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Deterioration, decay and identification of fungi isolated from wooden structures at the Humberstone and Santa Laura saltpeter works: A world heritage site in Chile

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ABSTRACT

The use of wood in construction has been part of mankind's history but wood placed into the environment is affected by biotic and abiotic agents and is degraded over time. Even in extreme environments, such as dry desert sites, deterioration of wood can take place. One site located in the Atacama Desert in northern Chile is the Humberstone and Santa Laura saltpeter works where offices and other structures were built of wood. Founded in 1872, the Humberstone and Santa Laura Saltpeter Works was designated a UNESCO World Heritage Site in 2005 for its historic significance. Since significant deterioration in the wooden buildings has taken place, investigations were initiated to better understand the degradation underway so conservation efforts to protect the historic buildings can be developed. The objectives of this study were to identify the type of deterioration and decay taking place and to isolate and identify fungi from wood samples of structural elements at both sites. Samples of deteriorated wood showed extensive degradation that resulted in a defibration of the wood. The middle lamella between cells was degraded and remaining secondary walls separated due to high concentrations of salts. This resulted in a serious corrosion of the exterior layers of wood cells. Although high salts inhibit fungi, many different fungi were isolated. Sequencing of the ITS region of the rDNA was used and fungi were identified as *Penicillium chrysogenum*, *Engyodontium album*, *Eupenicillium tropicum*, *Penicillium digitatum*, *Pseudotaeniolina globosa*, *Cladosporium phaenocomae*, *Aureobasidium pullulans*, *Penicillium virgatum*, *Coprinopsis sp.* and *Phanerochaete sordida*. Several of these fungi appear to be halophilic.

1. Introduction

Nitrate or saltpeter exploitation, which is still carried out today, began long ago in Chile during the pre-Colombian era (Minvu, 2003). The 'República' was a period of time for very active mining (Vial, 1987), and these mining activities were located mainly in Arica and Parinacota, Tarapacá, Antofagasta, and Atacama regions of Chile, which are desert areas with limited farming and forestry activities. This mineral, used as fertilizer and raw material for making explosives, made Chile the only manufacturer and exporter in the world at the end of War of the Pacific (1879–1883) because it had vast areas with high concentrations in its deposits (Vial, 1987). Saltpeter became Chile's major mining export product until the first decades of the 20th century. Mining remained a strong industry until the great depression of 1930's and the development of synthetic products of similar characteristics that eventually decreased the demand for Chilean nitrates (Culverwell, 2000).

The Humberstone and Santa Laura saltpeter works are a remarkable example of an industry that left a deep mark in Chilean history (Fig. 1). The sites (20°S 12′ 20.9″, 69°W 47′ 38.6″, and 1050 masl), are located in the so-called 'Pampa del Tamarugal', 47 km

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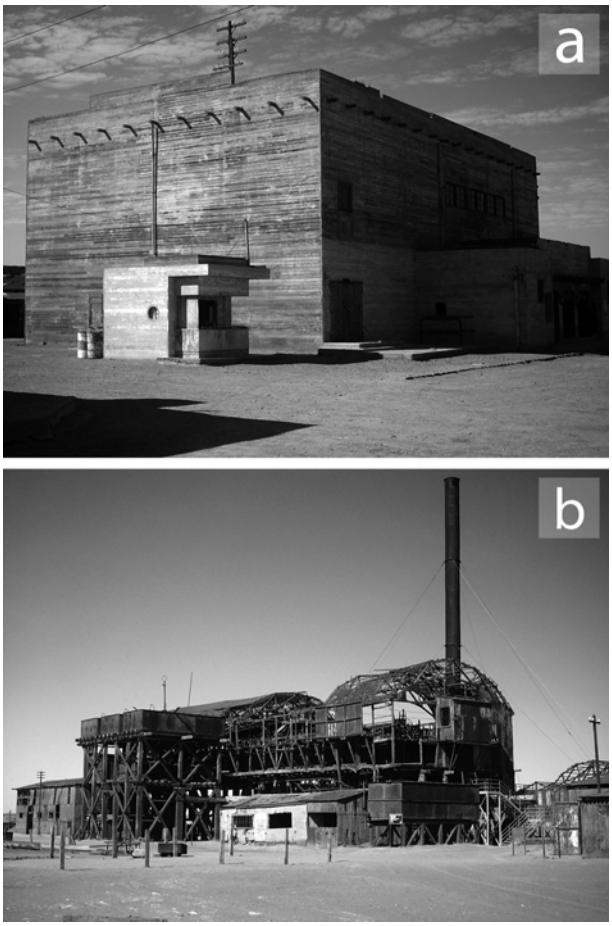

Fig. 1. Examples of the wooden historic structures at Humberstone (a) and Santa Laura (b) saltpeter offices in the Atacama Desert, Chile.

East of Iquique, in the Tarapacá region of Chile (Fig. 2). Currently, both sites make up an historic industrial complex which represents the mining activity of saltpeter extraction (Minvu, 2003); Santa Laura possesses the essential features related to industrial material processing in the area, and Humberstone has the structures related to the community aspects of the mining village. The facilities of these old saltpeter sites, which have now been declared National Monuments and UNESCO Cultural Heritage Sites of Humanity in 2005, possess many buildings made from a variety of materials including corrugated zinc sheets, concrete 'pampino', and wood.

The structures at the sites have become important for their historical significance providing valuable information about the past mining heritage of Chile. However, as wood is exposed to the environment it can be vulnerable to deterioration and decay. Historic and archaeological woods, even in desert regions, are subjected to attack and their degradation raises serious conservation concerns for preserving the wood (Blanchette, 2000, 2010).

Butin and Peredo (1986) and Furci (2008) have indicated that Chile is a relatively rich country in terms of fungal flora. However, only about 3300 fungi species are known. According to Gamundi (2003), the most important collection of fungi was conducted by Mujica et al. (1980) in his work called 'Flora Fungosa Chilena'. Respect to investigations of decay in historical buildings and in archaeological wood the information is very limited (Ortiz et al., 2011, 2012).

The historic wooden buildings at the Humberstone and Santa Laura saltpeter works, have evidence of serious deterioration and decay but no information is known about the type of degradation taking place, the microorganisms involved or the deterioration processes that are occurring in this unusual environment. Because of their important World Heritage Site status, every weakness that might be affecting their structure needs to be understood in order to make decisions leading to their successful preservation over time and establishing plans to prevent loss. The purpose of this study was to investigate the types of deterioration and decay occurring in wood at Humberstone and Santa Laura saltpeter works and identify the fungi isolated from the wooden structures.

2. Materials and methods

2.1. Wood sample collection

Wood samples with different stages of deterioration and decay were collected from Humberstone and Santa Laura saltpeter works buildings located in Tarapacá region, Province of Iquique, commune of Pozo Almonte, Chile. In addition, wood samples showing no degradation were also collected to identify the types of wood used in the structures. The samples of wood were placed in sterile plastic bags and taken to the laboratory where they were stored at 4 °C.

Micromorphological evaluations were done to identify the type of deterioration and decay in the wood samples using previously published methods that characterize decay types and deterioration processes (Blanchette et al., 1994; Blanchette, 2000; Blanchette et al., 2004; Blanchette et al., 2010). Wood samples were prepared for scanning electron microscopy (SEM) using techniques described previously by Blanchette and Simpson (1992). Observations and photographs were made using a Carl Zeiss, model EVO – MA10 Scanning Electron Microscope. Wood species identification was made, through the use of keys, as reported by Diaz Vaz (1979). Elemental analyses of samples from wood timbers were done by multi-elemental inductively coupled plasma atomic emission spectroscopy (Blanchette et al., 2002).

2.2. Inoculation and culturing

Small wood segments were aseptically cut from the collected samples and placed in Petri plates containing culture medium for isolating fungi. The culture medium was malt extract agar (in g l^{-1}: Difco-agar 15, Bacto-malt extract 10), potato dextrose agar (in g l^{-1}: Difco-agar 15, glucose 20, and potato infusion 4), or a selective medium for basidiomycetes fungi were used (in g l^{-1}: Difco-agar 15, Bacto-malt extract 15, yeast extract 2, benlate (Methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate) 0.06, streptomycin sulfate 0.01 and lactic acid 2.5). The media was sterilized at 121 °C for 20 min. The culture media MEA and PDA were prepared with pH 5.6 and 6.5. The pH of these media was adjusted using NaOH or HCl. Plates were incubated at approximately 24 °C ± 2 °C.

2.3. Identification of fungi

The liquid medium for obtaining the dry mycelium for DNA extraction was prepared with (g l^{-1}): Bacto-malt extract 10. The obtained suspension was sterilized at 121 °C for 25 min. Erlenmeyer flasks (500 ml) containing 125 ml were inoculated with pure cultures of mycelium to be identified and were incubated at room temperature in a shaker at 150 rpm for 7 days. The mycelium was filtered and washed according to the protocol described by Montiel (2005). After washing, the mycelium was dried in an oven at 45 °C for 12 h.

DNA extraction was carried out using the previous published protocol of Cubero et al. (1999). Integrity of extracted DNA was

Fig. 2. Location of the Humberstone and Santa Laura historic site in northern Chile.

determined by gel electrophoresis, formed by 1% (w/v) agarose dissolved in a TAE Buffer 0.5× with 5 μl of ethidium bromide (10 mg ml^{-1}). Loading buffer was prepared with 1 μl of Gel Loading Dye 6× for every 5 μl of DNA solution. The electrophoretic run was performed at 90 V for 40 min. DNA observation was done by using a UV transilluminator. DNA amplification was performed through PCR in a Thermal Cycler Biorad. Complete ITS rDNA were amplified using fungal specific primers ITS1F and ITS4.

The PCR reaction mix was prepared with 100 ng of genomic DNA, 1× Paq5000 Reaction Buffer, 0.8 mM of dNTPs mix (0.2 mM of each dNTP), 2.5 U Paq5000 DNA polymerase, 0.2 μM of forward primer, 0.2 μM of reverse primer and MQ H_2O to complete a final volume of 50 μl. The PCR reaction considered an initial denaturation at 95 °C for 2 min, 30 cycles of amplification, and a final extension at 72 °C for 5 min. Each amplification cycle considered a denaturation at 95 °C for 20 s, alignment at 60 °C for 20 s, and an extension at 72 °C for 30 s. The fungus *Candida dubliniensis* CD36 ATCC, provided by the Oral Biochemistry and Biology Laboratory from Universidad de Chile, was used as a positive control of the reaction. PCR products, prior performing the sequencing reactions, were purified by using the E.Z.N.A.® Cycle-Pure Kit commercial kit (Omega-Biotech).

3. Results

The collected wood samples obtained belonged to two tree species: *Pseudotsuga menziesii* (common name Douglas-fir or Pino Oregón) and *Nothofagus obliqua* (commonly called Roble beech). The most prevalent form of degradation observed was a defibration of the wood (Fig. 3). Scanning electron microscopy showed that the middle lamella between cells was degraded and secondary walls remained after the chemical attack. The loss of the middle lamella caused the remaining cells wall layers to separate. In advanced stages of defibration, the secondary walls were also degraded.

Chemical analyses of wood samples with defibration confirmed exceedingly high concentrations of salts were present (Table 1). In defibrated wood samples, concentrations of sodium ranged from 27,000 mg/kg in early stages of defibration to 116,000 mg/kg in advanced stages. Other elements associated with various salts, including potassium, calcium, magnesium, were also elevated with increasing concentrations found in samples with advanced defibration.

Evidence of some fungal decay was also found at the sites but the amount was limited. Samples collected and examined by scanning electron microscopy showed typical characteristics of a brown rot (Fig. 4). A diffuse attack on the cellulose resulted in cell

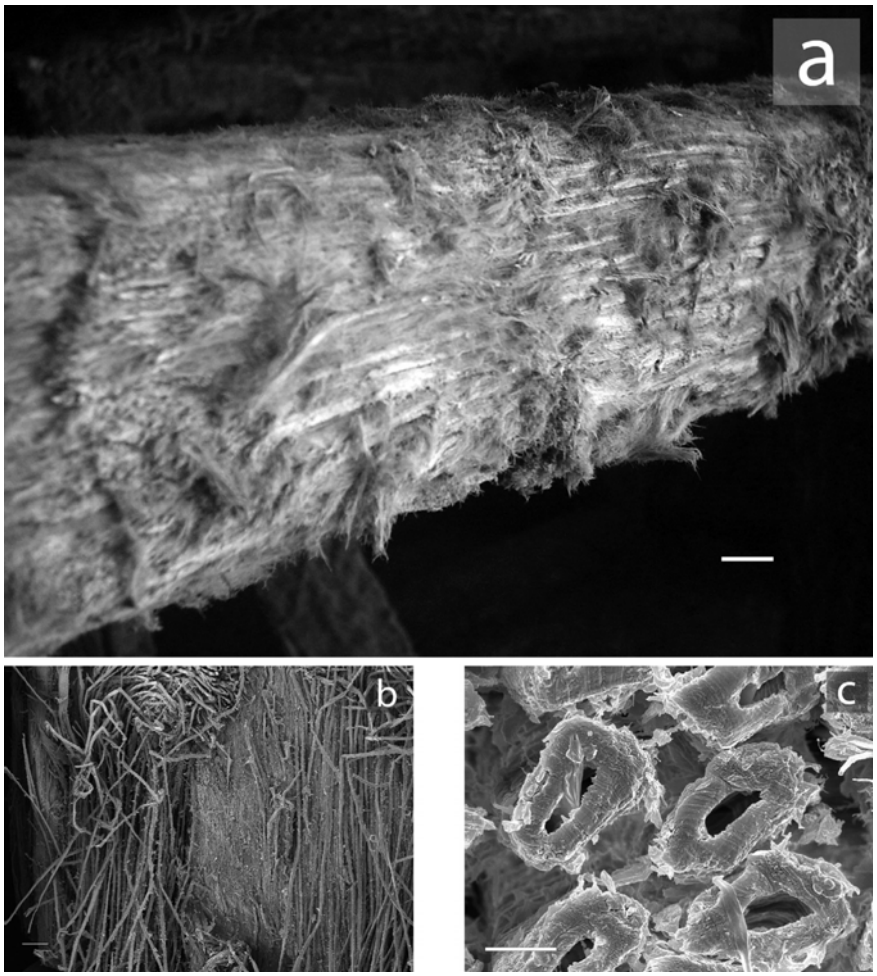

Fig. 3. Severe defibration in wood timber caused by high concentrations of salt (a) and scanning electron micrographs showing the attack on cell walls in wood (b and c). The corrosive actions of the salt degraded the middle lamella causing wood cells to separate (b). The middle lamella beween cells is completely degraded and the cellulose rich secondary walls remain (c). Bar in $a = 1$ cm, $b = 400$ μm and $c = 20$ μm.

deformation and a decrease in the rigidity of the wall structure. This type of attack was localized to the inner parts of Douglas-fir timbers and not associated with the areas of defibration. In areas where defibration occurred, some fungal mycelia were found on the surfaces of wood where salts had degraded the wood cells (Fig. 5).

Although many isolations were made from the affected wood samples showing decay, only ten different pure cultures of fungi were obtained from the isolations. Seven were isolated from wood samples obtained from the Humberstone site and three from the Santa Laura site. After rDNA extraction, the nucleotide sequences and BLAST analysis, identified, 2 cultures of Basidiomycota and 8 cultures of Ascomycota distributed in 4 classes, 6 orders, and 6 families (Table 2). Fungi that were found included, *Penicillium chrysogenum* (H 11-2), *Engyodontium album* (H 30-3), *Eupenicillium tropicum* (H 23-1), *Penicillium digitatum* (H 16-B), *Pseudotaeniolina globosa* (H-19), *Cladosporium phaenocomae* (S.L 15), *Aureobasidium pullulans* (H 30-1), *Penicillium virgatum* (H1) and *Phanerochaete sordida* (S.L. 8). *Coprinopsis sp.* (S.L. 16) was also found but with a BLAST match of only 94%. No brown rot fungi were isolated from the decayed wood found in the structures.

Table 1
Elemental analyses of historic woods from the Humberstone and Santa Laura Saltpeter Works. Results are in mg/Kg on a dry weight basis.

Sample	P	K	Ca	Mg	Mn	Al	Fe	Na
Zone 7 outer	126	7998	17,514	7934	80	3756	2146	48,846
Defibrated wood Santa Laura Offices								
Zone 2 outer	64	7062	8967	7873	52	2124	1179	50,682
Moderately defibrated wood Humberstone Offices								
Zone 2 inner	9	7372	1444	7567	25	188	127	35,447
Wood timber Humberstone Offices								
Zone 2 outer	37	12,560	2829	7011	92	281	341	115,590
Severely defibrated wood Santa Laura Offices								
Zone 1 outer	84	5671	3888	4688	97	651	1534	27,382
Wood early stages of defibration Humberstone Offices								
Sound modern pine wood	102	527	403	133	51	5	10	7

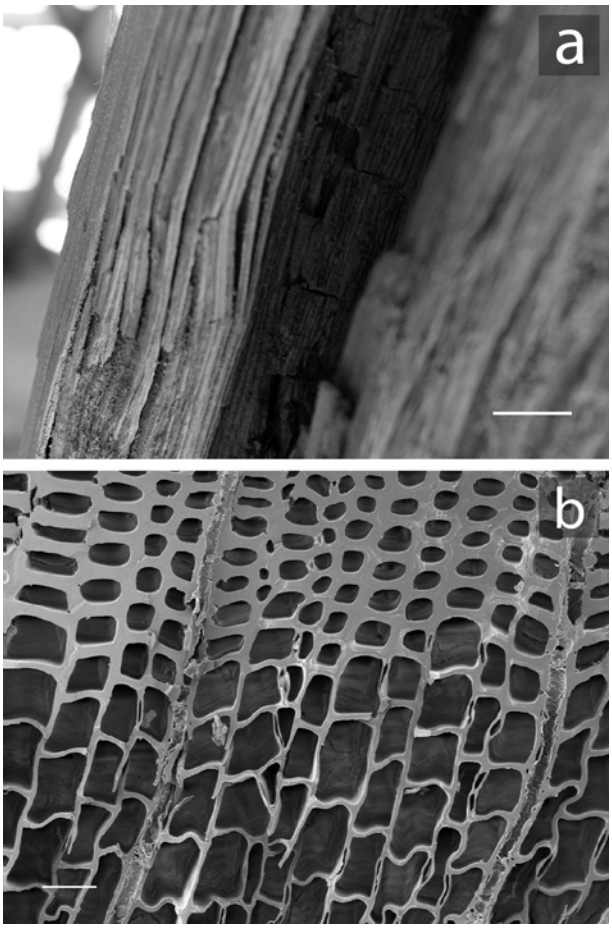

Fig. 4. Brown rot (dark area) in timber from leaching tower at the Santa Laura saltpeter works (a). Scanning electron microrgraph showing the brown rot attack on wood cells with a depolymerization of cellulose resulting in cells that have lost their original shape (b). Bar in $a = 1$ cm and $b = 40$ μm.

4. Discussion

The corrosive attack observed in this study was similar to deterioration previously reported by Parameswaran (1981), Blanchette et al. (2002) and Blanchette et al. (2004). This wood deterioration phenomenon is produced by the dissolution of lignin in the middle lamella due to chemical damage and the corrosive activity of salts. Johnson et al. (1992) and Kučerová et al. (2008) suggest that during this type of corrosive degradation, chemicals penetrate wood as solutes in water, thus forming crystals when water evaporates. The crystals cause cracks in the cell wall layers that lead to the disintegration of the wood cells. Blanchette et al. (2002), however, have shown that the formation of salt crystals are not the reason for the defibration. Instead, a chemical attack on lignin appears to take place in wood subjected to high salt concentrations. Moisture with dissolved salts is absorbed by wood and as evaporation takes place the salts accumulate. If these salts are not removed by rain or leeched out of the wood they gradually accumulate to exceedingly high concentrations. As subsequent moisture is introduced, the dissolved salts would cause a high pH solution to occur in the wood. Parameswaren (1981) suggests the alkali conditions found in wood of a potash storehouse where defibration was found were similar to an alkali pulping process which causes the degradation of lignin in the middle lamella of wood cells. Since moisture is apparently present only intermittently in the wooden structures at the Humberstone and Santa Laura Saltpeter Works, the alkali delignification process is slow and takes place over a long period of time. However, the nature of the chemical attack is comparable to the process of alkali pulping of wood. Preliminary observations by other investigators of wood from Humberstone and Santa Laura saltpeter works were made by Bahamóndez and Villagrán (2009). These authors suggested that deterioration in the woods could be resulting from spills of salt solutions that occurred in the production process of saltpeter, and whose impregnation into the wood with the constant cycles of wetting and drying submitted the wood to severe mechanical stress. They also suggested that some surface damage to the wood was likely attributed to high temperatures that existed during the production process. The results reported in our study demonstrate the presence of high concentrations of many elements that are components of salts within the deteriorated woods and elucidate the chemical attack that has taken place in the wood from these salts. The major cause of the defibration appears to be from this corrosive chemical degradation.

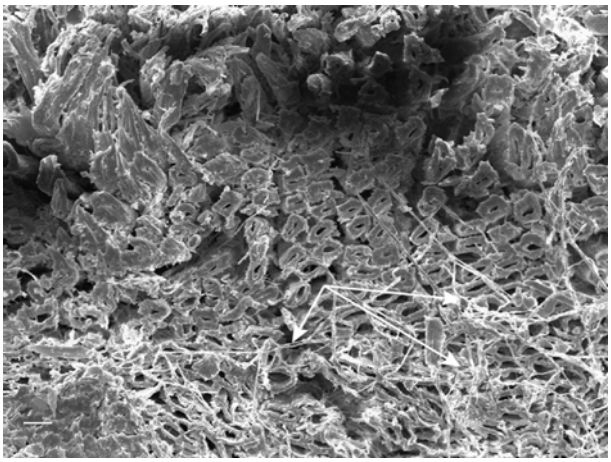

Fig. 5. Scanning electron micrograph showing defibrated wood cells and fungal mycelia growing on the degraded wood surface (arrows). Bar = 40 μm.

Several cultures had a BLAST match of 97% or greater to species designation. These included *P. chrysogenum* (H 11-2), *E. album* (H 30-3), *E. tropicum* (H 23-1), *P. digitatum* (H 16-B), *P. globosa* (H-19), *C. phaenocomae* (S.L 15), *A. pullulans* (H 30-1), *P. virgatum* (H1), and *P. sordida* (S.L. 8). The *Coprinopsis sp.* (S.L. 16) found had a BLAST match of only 94% which indicates sufficient variation in the sequence exists in comparison to those in the database and further studies using other genes (e.g. those related to the intergenic space (IGS1), ribosomal genes 18S and 28S, or mitochondrial genes) (Zhou et al., 2000; Rodriguez et al., 2004) with phylogenetic analyses are needed to determine more precisely what species this fungus may be. No isolates of brown rot basidiomycetes were isolated suggesting that this decay may not be active. The results presented here provide identifications for only the fungi that were cultured. Information on the past occurrence of fungi in the woods may be possible by DNA extraction directly from the wood samples and the use of fungal specific primers.

Salt can have an inhibitory effect on microbial growth, and wood decay causing basidiomycetes would not be expected to tolerate high concentrations of salt in wood (Meseguer, 2004). The attack observed in this investigation by brown rot fungi is an exception but apparently resulted inside the timber and was not directly associated with wood containing the high salt concentrations. It is also possible that the brown rot attack took place before concentrations of salts accumulated. Salt accumulation in these woods would be a slow process requiring long periods of time for low

Table 2
Fungal taxa identified from wood samples with comparisons (% identity) to the best BLASTn match with the NCBI GenBank database. Classification and previous reference to being found in Chile is noted.

Code	ITS	Clasification	Best Blast Match	Porcentage Identity	References to Being Found in Chile	Accession Number
H 11-2	ITS1 – ITS4	*Eurotiomycetes Eurotiales Trichocomaceae*	*Penicillium chrysogenum* JQ015265.1	99	Mujica et al., 1980; Donoso and Latorre, 2006; Hormazabal and Piontelli, 2009	KF578432
H 30-3	ITS1 – ITS4	*Sordariomycetes Hypocreales Cordycipitaceae*	*Engyodontium album* KC311469.1	98	none	KF578433
H 23-1	ITS1 – ITS4	*Eurotiomycetes Eurotiales Trichocomaceae*	*Eupenicillium tropicum* AY232277.1	99	none	KF578434
H 16-B	ITS1 – ITS4	*Eurotiomycetes Eurotiales Trichocomaceae*	*Penicillium digitatum* AB479307.1	99	Mujica et al., 1980; Lazo, 1996; Minter and Peredo, 2006	KF578435
H 19	ITS1 – ITS4	*Dothideomycetes Capnodiales Not assigned*	*Pseudotaeniolina globosa* HQ115663.1	100	none	KF578436
S.L 15	ITS1 – ITS4	*Dothideomycetes Capnodiales Davidiellaceae*	*Cladosporium phaenocomae* JF499838.1	99	none	KF578437
S.L 8	ITS1 – ITS4	*Agaricomycetes Corticiales Corticiaceae*	*Phanerochaete sordida* FN812727.1	97	Ortiz et al. 2013	KF578438
S.L 16	ITS1 – ITS4	*Agaricomycetes Agaricales Psathyrellaceae*	*Coprinopsis sp* JF681946.1.	94	none	KF578439
H 30-1	ITS1 – ITS4	*Dothideomycetes Dothideales Dothioraceae*	*Aureobasidium pullulans* JF817344.1	100	Hormazabal and Piontelli, 2009	KF578440
H 1	ITS1 – ITS4	*Eurotiomycetes Eurotiales Trichocomaceae*	*Penicillium virgatum* JF439503.1	97	none	KF578441

concentrations of salts to infiltrate with moisture followed by evaporation. The precipitation of the salts on the wood surfaces leads to accumulation in high concentrations that causes the corrosive deterioration. Although, wood decaying Basidiomycetes may not be expected to grow in wood with high salt concentrations, other microorganisms are capable of living in the presence of high salt concentrations. According to Castro et al. (2011), halophile microorganisms have developed several mechanisms of adaptation and have evolved to grow in extreme environments of high temperatures and salinity. Oren (2008) indicates that the fundamental characteristic of all halophile microorganisms is the fact that their cytoplasm has to be at least isosmotic in comparison with the surrounding environment. In regards to adaptation mechanisms of halophile microorganisms, there are two different strategies used to create an isosmotic state between their cytoplasm and their medium (Oren, 2008). The first one involves the accumulation of molar concentrations of potassium and chloride. This strategy requires a complete adaptation of the intracellular enzymatic machinery. The second strategy, highly used by microorganisms, is based on the biosynthesis and/or accumulation of organic osmotic solutes such as amino acids and amino acid by-products, sugar, or sugar–alcohols, which do not significantly participate in normal enzymatic activity. *Penicillium* and *Cladosporium* are two genera that have been considered to have species that are halophilic and there are reports of *A. pullulans* and *E. album* that have this characteristic as well (Attaby, 2001; Turk et al., 2004; Cantrell et al., 2006; Gaur et al., 2010; Jasmin et al., 2010; Gonsalves et al., 2012).

Many studies have been completed on the characterization of decay and identification of fungal species associated with historical buildings in different parts of the world (Blanchette and Simpson, 1992; Blanchette et al., 1994; Blanchette, 2000; Filley et al., 2001; Blanchette, 2003; Blanchette et al., 2004; Arenz et al., 2006). However, in Chile there is very limited information related to the various deterioration and decay processes that may occur in wooden constructions and no information about the fungi that may be involved. This study represents the first effort toward the identification of microorganisms isolated from Humberstone and Santa Laura saltpeter works. Although the fungi obtained appear to be able to tolerate the unusual conditions found at these sites, their role in the degradation of the woods is not known. The major degradative action that was observed appears to be due to the chemical attack of the wood by the salts but results demonstrate that there are diverse fungi present in the affected woods (Fig. 5). These fungi may have a role as secondary degraders utilizing the altered wood cell wall components that have been made available from the corrosion degradation processes.

Previous studies on fungi associated with wood in Chile have identified organisms using morphological characteristics of fungal fruiting bodies or cultural characteristics. However, Raberg et al. (2005) suggest that more precise methods should be used since it is very difficult to identify many of these fungi at the species level. According to Blanchette et al. (2005), improvements in molecular biology techniques have provided new tools for identifying microorganisms in wood. Particularly, the use of DNA sequencing and phylogenetic analyses that have successfully been used to identify microorganisms associated with degradation of wood products in service (Schmidt and Moreth, 2002; Kim et al., 2005; Lim et al., 2005; Moreth and Schmidt, 2005; Jacobs et al., 2010) and microbial diversity (Vasiliauskas and Stedlin, 1998; Adair et al., 2002; Jellison et al., 2003; Vasiliauskas et al., 2004; Mohomara et al., 2010; Ortiz et al., 2013). Results of the research we report in this study is also important, since several fungi, *E. album*, *E. tropicum*, *P. globosa*, *C. phaenocomae*, and *P. virgatum*, are reported from Chile for the first time.

The deterioration of wood caused by salts is a difficult issue that conservators must address for successful preservation of these historic sites. This problem appears to be most significant in regions

where rainfall is limited and the removal of salts from the surfaces of wood does not occur. Although rainfall may be limited, some moisture is necessary for the migration of salts and accumulation on surfaces as moisture evaporation takes place. There are very few examples of salt defibration that have been investigated, but in a previous study on salts affecting the historic wooden huts from early explores of Antarctica, these researchers suggested that the most effective conservation methods would be to reduce moisture with dissolved salts from entering the structures. In the dry deserts of northern Chile, rainfall is very limited but some moisture during certain times appears to be a source of salt migration into the wood. Although Bahamóndez and Villagrán (2009) suggest that salts entered the wood from spills that occurred during processing many years ago, the continued deterioration and severe defibration observed in the wood suggests the degradation process is still actively taking place and moisture with migrating salts is still a problem. With exceedingly high concentrations of salts present in many of the timbers, removal of these salts to avoid further defibration is warranted. Additional research, however, is essential to better understand the chemical reactions involved in the defibration process and for obtaining conservation treatments that can help remove the salts to preserve these historic buildings.

5. Conclusions

This study represents the first effort directed toward the identification of the types of deterioration and decay as well as the microorganisms isolated from Humberstone and Santa Laura saltpeter works, which exist in an extreme desert environment and unusually high salt concentrations. Extensive degradation caused by salt deterioration was found in the wooden structures. This attack was characterized by a degradation of the middle lamella in wood cells and a separation of the cells which caused a defibration of the wood. No other forms of biological attack were found in areas with the salt deterioration. However, inside some timbers and away from the salt accumulation, evidence of brown rot was observed. The brown rot fungus or fungi responsible for this degradation were not isolated. Fungi were isolated from the defibrated woods and nine cultures of the phylum Ascomycota were isolated and identified with a BLAST match of 97% or greater. Of all the pure cultures under study, five species have been reported in Chile for the first time, including *E. album*, *E. tropicum*, *P. globosa*, *C. phaenocomae* and *P. virgatum*. Additional studies are needed with isolate S.L 16 since sequencing the ITS region did not reveal its identity. This study does not provide direct evidence of deterioration caused by microorganisms, but the dissolution of lignin in the middle lamella due to the corrosive activity of salts and released cellulosic cell wall layers could serve as a substrate for these fungi. The presence of these various microorganisms, including several species considered as halophiles, provides a better understanding of the fungal diversity associated with such harsh environmental conditions. Currently there is an incomplete understanding of the chemical processes that cause the defibration in wood and little information on other factors that may influence this type of deterioration. The increasing number of historic structures being found affected by this type of degradation (Blanchette et al., 1994, 2002, 2004) reinforces the need for new investigations that can provide conservation strategies to help preserve the wooden buildings and other wooden artifacts at these important cultural heritage sties.

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References

Adair, S., Kim, S., Breuil, C., 2002. A molecular approach for early monitoring of decay basidiomycetes in wood chips. FEMS Microbiology Letters 211, 117–122.

Arenz, B., Held, B., Jurgens, J., Farrell, R., Blanchette, R.A., 2006. Fungal diversity in soils and historic wood from the Ross Sea Region of Antartica. Soil Biology and Biochemistry 38, 3057–3064.

Attaby, H., 2001. Influence of salinity stress on the growth, biochemical changes and response to gamma irradiation of *Penicillium chrysogenum*. Pakistan Journal of Biological Sciences 4, 703–706.

Bahamóndez, M., Villagrán, A., 2009. Elaboración de expedientes y estudio, segunda etapa del plan de intervenciones prioritarias para las oficinas salitreras Humberstone y Santa Laura online at https://www.dropbox.com/s/58758vugpxu81qq/Informe%20Estudio%20de%20Materiales.pdf.

Blanchette, R.A., Simpson, E., 1992. Soft rot decay and wood pseudomorphs in an ancient coffin (700 BC) from tumulus MM at Gordion, Turkey. International Association of Wood AnatomistsBulletin 13, 201–213.

Blanchette, R.A., Haight, J., Koestler, R., Hatchfield, P., Arnold, D., 1994. Assessment of deterioration in the archeological wood from ancient Egypt. Journal of the American Institute for Conservation 33, 55–70.

Blanchette, R.A., 2000. A review of microbial deterioration found in archeological wood from different environments. International Biodeterioration & Biodegradation 46, 189–204.

Blanchette, R.A., Held, B., Farrell, R., 2002. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. Polar Record 38, 313–322.

Blanchette, R.A., 2003. Deterioration in historic and archaeological woods from terrestrial sites. In: Koestler, R., Koestler, V., Charola, A., Nieto-Fernández, F. (Eds.), Art, Biology, and Conservation: Biodeterioration of Works of Art. The Metropolitan Museum of Art, New York, pp. 328–347.

Blanchette, R.A., Held, B., Jurgens, J., Haight, J., 2004. Wood deterioration in Chacoan great houses of the southwestern United States. Conservation and Management of Archeological Sites 6, 203–212.

Blanchette, R.A., Jurgens, J., Held, B., Arenz, B., Smith, J., 2005. Decay of historic and archeological wooden structures: degradation processes and molecular characterization of wood destroying fungi. In: Meeting of International Academy of Wood Science and of the 10th Meeting on Wood Products Research and Developments, Concepción, Chile, November 14–17.

Blanchette, R.A., Held, B.W., Arenz, B.E., Jurgens, J.A., Baltes, N.J., Duncan, S.M., Farrell, R.L., 2010. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Microbial Ecology 60, 29–38.

Blanchette, R.A., 2010. Microbial degradation of wood from aquatic and terrestrial environments. In: Mitchell, R., McNamara, C.J. (Eds.), Cultural Heritage Microbiology: Fundamental Studies in Conservation Science. ASM Press, Washington DC, pp. 179–190.

Butin, H., Peredo, H., 1986. Hongos parásitos en coníferas de América del Sur con especial referencia a Chile. Berlin, Stuttgart.

Cantrell, S., Casillas-Martinez, L., Molina, M., 2006. Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques. Mycological Research 110, 962–970.

Castro, L., Flores, A., Rodriguez, A., Aguilar, M., Aguilar, C., Rodriguez, R., 2011. Aislamiento y caracterización de microorganismos halófilos de suelos salinos de cuatro ciénagas Coahuila, México. Revista Científica de la Universidad Autónoma de Coahuila 3, 33–43.

Cubero, O., Crespo, A., Fatehi, J., Bridge, P., 1999. DNA extraction and PCR amplification method suitable for fresh, herbarium stored, lichenized, and other fungi. Plant Systematics and Evolution 216, 243–249.

Culverwell, M., 2000. The Mining Cluster in Antofagasta. INtegrating Small and Medium Supplier into the Productive Chain online at http://www.aia.cl/ingles/archivos/cluster/MalaikaCulverwell.pdf.

Díaz-Vaz, J., 1979. Claves para la identificación de maderas de árboles nativos y cultivados en Chile. Bosque 3, 15–25.

Donoso, A., Latorre, B., 2006. Caracterización del moho azul causado por *Penicillium* spp. en uva de mesa almacenada en frío. Ciencia e investigación Agraria 33, 143–155.

Filley, T., Blanchette, R.A., Simpson, E., Fogel, M., 2001. Nitrogen cycling by wood decomposing soft-rot fungi in the "King Midas tomb" Gordion, Turkey. Proceedings of the National Academy of Sciences 98, 13346–13350.
Furci, G., 2008. Diversidad de especies Hongos. In: Ocho Libros (Ed.), Biodiversidad de Chile: Patrimonio y Desafíos, pp. 366–375. Chile.
Gamundi, I., 2003. Discomycetes (Fungi, ascomycota) de Chile Austral I. Darwiniana 41, 29–36.
Gaur, R., Singh, R., Gupta, M., Kumar, M., 2010. *Aureobasidium pullulans*, an economically important polymorphic yeast with special reference to pullulan. African Journal of Biotechnology 9, 7989–7997.
Gonsalves, V., Nayak, S., Nazareth, S., 2012. Halophilic fungi in a polyhaline estuarine habitat. Journal of Yeast and Fungal Research 3, 30–36.
Hormazabal, E., Piontelli, E., 2009. Endophytic fungi from Chilean native gymnosperms: antimicrobial activity against human and phytopathogenic fungi. World Journal of Microbiology and Biotechnology 25, 813–819.
Jacobs, N., Scheiding, W., Weiss, B., Müller, D., Hiller, C., Brabetz, W., 2010. Detection of Wood Destroying Fungi Using DNA Microarray Technology. IRG/WP10-20435. The International Research Group on Wood Protection, Stockholm.
Jasmin, C., Chellappan, S., Sukumaran, R., Elyas, K., Bhat, S., Chandrasekaran, M., 2010. Molecular cloning and homology modelling of a subtilisin-like serine protease from the marine fungus, *Engyodontium album* BTMFS10. World Journal of Microbiology and Biotechnology 26, 1269–1279.
Jellison, J., Jasalavich, C., Ostrofsky, A., 2003. Detecting and identifying wood decay fungi using DNA analysis. In: Goodell, B., Nicholas, D., Schultz, T. (Eds.), Wood Deterioration and Preservation: Advances in Our Changing World, pp. 346–357. Washington.
Johnson, B., Ibach, R., Baker, A., 1992. Effect of salt water evaporation on tracheid separation from wood surfaces. Forest Product Journal 42, 57–59.
Kim, G., Lim, Y., Song, Y., Kim, J., 2005. Decay fungi from playgound wood products in service using 28S rDNA sequence analysis. Holzforschung 59, 459–466.
Kučerová, I., Ohlídalová, M., Frankl, J., Kloiber, M., Michalcova, A., 2008. Defibring of historical roof beam caused by ammonium sulphate and ammonium phosphates based fire retardants. In: International Conference on Wood Science for Preservation of Cultural Heritage: Mechanical and Biological Factors: Braga, Portugal: 2008, 5–7 November.
Lazo, W., 1996. Algunos *Ascomycetes* y *Basidiomycetes* interesantes de Chile. Boletín Micológico 11, 99–102.
Lim, W., Kim, J., Chedgy, R., Breuil, C., 2005. Fungal diversity from western red cedar fences and their resistance to B-thujaplicin. Antonie van Leeuwenhoek 87, 109–117.
Meseguer, I., 2004. Los microorganismos halófilos y su potencial aplicado en biotecnología. Ciencia e Investigación 7, 13–17.
Minter, W., Peredo, H., 2006. Hongos de Chile online at http://www.cibertruffle.org.uk/chilfung.
Minvu, 2003. Postulación de las oficinas salitreras Humberstone y Santa Laura para su inclusión en la lista del Patrimonio Mundial/UNESCO online at http://www.archivochile.com/Historia_de_Chile/sta-ma2/4/stamacult00003.pdf.
Momohara, I., Ota, Y., Nishimura, T., 2010. Assessment of decay risk of airborne wood-decay fungi. Journal of Wood Science 56, 250–255.
Montiel, A., 2005. Identificación de la enzima responsable de la degradación de pentaclorofenol (PCF) en *Amylomyces rouxii* y optimización del mecanismo de degradación mediante la expresión heteróloga de peroxidasas. Thesis. Universidad Autónoma Metropolitana, México.
Moreth, U., Schmidt, O., 2005. Investigations on ribosomal DNA of indoor wood decay fungi for their characterization and identification. Holzforschung 59, 90–93.
Mujica, F., Vergara, C., Oehrens, E., 1980. Flora fungosa chilena. Universidad de Chile, Santiago.
Oren, A., 2008. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems 4, 2.
Ortiz, R., Moreno, L., Ramirez, R., Olivero, P., 2011. Determination of the models of biodeterioration in elements of wood produced by rot fungi in building of the zone of historic conservation of Valparaíso, Chile. Revista de la Construcción 10, 82–89.
Ortiz, O., Navarrete, H., Honorato, A., Párraga, M., Carrasco, I., De La Vega, E., Ortiz, M., Herrera, P., Blanchette, R.A., 2012. Identificación de hongos de pudrición de maderas aislados desde tres sitios declarados como Patrimonio de la Humanidad en Chile. In: 3rd Iberoamerican Congress on Wood Protection, Concepción, Chile, November, 26–28.
Ortiz, R., Navarrete, J., Oviedo, C., Parraga, M., Carrasco, I., De La Vega, E., Ortiz, M., Blanchette, R.A., 2013. White rot Basidiomycetes isolated from Chiloe National Park in Los Lagos Region, Chile. Antonie van Leeuwenhoek Journal of Microbiology. http://dx.doi.org/10.1007/s10482-013-0041-z.
Parameswaren, N., 1981. Micromorphology of spruce timber after long-term service in a potash store house. Holz als Roh- und Werkstoff 39, 149–156.
Raberg, U., Hogberg, N., Johan, C., 2005. Detection and species discrimination using rDNA T-RFLP for identification of wood decay fungi. Holzforschung 59, 696–702.
Rodriguez, A., Xoconostle, B., Valdes, M., 2004. Ecología molecular de los hongos ectomicorrizicos. Revista Filotecnia Mexicana 27, 267–278.
Schmidt, O., Moreth, U., 2002. Data bank or rDNA-ITS sequences from building-rot fungi for their identification. Wood Science and Technology 36, 429–433.
Turk, M., Méjanalle, L., Sentjurc, M., Grimalt, J., Gunde-Cimerman, N., Plementina, A., 2004. Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. Extremophiles 8, 53–61.
Vasiliauskas, R., Stedlin, J., 1998. Fungi inhabiting stems of *Picea abies* in a managed stand in Lithuabia. Forest Ecology and Management 109, 119–126.
Vasiliauskas, R., Larsson, E., Larsson, K., Stedlin, J., 2004. Persistance and long-term impact of rotstop biological control agent on mycodiversity of *Picea abies* stumps. Biological Control 32, 295–304.
Vial, G., 1987. Panorama histórico del sector minero en Chile. Ambiente y Desarrollo, pp. 21–27.
Zhou, G., Whong, W., Ong, T., Chen, B., 2000. Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment. Molecular and Cellular Probes 14, 339–348.