

Analysis of membrane fatty acids by gas chromatography biology essay

[Science](#), [Biology](#)



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ABSTRACT

Acidobacillus is a genus of Proteobacter, a gram negative organism containing fatty acids in its membrane. It is the most important genus of chemolithotrophs. Acidithioacillus ferrooxidans is an autotrophic, acidophilic, mesophile occurring in single or occasionally in pairs or chains, depending on growth conditions. These acidophilic bacteria thrive in optimal pH level of 1.5 - 2.5 where they convert insoluble metals to their soluble state. The main objective of this work is to analyse membrane fatty acids by Gas chromatography. Gas chromatographic analysis of volatile fatty acids for identification of bacteria and for presumptive diagnosis of anaerobic infections is now widely practiced. Gas Chromatographic methods with high-quality capillary columns allow sensitive and reproducible fatty acid analyses, as well as the characterization of complex mixtures of geometric isomers when combined with other chromatographic separations and spectroscopic identification. Keywords: Acidithioacillus ferrooxidans, Fatty acids, Gas chromatography, Chemolithotrophs .

INTRODUCTION

Bacteria constitute a large domain of prokaryotic microorganisms. Almost all bacteria are invisible to the naked eye, with a few extremely rare exceptions. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals.(Cavalier-Smith T 2006). Bacteria

were among the first life forms to appear on Earth, and are present in most habitats on the planet, growing in soil, water, acidic hot springs, radioactive waste, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals, providing outstanding examples of mutualism in the digestive tracts of humans, termites and cockroaches (Madigan M 2006). Bacteria have different groups. Proteobacter is one among them to which the species *Acidithiobacillus* fall. Proteobacteria are a major group (phylum) of bacteria. They include a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, and many other notable genera (Madigan M 2005). All proteobacteria are Gram-negative, with an outer membrane mainly composed of lipopolysaccharides. There are five classes of Proteobacter (Lee et al 2005).. They are Alpha Proteobacteria (Caulobacterales), Beta Proteobacteria (Burkholderiales) Gamma Proteobacteria (Acidithiobacillales) Delta Proteobacteria (Bdellovibrionales) Epsilon Proteobacteria (Campylobacterales) The Gammaproteobacteria comprise several medically and scientifically important groups of bacteria. *Acidobacillus* is a genus of Gamma Proteobacteria. *Acidithiobacillus ferrooxidans* is a Gram negative rod shaped bacterium that is commonly found in deep caves or acid mine drainage, such as coal waste (Yu Yang et. al 2007). The members of this genus used to belong to *Thiobacillus*. These acidophilic bacteria thrive in optimal pH level of 1.5 - 2.5 where they convert insoluble metals to their soluble state (Yu Yang 2007). Even low concentrations (ppm) of these metallic ions would be extremely toxic to other bacteria (Rawlings 1947). In addition, these bacteria have been utilized in industrial bioleaching efforts to extract otherwise

unobtainable metals (Colmer 1986). *Acidithiobacillus ferrooxidans* is commonly found in diverse mining environments and several studies have isolated psychrotrophic strains capable of iron and sulfide oxidation at temperatures as low as 25°C while mesophilic strains of this species have not been shown to grow below 10°C (Ferroni et al. 1986; Leduc et al. 1993; Kupka et al. 2007). *Acidithiobacillus ferrooxidans* is a chemolithoautotrophic acidophilic bacterium that obtains its energy from the oxidation of hydrogen, ferrous iron, elemental sulfur, or partially oxidized sulfur compounds. This ability makes it of great industrial importance due to its applications in biomining (Johnson 1998). During these industrial processes, microorganisms are normally subjected to stressing circumstances in their environment, such as temperature and pH changes, nutrient starvation, and the presence of toxic heavy metals, which can affect their physiological conditions (Raquel Quatrini 2009). A primary component of bacterial adaptation to various stresses is the cytoplasmic membrane. The role of membrane lipids in modulating membrane structure and fluidity is a primary stress response mechanism (Rilfors et al. 1984; Hazel 1995) and determining changes in fatty acid and/or lipid composition assists in defining the link between strain variation and biophysical and biochemical characteristics of the cytoplasmic membrane. In different bacterial studies, cold temperature adaptation typically involves increasing the degree of fatty acid unsaturation and branching which in turn increase membrane fluidity and depress the phase transition to compensate for the cold-induced increases in membrane order (Hazel and Williams 1990). *Acidithiobacillus ferrooxidans* is an obligate acidophile used as a bioleaching agent in the recovery of metals (Leduc and

Ferroni 1994). Probably the most widely studied obligate acidophile, *A. ferrooxidans* has been described as tolerating conditions as low as pH 1.0 and as high as pH 6.0, although it is generally agreed that the optimal pH for growth of *A. ferrooxidans* is about 2.0 (Leduc and Ferroni 1994). The variation in range is due to strain heterogeneity and specific growth conditions and strains are also known to adapt rapidly to sub-optimal conditions. In both uncontrolled/natural (tailing, mines and mine waste) and bioleaching systems, the pH can affect the overall bacterial activity and resulting leaching rates (Kondrat'eva and Karavaiko 1997). Improved knowledge of *A. ferrooxidans* strain adaptation is an important aspect in understanding the intrinsic chemiosmotic kinetics of bacterially mediated bioleaching. The membrane of *Acidithiobacillus* contains fatty acids whose major role is to modulate membrane structure and fluidity (Rilfors et al. 1984). Branched-chain fatty acids are common constituents of the lipids of bacteria and animals. These fatty acids help to maintain body temperature. The membrane fatty acid composition under different growth condition can be determined using Gas Chromatography (Fernandez, 2008)

MATERIALS AND METHOD

Media : 9-K media; pH 2.3
Organism : *Acidithiobacillus ferrooxidans*
Chemicals : Acetone, Acetic acid, Distilled water
TLC sheets

Media Preparation

9K media was prepared using solution A and solution B. Solution A contains:
Ammonium sulphate - 3g/L
Dipotassium phosphate - 0.5g/L
Magnesium sulphate - 0.5g/L
Potassium chloride - 0.1g/L
Calcium nitrate - 0.0.

1g/L Solution A was prepared by mixing the chemicals and the volume was made up to 700ml. It was then autoclaved at 121°C for 1 hour at 15lbs pressure. Solution B was prepared by dissolving 44.7g of ferrous sulphate in 300ml of distilled water. The pH was adjusted to 2.3. It was stored in a reagent bottle to prevent the oxidation of ferrous sulphate. 70ml of solution A and 30ml of solution B was mixed, to which the organism was inoculated.

Initialization of culture

The organism was subcultured onto freshly prepared 9K media. It was incubated in a shaker at 30°C for 2-3 days at 250 rpm until a dirty brown media was obtained. It was then again subcultured onto 9-K media after every 3 days.

Extraction

The culture was filtered using Whatmann filter paper to remove the sedimented FeSO_4 . 10ml of the culture was transferred into a centrifuge tube. It was then centrifuged at 10000rpm for 20 minutes. The filtrate was then transferred into another centrifuge tube and was suspended in 10ml of distilled water. To obtain a monophasic system, 3.75 mL of a methanol:chloroform (2: 1, v/v) mixture was added for each mL of bacterial suspension in Milli Q water. Samples were kept at room temperature for 1 h with shaking. In order to convert the system into a diphasic state, 2.5 mL of a chloroform:distilled (1: 1 v/v) mixture was added for each mL of bacterial suspension and samples were again kept at room temperature for 1 h with shaking. Afterwards, samples were centrifuged at 1000 G for 5 min and the

chloroform-rich phase (bottom) containing the cellular lipids was removed and the solvent was evaporated. (Bligh and Dyer 1959).

Thin Layer Chromatography

Thin layer chromatography was performed. Acetone, acetic acid and water was mixed in the ratio 4: 1: 0. 5. A line was drawn 1cm from the bottom of thin layer plate. The sample was spotted on the line with the help of a micro pipette. It was then introduced into the beaker containing the solvent. The sample moved along with the solvent. Once the solvent moved up it was then removed from the beaker and the solvent front was marked.

RESULT

Acidothiobacillus ferrooxidans were found to grow at an optimum pH of 2. 3 in 9-K media with the substrate ferrous sulphate. EXTRACTION:

UsersOwnerDocumentsBluetooth FolderPhoto0443. jpgAfter centrifugation a bilayer was obtained. A chloroform rich fatty acid layer was seen at the bottom. THIN LAYER CHROMATOGRAPHYThin layer chromatography of fatty acids was performed. Fatty acids and cholesterol migrate to unique positions in the upper half of the chromatogram, (Dr. Gary Witman 1991)GAS CHROMOTOGRAHYPEAK NORTH EIGHT AREA AMOUNT % AREA100. 3293. 409418. 214100Total area : 418. 214 mV-Sec