Author Contributions

Olvera-Ramírez Roxana: Investigation, formal analysis, writing—original draft. Rodríguez-Dorantes Angélica María: Validation, methodology, writing—reviewing. Medina-Jaritz Nora Beatriz:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.
Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Ahmed, E. and Holmström S. J. M. 2014. Siderophores in environmental research: roles and applications. Microb. Biotechnol., 7(3), 196-208. https://doi.org/10.1111/1751-7915.12117

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990 Oct 5;215(3):403-10. https://doi.org/10.1016/S0022-2836(05)80360-2

Berdoulay, M. and Salvado, J. C. 2009. Genetic characterization of microbial communities living at the surface of building stones. Lett. Appl. Microbiol., 49, 311-316. https://doi.org/10.1111/j.1472-765X.2009.02660.x

Crispim, C. A., Gaylarde, P. M., Gaylarde, C. C. 2003. Algal and cyanobacterial biofilms on calcareous historic buildings. Curr. Microbiol., 46(3), 79-82. https://doi.org/10.1007/s00284-002-3815-5

Dybas, M., Tatara, G., Criddle, C. S. 1995. Localization of carbon tetrachloride transformation activity of *Pseudomonas* sp. strain KC. Appl. Environ. Microbiol., 61, 758-762. https://doi.org/10.1128/aem.61.2.758-762.1995

Echevarría-García, L. 2013. Técnicas y métodos de uso de las biopelículas en la búsqueda de procesos de biorremediación. Sci. Int. J., 10(3), 32-41.

Foster, J. W. and Davis, H. 1949. Detection and ocurrence of acid-producing fungi. Bull. Torrey Bot. Club, 3, 174-176.

Galván, B. J. 1984. Aspectos generales de la arqueología

de Malinalco, Estado de México. México, D. F., México: Instituto Nacional de Antropología e Historia.

García, P. J. 1958. Malinalco. Guía Oficial. México, D. F., México: Instituto Nacional de Antropología e Historia.

Gorbushina, A. A. 2007. Life on the rocks. Environ. Microbiol., 9(7), 1613-1631. https://doi.org/10.1111/j.1462-2920.2007.01301.x

MacFaddin, J. F. 1990. Pruebas bioquímicas para la identificación de bacterias de importancia clínica. México, D. F., México: Panamericana.

McNamara, C. J., Perry, T. D., Zinn, M., Breuker, M., Hernandez-Duque, G., Mitchell, R. 1972. Microbial processes in the deterioration of Mayan archaeological buildings in southern Mexico. In: Miller, J.H. Experiments in Molecular Genetics. Cold Spring Harbor, Nueva York, E. U. A.: Cold Spring Harbor Lab. Press.

Miller, G.L. (1972) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars. Analytical Chemistry, 31, 426-428. http://dx.doi.org/10.1021/ac60147a030

Morrissey, J. A., Cockaine, A., Hill, P. J., Williams, P. 2000. Molecular cloning and analysis of a putative siderophore ABC transporter from *Staphylococcus aureus*. Infect. Immun., 68(11), 6281-6288. https://doi.org/10.1128/iai.68.11.6281-6288.2000

Noguez, X. 2006. El Templo Monolítico de Malinalco, Estado de México. Arqueol. Mex., 78.

Ortega-Morales, B. O., Narváez-Zapata, J. A., Schmalenberger, A., Sosa-López, A., Tebbe, C. C. 2004. Biofilms fouling ancient limestone Mayan monuments in Uxmal, Mexico: a cultivation-independent analysis. Biofilms, 1, 79-90. https://doi.org/10.1017/S1479050504001188

Pandey, A. B., Bringel, F., Meyer, J. M. 1994. Iron requirement and search for siderophores in lactic acid bacteria. Appl. Environ. Microbiol., 40, 735-739. http://dx.doi.org/10.1007/BF00173337

Relman, D. A. 1993. Universal bacterial 16S rRNA amplification and sequencing. In: Persing, D. H. editor. Diagnostic Molecular Microbiology: Principles and Applications. Washington D. C., E. U. A.: ASM Press.

Scheerer, S., Ortega-Morales, O., Gaylarde, C. 2009. Microbial Deterioration of Stone Monuments - An updated overview. Adv. Appl. Microbiol., 66, 97-139. https://doi.org/10.1016/s0065-2164(08)00805-8

Sugumaran, P. J. and Janarthanam, B. 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. W. J. Agric. Sci., 3(3), 350-355.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. C. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Res., 24, 4876-4882. https://doi.org/10.1093/nar/25.24.4876

Torres, M. L., Álvarez, G. D., Reves, G. M., Hernández, R. J. 1989. Informe del deterioro y propuesta para la conservación de la zona arqueológica de Malinalco-Estado de México. Rev. Inst. Inv. Antropol., 26, 107-128. https://doi.org/10.22201/iia.24486221e.1989.1.13031

Warscheid, T. and Braams, J. 2000. Biodeterioration of stone: a review. Int. Biodeterior. Biodegrad., 46, 343-368. https://doi.org/10.1016/S0964-8305(00)00109-8

White, O. L., Zepeda, G. C. 2005. El paraíso botánico del convento de Malinalco, Estado de México. México, D. F., México: Universidad Nacional Autónoma de México.

How to cite this article:

Olvera-Ramírez Roxana, Rodríguez-Dorantes Angélica María and Medina-Jaritz Nora Beatriz. 2024. Heterotrophic Bacteria from an Aztec Archaeological Zone. *Int.J.Curr.Microbiol.App.Sci.* 13(12): 284-291.
doi: https://doi.org/10.20546/ijcmas.2024.1312.030