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Deciphering archaeal glycolipids of an extremely halophilic archaeon of the genus *Halobellus* by MALDI-TOF/MS

Simona Lobasso [a], Azahara Pérez-Davó [b], Rita Vitale [a], Mercedes Monteoliva -Sánchez [b], Angela Corcelli [a,*]

[a] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari "Aldo Moro", Bari, Italy
[b] Department of Microbiology, Faculty of Pharmacy, University of Granada, Granada, Spain

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ABSTRACT

Polar membrane lipids of an archaeal microorganism recently isolated from the natural salt lake Fuente de Piedra (Málaga, Spain) have been studied by means of TLC in combination with MALDI-TOF mass spectrometry. The major phospholipids are the ether lipids phosphatidylglycerophosphate methyl ester and phosphatidylglycerosulfate, while phosphatidylglycerol is barely detectable; in addition the bisphosphatidylglycerol (archaeal cardiolipin) has been detected for the first time in a representative of the genus *Halobellus*. The structures of glycolipids, including a glycosyl-cardiolipin, have been elucidated by post source decay (PSD) mass spectrometry analysis. Besides the monosulfated diglycosyl diphytanylglyceroldiether, two variants of a bis-sulfated diglycosyl diphytanylglyceroldiether have been identified; furthermore the glycosyl-cardiolipin is found to have the same structure of the analogue present in *Halorubrum trapanicum* and *Haloferax volcanii*. The role of the abundant sulfated glycolipids in facing high extracellular salinity is discussed.

1. Introduction

Extremophile microorganisms are well known for their ability to populate extreme environments characterized by severe conditions such as extreme salinity, where the values of water activity are low and there is an extreme stress due to desiccation, high pressure, high temperature or high UV irradiation levels (Canganella and Wiegel, 2011; Jaenicke, 1991).

Halophiles are extremophile organisms well adapted to live in higher salt concentration environments. They are widely distributed in natural habitats such as solar salt lakes, saline soils, sea water, highly mineralized hot springs, groundwater, rivers and marshes where salinity conditions are suitable for their development and growth (Oren, 2011; Ramos-Cormenzana, 1991; Rodríguez-Valera et al., 1981).

The salt-loving archaeal microorganisms, typically found in coastal saltern ponds and hypersaline lakes, are grouped in the family Halobacteriaceae, encompassing a constantly increasing number of representatives (Oren, 2012).

The unique structural characteristics of the archaeal polar membrane lipids, that is, the *sn*-glycerol-1-phosphate backbone, isoprenoid hydrocarbon chains and ether linkages, are in striking contrast to the bacterial and eukaryotic characteristics of the *sn*-glycerol-3-phosphate backbone, fatty acid chains and ester linkages. The biosynthetic pathways and genes involved in the synthesis of the archaeal membrane lipids have been described in recent studies (Lombard et al., 2012; Pearson, 2014; Villanueva et al., 2014).

Most of the phospholipids and glycolipids of extreme halophiles are anionic, so that their negatively charged groups

Abbreviations: 9-AA, 9-aminoacridine; BPG, bisphosphatidylglycerol (diphytanylglycerol ether analogue) or ether lipid cardiolipin; ESI, electrospray ionization; HPTLC, high-performance thin-layer chromatography; MALDI-TOF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PA, phosphatidic acid (diphytanylglycerol ether analogue); PGP-Me, phosphatidylglycerophosphate methyl ester (diphytanylglycerol ether analogue); PGS, phosphatidylglycerosulfate (diphytanylglycerol ether analogue); S-DGD, sulfated diglycosyl diphytanylglycerol diether; S-DGD-PA, glycosyl-cardiolipin or 2-HSO_3-Man*p*-α1,2-Glc*p*-α1,1-[*sn*-2,3-di-*O*-phytanylglycerol]-6-[phospho-*sn*-2,3-di-*O*-phytanylglycerol]; S_2-DGD, bis-sulfated diglycosylglycerol diether; S_2-DGD_{OH}, hydroxylated bis-sulfated diglycosylglycerol diether; PSD, post source decay.

* Corresponding author at: Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari "Aldo Moro", piazza G. Cesare 11, 70124 Bari, Italy. Tel.: +39 0805448530; fax: +39 0805448538.
E-mail address: angela.corcelli@uniba.it (A. Corcelli).

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would impart a high negative charge density to the halophile membranes. No nitrogenous base-containing phospholipids, such as phosphatidylserine or phosphatidylethanolamine, are present in extreme halophiles, and this may be characteristic of the halophilic archaea, in contrast to the methanogenic and thermophilic archaea.

The diether lipid core that forms the basis for most polar lipid structures present in the family Halobacteriaceae is 2,3-di-*O*-phytanyl-*sn*-glycerol (C_{20}, C_{20}), also called archaeol (Kates, 1972); in some haloalkaliphile and *Halococcus* species C_{20}, C_{25}- and C_{25}, C_{25}-diether variants of the diphytanylglycerol diether lipid core were also identified (Kates, 1993).

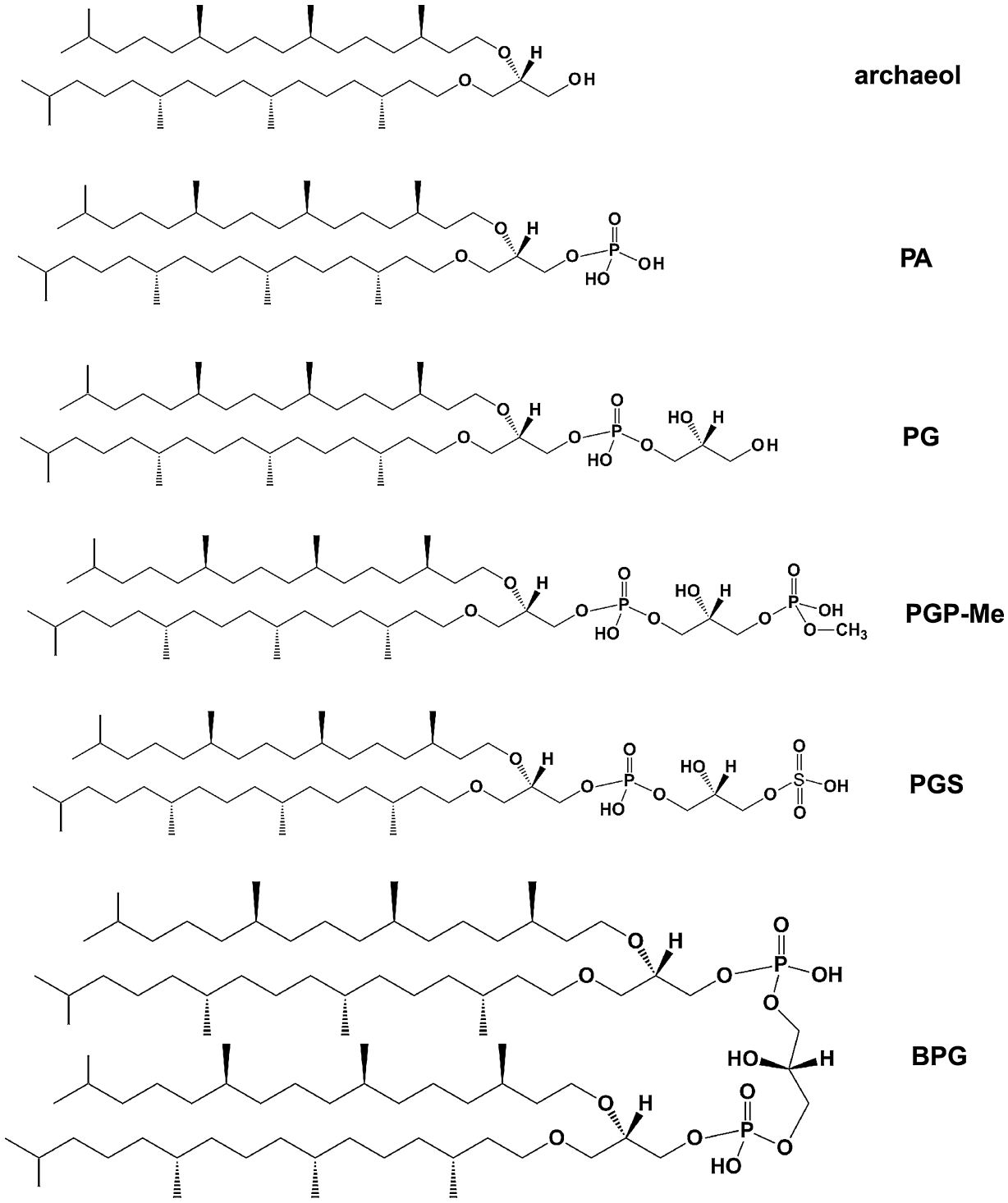

Fig. 1. Structures of archaeol and archaeol-derived phospholipids of extremely halophilic archaea. Archaeol, 2,3-di-*O*-phytanyl-*sn*-glycerol; PA, phosphatidic acid (diphytanylglycerol ether analogue); PG, phosphatidylglycerol (diphytanylglycerol ether analogue); PGP-Me, phosphatidylglycerophosphate methyl ester (diphytanylglycerol ether analogue); PGS, phosphatidylglycerosulfate (diphytanylglycerol ether analogue); BPG, bisphosphatidylglycerol (diphytanylglycerol ether analogue) or ether lipid cardiolipin.