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Bacillus species as an intrinsic controller of fungal deterioration of archival documents

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ABSTRACT

The present study samples 19th century maps (belonging to India's Great Trigonometrical Survey) and daptars (official papers of Maratha history) to isolate the microflora and explore their interactions within that niche. Amongst the array of microbial isolates, the work successfully isolates a novel strain of *Bacillus,* producing a potent, low molecular weight, broad spectrum antagonistic protein fraction. The predominance of this species rendered the documents sampled resistant to biodeterioration in comparison to others. The antifungal activity of the isolate was attributed to a low molecular weight, heat, pH stable, extracellular protein fraction that was isolated with 30% and 60% ammonium sulfate precipitation. The activity of this fraction was established by bio-autography. Its stability upto 50 °C and resistance to proteolytic cleavage makes this protein fraction a novel broad spectrum potent antifungal candidate.

1. Introduction

Archival documents allow us to establish communications between past and future generations. A collection of such documents is a priceless treasure for every country. The risk of biodeterioration in indoor environments, such as libraries and repositories, of archival documents all over the world has been a great cause of concern (Pasquarella et al., 2012).

Paper was made from rags in the beginning and later, due to scarcity as well as the technological advances, rags were substituted by wood pulp treatment involving acidic or alkaline methods (Baty et al., 2010). The documents were bound using gum or glue from natural sources as also the pigments and dyes used for writing or printing. In order to improvise on the printing, sizing was done using gelatin from animal sources as well other equivalent alternative plant derivatives like rosin and starch (Zottia et al., 2008; Pinzari et al., 2010; Benetti et al., 2011; Harkawy et al., 2011). These chemical characteristics of the paper along with environmental as well as biological factors have been held responsible for the archival degradation, characterized by foxing, discoloration and finally loss of paper structure (Baty et al., 2010; Espejo et al., 2010).

Over 200 fungal spp. are known to damage both wood and cellulose by physical insertion of hyphae and production of primary and secondary metabolites (Zyska, 1997; Bennett et al., 2009; Sterflinger, 2010). These metabolites include fungal pigments and various extracellular hydrolytic enzymes like cellulases, amylases and proteases etc. Fungi are thus directly responsible for damage to the structure of paper (Sterflinger, 2010). Following fungal colonization, degrading cellulosic material provides an enriched medium for bacteria (Michaelsen et al., 2010). Numbers of bacterial spp. have been reported to colonize and damage paper however *Bacillus* spp are one of the most predominant causes of paper deterioration (Olubanke, 2010). It may be however argued that these bacteria may have arrived on the surfaces of paper as contaminants from dust, and that they may not play an important role as causative agents of degradation.

Bacillus licheniformis, is one such saprophytic bacterium, known to produce bacteriocin-like peptides showing antagonistic activity against many gram positive and few gram negative organisms, but not fungi (Pattnaik et al., 2001; Martirani et al., 2002; Cladera-Olivera et al., 2004; Cui et al., 2012). Literature also reports on a low molecular weight bacteriocin-like protein from *B. licheniformis* MKU3 having a wide spectrum of antibacterial as well as antifungal activity (Lili et al., 2006). On the other

Abbreviations: CFS, Cell Free Supernatant; CMC, Carboxy Methyl Cellulose; GTS, Great Trigonometrical Survey.

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hand, Lebbadi et al., 1994 and Tendulkar et al., 2007 isolated two low molecular weight peptides from *B. licheniformis* having exclusive anti fungal activity. All these peptides have different molecular mass, heat resistance and antagonistic spectrum (Pattnaik et al., 2001; Martirani et al., 2002; Cladera-Olivera et al., 2004).

The present work aims to understand archival deterioration or preservation in correlation to its microbial flora. The documents were selected by manual examination for signs of deterioration. The microflora was isolated and identified using standard microbial techniques. One of these isolates, belonging to *Bacillus* species, was found to produce a potent antifungal compound when tested against fungal genera *Aspergillus*, *Penicillium* and *Cladosporium* which are often associated with paper deterioration irrespective of geographical locations (Olubanke, 2010). This active compound was then characterized by partial purification, gel electrophoresis and bioautography.

2. Materials and methods

2.1. Sample selection

19thcentury documents i.e. Maps and Daptars, were used in the study (Fig. 1). Maps belonging to India's Great Trigonometrical Survey (GTS) were selected. India's GTS was a massive project that mapped India, as well as measured the curvature of the earth. Daptars (Sawantwadi, Patwardan, Menavati etc.) were also selected for the study. Daptars are official papers, diaries, accounts and various tables which form an important source material of Maratha history. All the samples were procured from the Maharashtra state archives, Mumbai. To determine the extent of deterioration of the manuscripts the documents were visually examined. Both maps and daptars were screened and categorized according to the degree of deterioration. Foxed, tattered and brittle documents along with better preserved documents were selected for the study for comparison (Fig. 1).

2.2. Isolation of microbial biodeteriogens

The documents were sampled using standard non invasive techniques like adhesive tapes and cotton swabs. The used cotton bud was then swabbed on to Nutrient agar for bacteria and Sabouraud agar for fungi. The colonies were screened for cellulolytic activity using Carboxy Methyl Cellulose (CMC) Congo-red agar (Huang et al., 2012). Cellulose degrading fungal and bacterial colonies, showing discoloration of Congo-Red on the solid medium (Lu et al., 2004; Gupta et al., 2012), were taken for further study. The adhesive tapes carrying the sampled organisms, both bacteria and fungi, were placed directly on CMC congo-red agar plates to obtain a replica of the microbial flora from the archival documents on to the plate. The fungal isolates showing cellulolytic activity, obtained from both these techniques, were identified based on their cultural and morphological characteristics (Ellis et al., 2007). The bacteria which showed cellulolytic activity were subjected to preliminary identification through gram staining and biochemical characterization. From the replica of the adhesive tapes on to CMC congo-red agar plates two bacterial isolates, which showed distinct zones of inhibition of fungi (Fig. 2) were isolated and characterized using

Fig. 1. Some Archival Documents selected for the study (A) Well preserved archival map (B) Foxed tattered brittle archival map (C) Zoomed Foxed area of map from B (D) Stained, foxed daptar (E) Foxed, stained, brittle, torn daptar (F) Well preserved daptar. (samples were procured from the Maharashtra state archives, Mumbai).