UNESCO SCIENTIFIC COLLOQUIUM ON FACTORS IMPACTING THE UNDERWATER CULTURAL HERITAGE
10th Anniversary of the Convention on the Protection of the Underwater Cultural Heritage

Royal Library of Belgium, Brussels, Belgium.
13th & 14th December, 2011.

New Bacterium Species Discovered on RMS Titanic Rusticles

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Introduction

On April 12, 1912 after a two year effort, involving more than 1,700 workers from Harland and Wolff ship builders, the Royal Mail Steamship (R.M.S.) Titanic was completed and ready to set sail. At that time it was the largest moveable object, measuring 28.7 m wide, 269.1 m long, by 53.3 m high from the keel to the top of the four funnels. At 12:00 h the R.M.S Titanic left port in Southampton, England. The first stop was France and then on to Ireland, with the final destination across the Atlantic Ocean to New York City, USA (Ballad, 1995). On the third day of the transatlantic voyage, at 23:35 h, the Titanic struck an iceberg. At 02:00h on April 15, 1912 the proclaimed “unsinkable” R.M.S Titanic sank.

The wreck of the Titanic was discovered in September 1985, 73 years after her sinking, by ocean scientists from America and France. The lead figures were Dr. R. D. Ballard, USA and Dr. Jean-Louis Michelle, France. The ship was scattered over the North Atlantic Ocean floor, 3.7 km deep.

In 1986 Dr. Ballard returned to the Titanic wreck site using a manned submersible, the Alvin, which enabled him and his team to view the sunken ship. The organic material had disappeared from the wreck, but the damage to the inorganic material varied, based on its elemental composition (Freemantle, 1994). Some objects such as dishes, copper and glass, however, were preserved. The scientists observed that the ferrous-iron structures, such as cast iron, wrought iron, and steel were corroded and covered, draped with rust-like precipitates. Ballard (1995) named them “rusticles” because they hung like icicles but looked like rust.

In June 1991, a group of Russian, Canadian, and American ocean scientists returned to the Titanic wreck site to produce the IMAX documentary movie “Titanica”. On this dive, they collected samples of water, sediment cores and rusticles. The water surrounding the wreck was 2.16 °C, the oxygen concentration was 7.35 % and the salt concentration was 3.5 % w/v (Wells & Mann, 1997). The Titanic wreck was, however, not covered everywhere by sediments. Some sediment was partially removed by wave currents from the Western Boundary Undercurrent (Hayson, 1993, Wells, & Mann, 1997).

A Short Discussion on Metallurgy

The Titanic was constructed primarily of steel. Rusticles grow on this metal. It is therefore important to understand the make-up of the various types of steel used in the Titanic because the type of steel affects both the types of rusticles and their chemical and crystallographic composition.

It is generally assumed that our ancestors found meteorites and, thinking they were ordinary stones, placed them around their wooden fires. After these fires cooled, however, the meteorites turned to iron sponge. This happened due to a reduction of iron oxide in the meteorite. The carbon from the wood had reduced the melting point of the iron within the meteorite. The resulting sponges were collected, hammered into different shapes and used as tools. By 1,500 BC, what was by then known as iron ore, was well established in the art of tool making (Borestain, 1984).

For about 3,000 years no change occurred in the use of iron ore. People reduced iron oxide into sponge and pounded it into different products to produce wrought iron. The Titanic had many pieces made by this technique ie. stairwells, ornamental railings, gates, etc.

In the middle ages, the use of blast furnaces was established. The hot air was forced into the iron ore rocks, thereby further reducing iron oxide, resulting in a change in the chemical reaction. This technique produced “pig” iron, which has the characteristics of being hard and brittle. It was therefore difficult to shape by hammering alone. Pig iron is primarily used for creating cast iron. The difference between wrought iron and pig iron is in the composition of carbon. Wrought iron has 0.005% or less carbon, while pig iron has 3% or more carbon (Borestain, 1984).

In the science of metallurgy, metal is defined as a pure element made up of only one type of atom and one crystal structure. It may, however, have some impure atoms within its structure.

An alloy is a combination of two or more chemical elements, one of which must be a metal. An alloy containing iron as the primary component, and small amounts of carbon as the major alloying element, is referred to as steel (Borenstain, 1984).

Alloy steels are, in metallurgical terms, steels that contain manganese silicon, chromium, molybdenum, nickel or copper in quantities greater than those listed for carbon steel. The alloying process enhances the mechanical properties of the steel, its fabricating characteristics and other features.

This paper will focus on two types of steel found in the Titanic, carbon steel and cast iron. Carbon steel was used to build the hull of the ship. It contains less than 1.65% manganese, 0.60% silicone, and 0.60 % copper with no minimum for other alloying elements.

Cast iron, used in the Titanic’s coal furnaces, is a generic name for a group of cast ferrous alloys in which the carbon content exceeds the solubility of carbon in austenite face-centered cubic crystal structure, a high temperature form of iron. The carbon is greater than 2% in cast irons, most being between 3.0 and 4.5 wt % C (Borenstain, 1994).

Biomineralization

If we simplify the biomineralization process, there are two categories: biologically induced mineralization and biologically controlled mineralization (Frankel & Bazylinski, 2003)

The induced mineralization process generally starts the extra-cellular growth of crystals, due to the metabolic activity of bacteria and the chemical reactions of its byproducts. The bacteria secrete metabolic products that react with ions or compounds in the environment around the Titanic, causing continuous decomposition of mineral particles, in this case, iron, manganese, nickel, etc.

Bacterially induced mineralization is almost equivalent to inorganic mineralization Fig 1. Under some environmental conditions, the minerals have crystal chemical characteristics, produced by inorganic chemical reactions. The bacteria surfaces either in the form of cell walls or sheaths, or as dormant spores, can act as inactive sites for absorption of ions and mineral nucleation and growth (Beveridge, 1989, Bauerlein, 2003).

There are two general types of cell wall surfaces: gram positive and gram negative. This is based on the staining techniques in light microscopy. The gram negative cell wall is different from gram positive in that it has a thinner peptidoglycan layer and does not contain secondary polymers Fig 1. It is sandwiched between two lipid/protein bilayers. This space is called periplasma Fig 2, 3 (Beveridge, 1981, Frankel & Bazylinski, 2003).

Iron and manganese minerals, often found in rusticles, are sometimes produced by bacteria. This is caused by the precipitation of oxides of both metals, at acidic and neutral pH conditions. The changes of pH in rusticles may occur due to the circulation of the water inside this formation, whereby some of their internal spaces may have less water, or, in some cases are cut off from water circulation altogether Fig 4. The rusticles contain sulfate-reducing bacteria as a part of the rusticle formation and are responsible for forming small niches in consortia, with almost or completely anerobic conditions.

Mineral formation results from the neutralization of chemically reactive sites on the cell, and the combination of additional metal ions, with the initially absorbed metals (Southam, 2000). The outer surface of the cell, the cell wall, is most active where mineralization takes place Fig 1. Non living cells can, however, also form minerals (Urrutia et. al, 1992).

Bacterially Induced Corrosion

The corrosion process by bacteria can be characterized as microbiological, metallurgical, electrochemical and aquatic chemistry. Microbiological corrosion depends on the diversity of microorganisms in the specific environment. Metallurgical corrosion depends upon the type, structure, and the process of how this metal was created. Electrochemical corrosion depends upon the mechanisms by which the corrosion process has occurred. In this case, the metal enters into a solution in the form of ions and rust forms by subsequent reactions (Borenstain, 1994). Aquatic chemistry is influenced by local substrate such as sediments, bed rock, water currents and waves.

As discussed above, samples from the Titanic site included water, sediment, rusticles, and metal. Sediments at the site also included a dense mud (Blasco personal communication, 1996).

Rusticles are made of a brittle 100-200 μm thick oxyhydroxide shell with a smooth , dark, red lepidocrocite (y-Fe0 (OH)) outer surface Fig 5. There is a rough, orange, geothite (α-Fe0 (OH)) inner surface Fig 6. Lepidocrocite is a stable, orthorhombic ferric hydroxide mineral which occurs as scaly aggregates or as occasional elongate crystals (Steffyn-Egli and Buckley, 1998). It is formed under oxidizing conditions by weathering, or by hydroternal alternation of iron bearing minerals, along with its polymorph, which is the same chemical composition but different structure of goethite and hematite. It also forms as a direct inorganic or biogenic precipitate from water. Geothite is also a stable orthorhombic ferric hydroxide mineral but it occurs as acicular (needle-like) reniform kidney-shaped, or botryoidal cauliflower shaped aggregate. Like its polymorph, lepidocroite, it is formed under oxidizing conditions by weathering or hydrothermal alternation of other iron bearing minerals and also forms as a direct inorganic or biogenic precipitate from water (Wells & Mann, 1997).

Bacteria are the most important group of microorganisms involved in iron mineralization. They are considered important in the transformation of iron at geological mineral formation sites, and mineral formation during corrosion of steel and other iron based structures as mentioned previously. Iron bacteria are also special in their ability to survive at a wide pH and temperature range.

Metal Samples Collection

Metallic samples were brought up from the Titanic wreck during the 1991 dive. These samples fall in three broad categories of ferrous materials: cast irons, wrought irons and steels. The research on these metals was conducted by Metals Technology Laboratories of CANMET in Canada (1992).

The first specimens, a bracket and a screw, were taken from the sediment ~ 200m east of the stern. They are a gray cast iron consisting of graphite flakes in a pearlitic matrix and show strong corrosion, called graphitization metallic matrix, whereby it is dissolved leaving behind only graphite flakes. This is typical of cast iron.

The second specimen was a foot casting coming from the same location as the first. This is pearlite gray cast iron showing extensive corrosion as in the first sample.

The third specimen was found on the deck near the starboard gunwale. It was made of gray cast iron, and showed graphitization from corrosion.

The fourth specimen was a rivet with red paint on the head, suggesting that it came from the hull plate. This confirms that hull plates were put together with wrought iron rivets.

The fifth and sixth specimen, a small hinge and a large hinge, were recovered from the forward section of the bow, starboard side, beside the officers' quarters on the top deck. Both were made of wrought iron, and show galvanic corrosion. This occurs when two different metals come in contact, as in this case bolts on screws.

The seventh hull plate is low carbon steel (<0.2% C). Its high sulfur content indicates it was formed using the basic open-hearth process.

The eighth specimen is sheet metal from the low section of the wreck, near the centre line of the ship which is the top deck. This steel sample is fully-billed and has a sulfur content within today's limits (Brigham and Lafreniere, 1992).

Description of the Rusticles

Rusticles are very complex structures. The main purpose of the rusticle is to supply nutrition by the circulation of water through the rusticle. A rusticle is a consortium of bacteria, fungus and marine snow. The consistency of rusticles is like a combination of sponge, fumicle and hard material made of iron flakes of varying sizes.   The rusticles have several different shapes, plate-like branching, oozing, clustered and folded (Pellegrino & Culimure, 1997).

The description of the small foot is provided. The width is 6 cm and the length is 5 cm. Fig 4. The colouration of the foot is like autumn leaves in Canada, varying from dark red to orange, different shades of brown and yellow and also black. The colour is in some instances tied to a crystallized or a morphic iron compound. eg, goethite, lepidocrocite.

The crystal shapes vary; some can be seen as cubic needle like or orthorhombic shapes. Besides the bio-organic occupants, sand and small pieces of glass, clay, and coal are buried within the rusticle. It, therefore also contains water channels.

Rusticles grow from exposure to outside water currents. The debris carried in the currents contributes to the variety of objects found in and on them. Sea shells, glass and sand found embedded on the outside of the rusticle provide a historical data of strong water current periods specific for that part of rusticles growth.

Rusticle Formation from a Bacterial Perspective

1) The microbial adhesion mast occurs as the first step of rusticle formation
2) Consortia are formed by two or more microorganisms with similar ecological niches, but different physiologies that complement each other (Marshall, 1984). These microorganisms stay together permanently with normal cell-to-cell contact. Since the members of the consortia stay together for a long time (as observed since 1985 on the Titanic) during their life cycle, beneficial interactions between them is assumed.
3) Forms similar to Caulobacter rosettes had been observed in the rusticles. As the rosette cells had stalks of equal length, the indication is that this could be the second species to appear on the Titanic wreck.

For a permanent consortium to become newly established, certain conditions have to be met:

1) The species must be in the same environmental location.
2) Both species should have a need for each other as in a stress situation or symbiosis. Extreme conditions often favour the production of a polymer on the cell surface.
3) The species will have to find each other. A motile central cell may be chemotactically attracted by excretion of “peripheral” cells.
4) The peripheral organisms may accidentally touch a surface polymer of a central cell and remain there permanently. Adhesiveness of at least one species must play an important role in the initial formation of consortia.
5) Chemical/biochemical interactions have to begin (Marshall, 1984).

Synchronous division may depend on syntrophic interactions on cooperative metabolisms of one substrate and on a constant environment. There must be some kind of symbioses of the rusticle consortium. There most likely is a bacterial species which excretes or produces only polymers formations, which attracts nutrients from the environment (Marshall, 1984). This is going to be studied further.

Characteristics of the New Bacterium *Halomonas titanicae*

The cells are rods, 0.5-0.8μm in diameter and 1.5-6.0 μm in length. They occur individually or in pairs, a gram negative, heterotrophic, aerobic, peritrichausly, flagellated and non-endspore forming. Most importantly they are mobile Fig 2.

The colonies are circular, smooth, convex, and white cream colour, when grown on agar. The liquid medium cells grow on the surface of the medium. Moderately halophilic optimal growth is at 2-8% (w/v) NaCl. The temperature range for growth is 4-42 °C, but optimal growth is at 30-37°C, and pH at a 5.5-7.5 range.

Phylogenetic analyses, based on 165 rRNA gene sequence comparison, indicated that bacterium clustered within the species of Halomonas. The nearest strain were *Halomonas neptunia* (98.6%) and the *Halomonas variablis* (98.4%). On the basis of phenotypic chemotaxonomic and phylogenetic data, this bacterium was considered as a novel species and named *Halomonas titanicae* sp.(Sanchez-Porro, et. al, 2010, Kaur & Mann, 2004).

Recommendation

Rusticles are unique. They contain different species of bacteria, fungi, marine snow, sand clay, small pieces of glass, and shells in some instances pieces of coal. The coal pieces are from the coal used to power RMS Titanic. The Titanic is not unique in the having rusticles. Other wrecks made of iron and steel, including oil rigs and gas pipe lines in the deep ocean also have rusticle formations.

Within a year or two of being installed, rusticles begin to form on the moorings of oil rigs. The corrosion caused by the formation of rusticles, bacteria and normal processes incurs costs in the millions of dollars to the marine industry.

The identification of the *Halomonas titanicae* bacteria helps shed light on rusticle formation. This knowledge can be used to assist the marine industry. These bacteria can also be used to dispose of old merchant and naval ships and oil rigs in the ocean after they have been cleaned of toxins and oil based products.

The story of the RMS Titanic is well known internationally. It has captured our imaginations throughout history. We have explored the wreck, taken artifacts and scientific samples. It is through these samples that we continue to learn so much about the deterioration of the Titanic wreck and how this knowledge can be used to help maintain other metal structures in the marine environment.

Unfortunately, due to rusticle consumption, the Titanic wreck cannot be preserved forever as an underwater heritage site. While it exists, however, we can visit it and continue to learn more about our marine environment through further scientific study. The story of this ship will live on long after it has become a rust spot on the ocean floor.

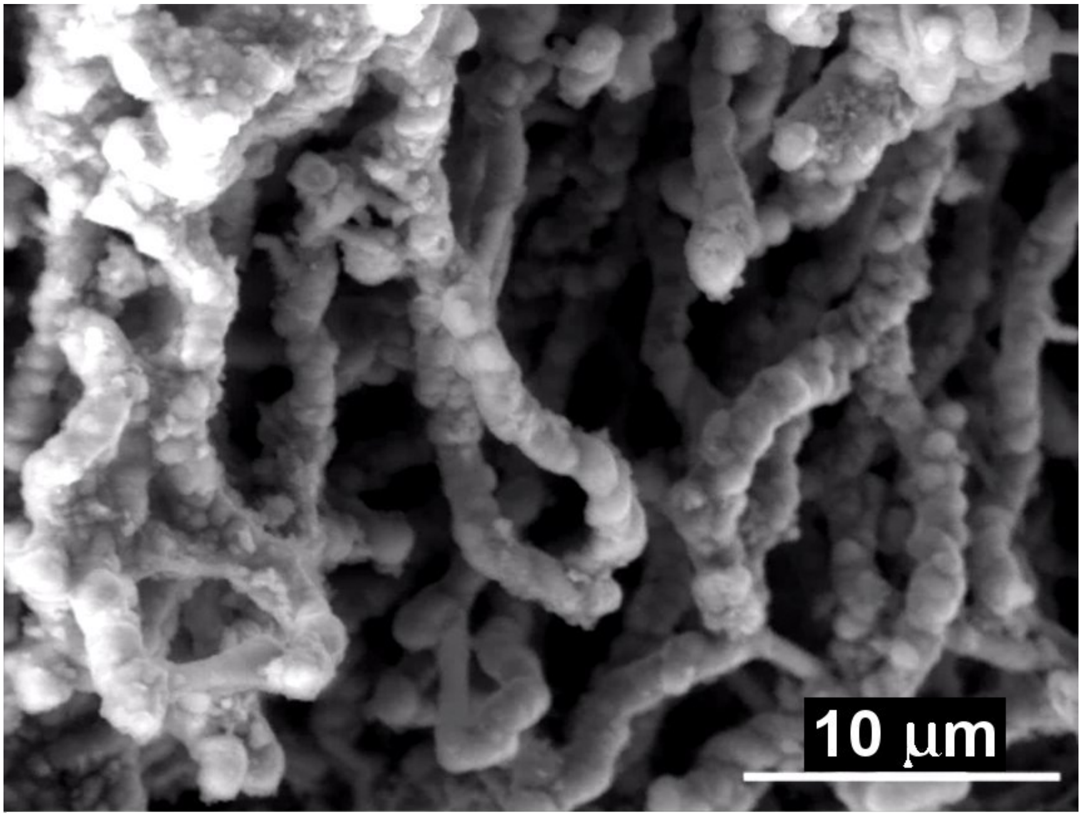

Figure 1. An ESM micrograph of bacteria in the initial stages attaching to the RMS Titanic's metallic structures and forming chain-like configurations. In this stage the bacteria is already biomineralized.

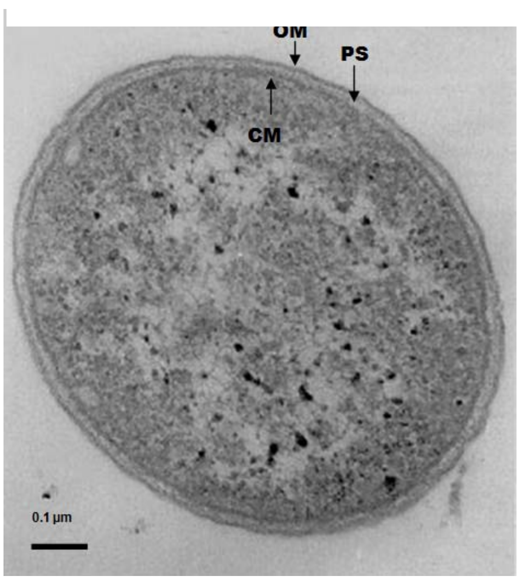

Figure 2. This TEM micrograph shows a thin section of a *Halomonas titanicae* cell with three distinct layers of the cell envelope: dense outer membrane [OM], middle light zone periplasmic space [PS] and inner dense cell membrane [CM].

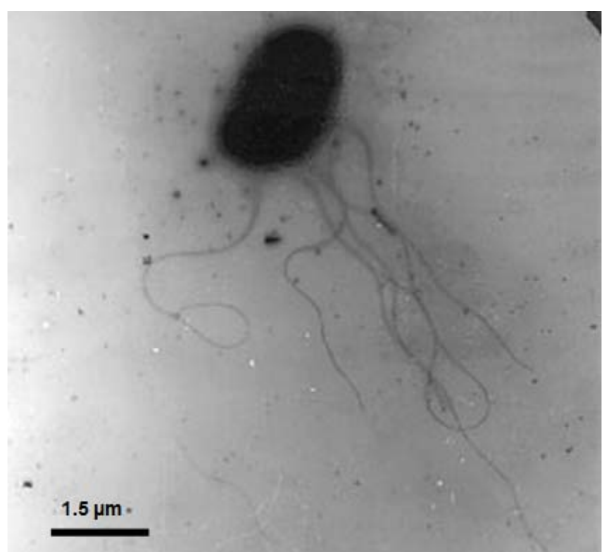

Figure 3. Negatively stained cells show varying numbers of unsheathed flagella, between 2 - 6. Length of flagella range from 9-12 µm and diameter from **1**5 - 18 nm.

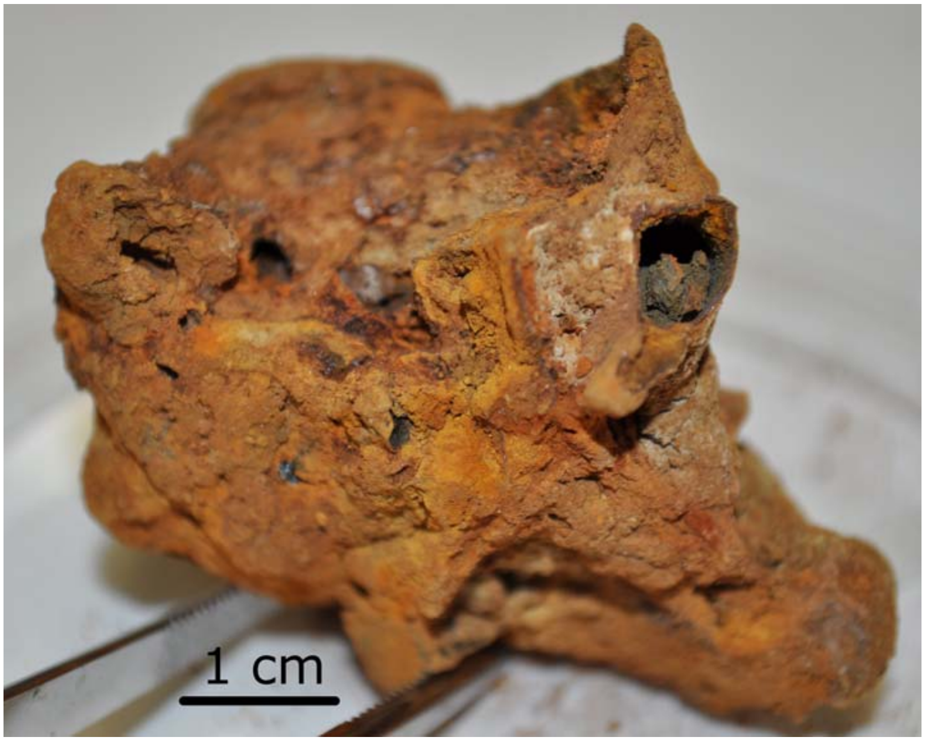

Figure 4. A foot shaped sample of rusticle taken from the RMS Titanic in 1991. The chemical reactions of iron oxide produce the autumnal colours of red, orange, yellow and brown. The large blackish opening on the upper right side of the sample is a channel that circulates water through the rusticle structure.

Figure 5. Lepidocrocite is an iron oxyhydroxide mineral occurring on the outside of the rusticle structure.

Figure 6. Goethe is an iron bearing oxide mineral occurring inside the rusticle structure.

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