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Pre-Columbian Archeological Textiles: A Source of *Pseudomonas aeruginosa* with Virulence Attributes

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Abstract: *Pseudomonas aeruginosa* is an opportunistic pathogen associated with a broad spectrum of infections in humans. However, the pathogenicity of environmental *P. aeruginosa* strains, especially isolates from museums and conservation laboratories, is not widely recognized. In this study, the virulence attributes of *P. aeruginosa* isolated from pre-Columbian textiles were compared to those of a clinical strain. Both genetically identified environmental strains (KP842564 and KP842565) exhibited a high ability to form biofilms on abiotic surfaces and high hemolytic activity. In addition, strain KP842564 was a moderate pyocyanin producer and showed proteolytic properties toward bovine serum albumin, fibrinogen, mucin, and casein. In contrast to the clinical isolate, the environmental strains were susceptible to all the tested antimicrobial agents. The strains also showed high bioadhesion and colonization capacity on archeological textile samples, in which wool fibers were the only source of nutrients, as confirmed by scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) analysis. This study highlights the need to identify microorganisms which inhabit historic objects, in order to avoid exposure to occupational hazards. Although the strain KP842565 exhibited only some of the examined virulence-related features, given that the production of pyocyanin and hemolysins as well as the formation of biofilm are important virulence factors of *P. aeruginosa*, the results indicate that these strains may present a potential health risk for humans.

Keywords: *Pseudomonas aeruginosa*; virulence; occupational hazard; archeological textile; SEM-EDX; FTIR

1. Introduction

Pseudomonas aeruginosa is one of the major opportunistic human pathogens. It is capable of infecting a range of tissues and is known to cause severe nosocomial infections. It is often associated with high morbidity and mortality, particularly in immunocompromised and vulnerable patients [1]. Infections by *P. aeruginosa* are particularly challenging, because of its high level of antibiotic resistance [2]. *P. aeruginosa* pathogenicity depends on various virulence factors, including cell-associated factors, i.e., flagella and pili, acting as both adhesins and motility factors, as well as adherence abilities and the production of lipopolysaccharides [3]. The second group comprises extracellular virulence

factors, such as the secretion of proteases, lipases, phospholipases, elastases and exotoxins, the production of pigments, especially pyocyanin, biofilm formation on biotic and abiotic surfaces, and the production of quorum sensing molecules [4]. Due to its virulence factors, after the initial step of colonization, *P. aeruginosa* can cause extensive tissue damage, dissemination in the bloodstream, and even life-threatening infections [5].

Although the threat posed by clinical *P. aeruginosa* isolates to humans is well documented, the potential virulence of environmental strains is still not widely recognized, especially in the case of strains isolated from museums and cultural heritage objects. This issue has been raised by recent reports regarding similarities between clinical and environmental *P. aeruginosa*. Grosso-Becerra et al. [6] found that *P. aeruginosa* isolates of clinical and non-human origin, collected from a plant, the ocean, a water-spring, and from the stomach of a dolphin, did not show genomic variability, and their genomes exhibited very high sequence similarity. Separate reports suggest that the genetic variability of *P. aeruginosa* is extremely low, but recombination events within the species occur at high levels [3]. Other studies have found that clinical *P. aeruginosa* isolates are genotypically, chemotaxonomically, and functionally indistinguishable from environmental isolates. Römling et al. [7] observe that the most frequently identified *P. aeruginosa* clone in cystic fibrosis patients was also detected with high frequency in aquatic environments across various geographic areas. Moreover, Foght et al. [8] report that *P. aeruginosa* isolated from clinical sources and from a gasoline-contaminated aquifer formed a coherent taxonomic group and were indistinguishable at the level of molecular taxonomy, although they showed a few distinguishing characteristics in terms of their adaptation to inhabitation conditions.

On the other hand, on the basis of biochemical, physiological, and metabolic tests, Bouhaddioui et al. [9] found that *P. aeruginosa* isolated from a hospital environment could be clearly differentiated from that found in marine water, whereas the mineral water strains were more closely related with clinical strains. Similarly, a clear distinction between clinical and environmental strains (isolated from soil, aliments, olive, rivers water, wells, and public swimming pools) was detected by Maroui et al. using pulsed field gel electrophoresis [10]. These findings are partially consistent with results reported by Grosso-Becerra et al. [6], who concluded that, although clinical and environmental *P. aeruginosa* strains are phenotypically diverse, this is not reflected in their genomic variability. Such conflicting reports call for further research regarding the virulence of environmental *P. aeruginosa* strains, including those isolated from archeological textiles.

The aim of the present study was to assess the virulence attributes of genetically identified *P. aeruginosa* strains isolated from archeological pre-Columbian textiles in comparison with a clinical isolate. The damage caused by the formation of biofilms by *P. aeruginosa* on the archaeological fabrics was also determined by means of scanning electron microscopy (SEM) and SEM with energy-dispersive X-ray spectroscopy (SEM-EDX). The textiles from which the *P. aeruginosa* strains originated dated back to the pre-Columbian archeological period (1250–1450 AD) and came from the Andean area in Argentina. They are currently deposited in the Archaeology Division of the Museum of La Plata, the main archeological repository of Argentina, which has a collection of about 700 textile pieces from various sites in Peru, Chile, and Bolivia, as well as from the provinces of Salta and Jujuy. The vast majority of the pieces were donated or sold to the museum in the nineteenth century by collectors. A small collection of textile debris came from the pre-Columbian city Santa Rosa de Tastil, located in the Quebrada del Toro, Rosario de Lerma Department, Salta Province, in northwest Argentina. This site has been studied for almost a century. The tested textile debris came from excavations directed by Rolandi de Perrot in the Puna Argentina performed between 1967 and 1969 [11]. To the best knowledge of the authors, there have been no previous in-depth studies on the virulence of microorganisms isolated from cultural heritage objects, and this research is the first to consider not only the biodeteriorative potential of these strains but also their potential threat to humans.

2. Materials and Methods

2.1. Characterization of Archeological Textiles

The textile samples came from the pre-Columbian period and are stored as Deposit 25 in the Archeological Division at La Plata Museum, Argentina. Observations of the surface morphology of the fibers in the archeological fabric samples and estimation of the elemental composition were carried out using scanning electron microscopy (SEM): Nova NanoSEM 230 microscope, SEDetector, HV: 5 kV, low vacuum (FEI, Hillsboro, Oregon, USA) equipped with an EDS X-ray micro-analyzer (EDAX, Mahwah, NJ, USA). The fibers of the archeological specimens were identified and their chemical structure was also examined by means of Fourier transform infrared spectroscopy (FTIR). A Nicolet 6700 FTIR reflection spectrophotometer (Thermo Scientific, Waltham, MA, USA) equipped with an attenuated total reflectance (ATR) accessory was used. The FTIR absorption spectra of the samples were recorded in the range of 600–4000 cm^{-1}, with a resolving power of 4 cm^{-1}. The results were analyzed with the software application OMNIC 3.2 (Thermo Scientific, Waltham, MA, USA).

2.2. P. aeruginosa Isolates

Two *P. aeruginosa* strains isolated from archeological textiles were used. The strains had previously been identified and the nucleotide sequences of their 16S rRNA gene deposited in the GenBank NCBI database with the accession numbers KP842564 and KP842565 [12]. To estimate the virulence-related parameters of the strains, *P. aeruginosa* isolated from a patient with an outer-ear infection was used as a reference. The clinical *P. aeruginosa* strain was identified on the basis of 16S rRNA gene sequence (GenBank accession number MN830401).

2.3. Biofilm Formation on Glass and Polypropylene

The biofilm-forming ability of the bacteria on glass and polypropylene was investigated according to a published method [13]. A 2 mL aliquot of bacterial suspension (10^8 cells/mL) was inoculated onto material slices (20 mm × 20 mm) placed in six-well tissue plates (Greiner Bio-One GmbH, Frickenhausen, Germany). After initial adhesion (90 min, 37 °C, with agitation), the plates were rinsed twice with 1 mL PBS to remove non-adherent cells. Afterwards, 2 mL of fresh TSB medium (pancreatic digest of casein 1.7%, peptic digest of soybean 0.3%, glucose 0.25%, NaCl 0.5%, K_2HPO_4 0.25%; pH 7.3) was added to each well. In the biofilm formation phase, the plates were incubated for 24 h at 37 °C. Slices without bacteria were used as a negative control. The adhered biofilms were carefully scraped off the surface of the materials using a sterile cell scraper. The number of cells was determined on TSA medium by the standard count plate method and the biofilm level was expressed as colony forming units per 1 cm^2 of material (CFU/cm^2).

2.4. Pyocyanin Quantification

Pyocyanin was extracted and quantified according to the methodology described by Essar et al. [14], with some modifications. The strains were cultured on PPGAS medium (NH_4Cl 0.11%, KCl 0.15%, Tris-HCl 0.19%, $MgSO_4$ 0.02%, glucose 0.5%, peptone 1.0%; pH 7.2) for 72 h at 37 °C. The bacterial culture was then centrifuged (6000 rpm, 4 °C, 10 min) and 3 mL chloroform was added to 5 mL supernatant, followed by centrifugation (4500 rpm, 5 min). The organic phase was transferred to another Falcon tube, mixed with 1 mL HCl (0.2 M) and centrifuged again. The concentration of pyocyanin (expressed as μg/mL) was determined by multiplying the absorbance of the upper phase at 520 nm by the constant 17.072.

2.5. Proteolytic Activity

Proteolytic activity was determined by the thin-layer enzyme assay (TEA) cultivation technique using bovine serum albumin (BSA, fraction V; Sigma-Aldrich, St. Louis, MO, USA), mucin (type

II, Sigma-Aldrich, St. Louis, MO, USA), fibrinogen (Merck Millipore, Darmstadt, Germany), and casein (BDH Chemicals, Poole, Dorset, UK). According to the TEA method, the inner surface of a polystyrene Petri dish was coated with a protein solution (1 mg/mL) and then covered with 9 mL of TSA medium. Ten microliters of the bacterial suspensions in saline (10^8 cells/mL) were spotted on the surface of the agar. After incubation (37 °C, 48 h) the agar was removed and the surface was rinsed with distilled water and exposed to water vapor. The diameters of the zones with decreased wettability were measured, indicating enzyme degradation of the protein [15].

2.6. Hemolysis

Hemolytic activity was determined on TSA medium containing 2% fresh sheep blood (GrasoBiotech, Poland) [16]. Bacterial suspensions in saline (10^8 cells/mL) were inoculated in a circular manner and the plates were incubated for 24 h at 37 °C in two gaseous conditions: aerobic and in 5% CO_2. Hemolysis was expressed by the hemolytic index, calculated by dividing the diameter of the colony plus the hemolysis zone by the colony diameter.

2.7. Antimicrobial Susceptibility Testing

P. aeruginosa strains were tested for their susceptibility using the reference disc diffusion method [17]. Colonies from the exponential phase of bacterial growth (24 h) on TSA were suspended in sterile saline, adjusted to 10^8 cells/mL and then plated in Mueller–Hinton agar (beef extract 0.2%, casein hydrolysate 1.75%, starch 0.15%, agar 1.7%; pH 7.3). After 15 min, antimicrobial discs containing amikacin (30 µg/disc), imipenem (10 µg/disc), cefepime (30 µg/disc), ciprofloxacin (5 µg/disc), aztreonam (30 µg/disc), polymyxin B (300 units/disc), and piperacillin–tazobactam (100/10 µg/disc) (Thermo Scientific™ Oxoid™) were placed on the plates, and incubation was performed at 37 °C for 18 h. The zones of inhibition around the discs were measured and interpreted following CLSI guidelines [18] (Table 1).

Table 1. Criteria for assessing *Pseudomonas aeruginosa* sensitivity to antimicrobial agents according to CLSI guidelines [18].

Antimicrobial Agent	Interpretive Categories		
	S—Susceptible	I—Intermediate	R—Resistant
	Zone Diameter (mm)		
amikacin	≥17	15–16	≤14
imipenem	≥19	16–18	≤15
cefepime	≥18	15–17	≤14
ciprofloxacin	≥21	16–20	≤15
aztreonam	≥22	16–21	≤15
polymyxin B	≥12	–	≤11
piperacillin–tazobactam	≥21	15–20	≤14

2.8. Bioadhesion and Biofilm Formation on Archeological Textiles

The *P. aeruginosa* strains were inoculated on a solid mineral medium [12] on agar slants with archeological textiles samples (10 mm × 10 mm) as the sole carbon source. The number of bacteria in the biofilm structure was determined after 3 and 5 days of incubation at 30 °C using the standard count plate method. The effects of biofilm formation on the textiles were also assessed using SEM microscopy, SEM-EDX, and FTIR analysis, as described above.

2.9. Statistical Analysis

All experiments were conducted in triplicate. Statistical analysis of means and standard deviations was performed using Statistica v.10.0 (StatSoft. Inc., Tulsa, OK, USA) software. To compare the virulence features of the environmental strains and clinical isolate, analysis of the statistical significance

of differences (one-way analysis of variance ANOVA) was conducted using ORIGIN v.6.1 (OriginLab Corporation, Northampton, MA, USA) software.

3. Results and Discussion

3.1. Characteristics of Archeological Fabrics

The cross-sectional view of the archeological textile samples obtained by SEM allowed its geometrical structure to be established. The plain-woven fabric was formed by warp and weft threads (Figure 1A,B). The wefts seen in the fabric structure were very tightly packed, which accounted for the high compressibility of this textile object. The weft threads on one surface of the fabric, probably on the right side, exhibited extensive damage (Figure 1E, arrows). The character of the thread damages indicates that fiber rupture had been caused by mechanical forces such as shear and friction, which are typical during intensive use of a textile product.

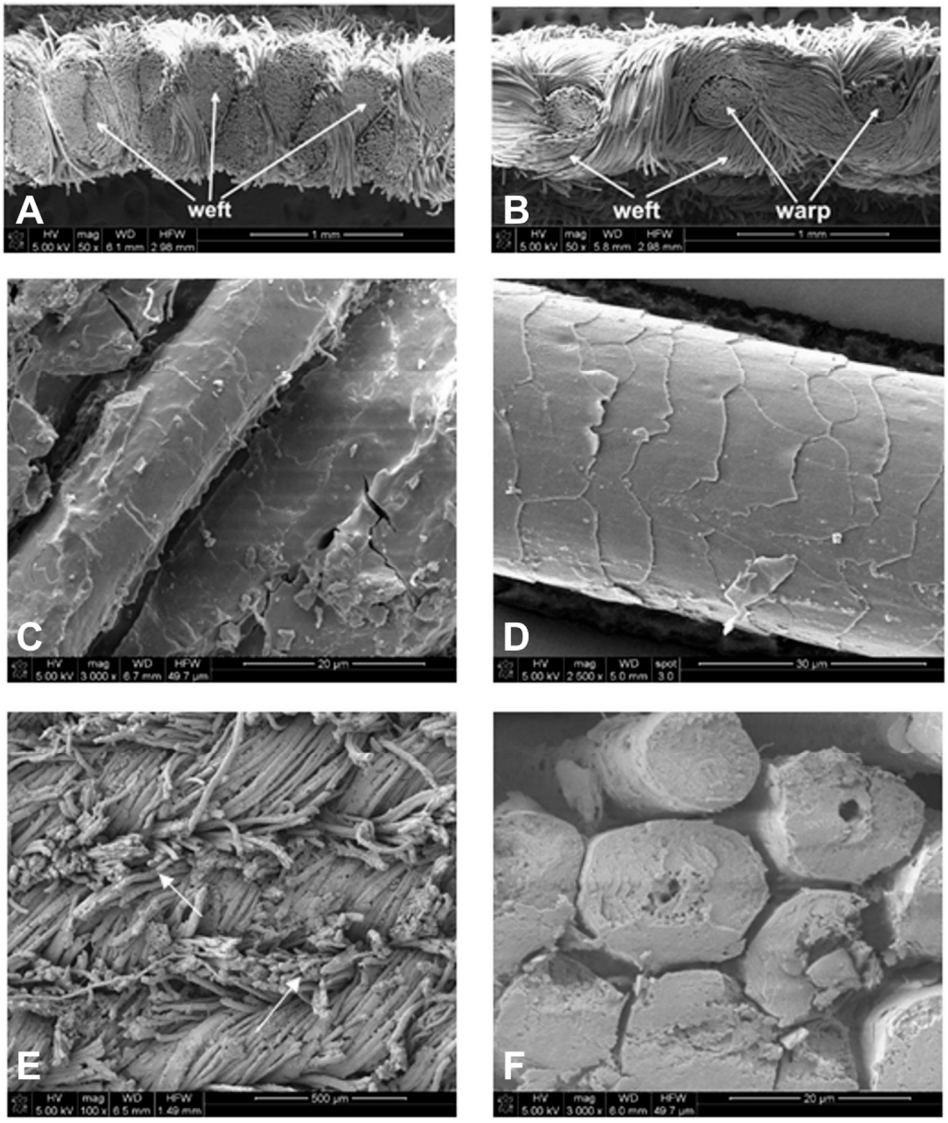


Figure 1. SEM images of the cross-section of the archeological fabric (**A,B**); fibers in archeological textile fabric (**C**); contemporary alpaca fibers (**D**); thread damages to archeological textile fabric (**E**); cross-section of the archeological fibers (**F**).

The morphological features of the fiber surface obtained by SEM revealed that the examined archeological object was made from fibers of animal origin, most probably from alpaca or llama fibers. A detailed comparison of archeological (Figure 1C) and contemporary alpaca fibers (Figure 1D) showed a close similarity in the surface topography. On these fibers surfaces (Figure 1C,D) thin scales which form an interrupted irregular wave pattern can be observed. Similar morphological features of the alpaca fibers surface were visible in the SEM images made by McGregor et al. [19]. The SEM cross-sectional view of the fibers forming the weft thread showed an almost circular outline of fibers with a diameter of 15–20 µm and small medulla (Figure 1F).

3.2. Virulence of P. aeruginosa Isolated from Pre-Columbian Textiles

Of the virulence-related parameters of *P. aeruginosa*, eight were examined for two *P. aeruginosa* strains isolated from the pre-Columbian textiles: biofilm formation on glass and polypropylene; pyocyanin production; proteolytic activity toward albumin, mucin, fibrinogen, and casein, and hemolysis. The antibiogram test was also performed to ascertain their susceptibility to seven different antimicrobial agents.

The environmental strains KP842564 and KP842565 showed a strong ability to form biofilms on glass and polypropylene (2.8×10^8–5.3×10^8 CFU/cm^2). Polypropylene was colonized by *P. aeruginosa* KP842565 and the clinical strain more intensively than glass, while biofilm formation by KP842564 was the same on both surfaces (4.1×10^8 CFU/cm^2). Although the strains isolated from the textiles showed good ability to inhabit both tested abiotic surfaces, the formation of biofilms was only statistically comparable to that of the clinical isolate on glass (Table 2).

Table 2. Characteristics of the clinical strain of *P. aeruginosa* and the environmental strains isolated from archeological textiles.

Feature		Environmental Strains		Clinical Strain
		KP842564	KP842565	
Virulence-related parameters				
Biofilm formation (log (CFU/cm^2)) ± SD	polypropylene	8.6 ± 0.4 [a]	8.7 ± 0.3 [a]	9.2 ± 0.4
	glass	8.6 ± 0.5 [b]	8.4 ± 0.6 [b]	8.9 ± 0.4
Pyocyanin production (µg/mL) ± SD		1.09 ± 0.3 [a]	0.39 ± 0.2 [a]	8.48 ± 0.8
Proteolytic activity—decreased wettability zone (mm) ± SD	bovine serum albumin	16.4 ± 1.1 [a]	0 [a]	20.8 ± 0.4
	mucin	22.0 ± 0.7 [a]	0 [a]	25.6 ± 0.5
	fibrinogen	12.6 ± 1.7 [a]	0 [a]	17.6 ± 1.1
	casein	14.4 ± 0.5 [a]	0 [a]	19.6 ± 1.1
Hemolytic index ± SD		1.50 ± 0.07 [b]	1.46 ± 0.05 [b]	1.41 ± 0.09
Susceptibility to antimicrobials—inhibition zone (mm)/category *	amikacin	23/S	28/S	22/S
	imipenem	33/S	32/S	27/S
	cefepime	34/S	31/S	11/R
	ciprofloxacin	37/S	33/S	33/S
	aztreonam	27/S	25/S	0/R
	polymyxin B	12/S	16/S	0/R
	piperacillin–tazobactam	22/S	21/S	20/I
Additional features				
Biofilm on archeological textile (log (CFU/cm^2))	after 3 days	5.8 ± 0.5	8.7 ± 0.8	nd
	after 5 days	6.8 ± 0.6	8.6 ± 0.7	nd
Sequence homology of 16S rRNA to the clinical strain (%)		99.7	99.5	–

SD—standard deviation; [a] statistically significant difference in comparison to clinical *P. aeruginosa* ($p < 0.05$); [b] no statistically significant difference in comparison to clinical *P. aeruginosa*; * category: S—susceptible, I—intermediate, R—resistant; nd—not determined.

The ability to form biofilm on both abiotic (e.g., polystyrene, polypropylene, silicone, glass) and biotic (e.g., tissue) surfaces is one of the virulence factors known to be directly related to the pathogenicity of *P. aeruginosa* [5]. Biofilm formation confers significant resistance to antimicrobials and

components of the host immune system, making biofilm-based infections a significant clinical problem. Furthermore, there is increasing evidence that biofilm-mediated infection facilitates the development of chronic and recurring infectious diseases [1,2] (Kerr and Snelling, 2009; Lister et al., 2009). In this context, the ability of *P. aeruginosa* strains originating from textiles to colonize glass and polypropylene is of major importance.

Among the arsenal of virulence factors produced by *P. aeruginosa* is pyocyanin, a blue–green pigment that alters the physiology and homeostasis of host cells by exposing it to oxidative stress. Pyocyanin modifies host cell responses by inhibiting generation of neutrophil superoxide and lymphocyte proliferation as well as by increasing interleukin-8 production by human airway epithelial cells [5]. Moreover, several host genes are induced in response to pyocyanin, including mucins, inflammatory cytokines, chemokines, and oxidative stress-responsive genes [5]. Clinical *P. aeruginosa* strains are able to secrete pyocyanin at various levels, ranging from 1.29 to 25.94 μg/mL [20]. Hunter et al. [21] reported less diversity in pyocyanin production in a group of clinical strains (1.62–9.83 μg/mL), but the concentrations were correlated with stages of disease severity. Pyocyanin secretion also depends on strain origin, with the highest amounts in *P. aeruginosa* isolated from the sputum and urine of patients with cystic fibrosis [20,21].

In our study, all the tested strains were able to secrete pyocyanin, but at different levels. *P. aeruginosa* KP842564 produced 1.09 μg/mL while *P. aeruginosa* KP842565 produced 0.39 μg/mL of pyocyanin (Table 2). These amounts were statistically lower than for the clinical *P. aeruginosa* strain (8.48 μg/mL). These findings are in agreement with Bouhaddioui et al. [9], who demonstrated the ability of all *P. aeruginosa* isolated in their study from mineral water to produce pyocyanin, whereas 13% of clinical strains were non-pyocinotypable. Moreover, environmental *P. aeruginosa* has been shown to secrete from 0.36 to 24.5 μg/mL pyocyanin at 37 °C, but much higher concentrations at 30 °C, ranging from 1.6 to even 197.2 μg/mL [6].

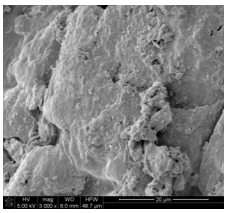
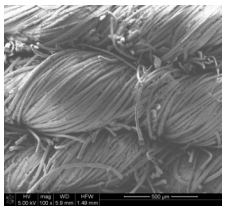
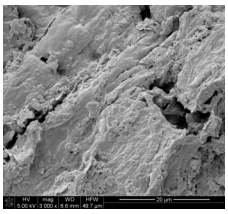
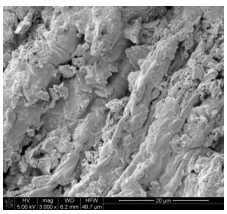
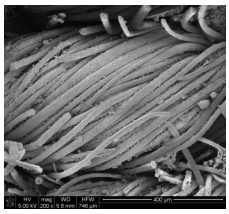
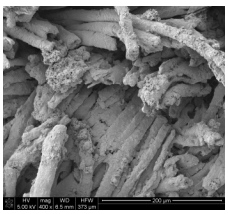
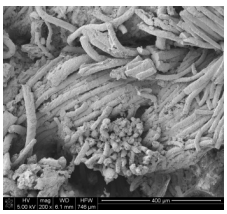
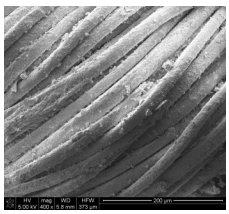
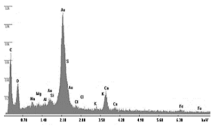
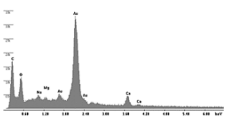
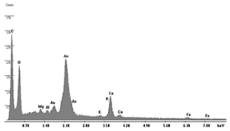
The virulence of *P. aeruginosa* is also determined by exoproducts, such as proteases which help establish and maintain infections by damaging host tissues and degrading proteins that function as part of the host defenses [5]. Protease-deficient strains are generally less virulent than protease producers in burned mouse models [22]. Since proteases play an important role in *P. aeruginosa* infections, they are typical features of clinical strains [15,23]. Among the strains tested in our study, environmental *P. aeruginosa* KP842564 and the clinical isolate were characterized by protease production. Both strains degraded bovine serum albumin, mucin, fibrinogen, and casein, whereas the environmental strain KP842565 revealed no proteolytic activity (Table 2). Proteolysis of proteins naturally occurring in the human body should be considered as virulence attributes, but degradation of casein is also associated with *P. aeruginosa* pathogenicity, since proteases typical for clinical isolates are capable of degrading casein [23]. The tested strains originating from textiles further exhibited hemolytic activity, forming beta hemolysins at a level statistically comparable to the clinical isolate (Table 2). Production of hemolysins indicates the ability of these strains to induce tissue damages and may play a role in spreading the infection.

Clinical *P. aeruginosa* strains are characterized by innate or acquired resistance to many antibiotic classes, including antipseudomonal penicillins, carbapenems, aminoglycosides, and ciprofloxacin [24]. The resistance of this bacterium to antibiotics is the result of a number of independent co-occurring mechanisms, including restricted uptake, efflux system, drug inactivation, and changes in targets [25]. Furthermore, due to the overuse of antibiotics, the resistance of *P. aeruginosa* is increasing globally [24]. In our study, we examined the susceptibility of the tested environmental and clinical *P. aeruginosa* strains to antimicrobial agents from seven classes, i.e., aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins and β-lactamase inhibitors, monobactams, and polymyxins. Only the clinical isolate showed resistance to cefepime, aztreonam, polymyxin B, and intermediate susceptibility to piperacillin–tazobactam (Table 2).

3.3. Degradation of Archeological Textiles by *P. aeruginosa*

The *P. aeruginosa* strains isolated from the textiles were also examined for their ability to form biofilms on archeological material samples, which served as the sole carbon source. Both strains colonized the textiles, forming biofilm after 3 days at the level 5.6×10^5 (KP842564) and 5.9×10^6 CFU/cm^2 (KP842565). Extending the incubation time to 5 days resulted in an increase in the number of biofilm cells by 1–2 log units (Table 2). SEM-EDX analysis of pre-Columbian textile colonized by both tested *P. aeruginosa* strains showed decreases in oxygen content of 12% in the case of colonization by KP842564 and 5% by KP842565. This may be the result of bacterial bioadhesion and biofilm formation on the textile samples (Table 3). The metabolism of *P. aeruginosa* inhabiting fabrics can cause changes in the content of elements, as has previously been reported for microbial communities growing on historical materials. Gutarowska et al. [26] observed the activation of numerous metabolic pathways, including those regulating the production of primary and secondary metabolites, including metabolites associated with the deterioration of organic compounds.

Table 3. Microscopic and chemical analysis of archeological textile samples after biofilm formation by *P. aeruginosa* isolates.

Sample		Textile Sample After Biofilm Formation by *P. aeruginosa* KP842564	Textile Sample after Biofilm Formation by *P. aeruginosa* KP842565		Control
SEM images				Right side	
					
				Left side	
Chemical analysis (SEM-EDX)	Spectrum				
	Element content (%)	C: 53.30, O: 20.85, Na: 1.54, Mg: 0.63, Al: 0.57, Si: 0.55, S: 6.71, Cl: 0.71, K: 0.77, Ca: 10.68, Fe: 3.66	C: 53.27, O: 28.04, Na: 2.40, Mg: 1.09, Ca: 15.20		C: 51.47, O: 33.42, Mg: 0.80, Al: 0.52, K: 0.66, Ca: 11.15, Fe: 1.97

Analysis of the infrared absorption spectra of the archeological fibers before and after biofilm formation in comparison to contemporary alpaca fibers revealed significant changes in the molecular structure of the keratin (Figure 2). The spectral range between 1000 and 1170 cm^{-1} is recommended for investigation of structural changes in wool fibers caused by various destructive factors, such as temperature, oxygen, light, and microorganisms [27,28].

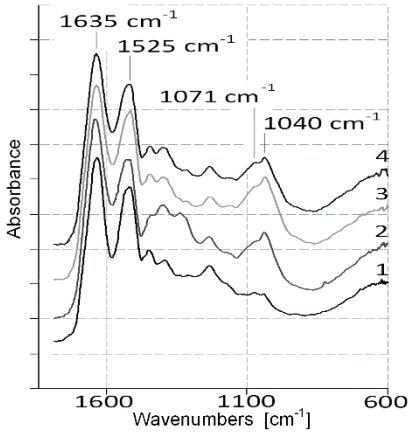


Figure 2. FTIR-ATR absorption spectra of fibers: contemporary alpaca fibers (**1**); archeological fabric (**2**); archaeological fabric after biofilm formation by *P. aeruginosa* KP842564 (**3**); archeological fabric after biofilm formation by *P. aeruginosa* KP842565 (**4**).

All spectra for the archeological fibers showed distinctly stronger absorption near 1040 cm^{-1} than in the case of the contemporary alpaca fibers, which may result from the oxidation of disulfide S–S bonds of cysteine and the formation of the two functional groups. The characteristic peak at 1040 cm^{-1} represents cysteic acid ($–SO_3^-$) and the spectral signal at 1071 cm^{-1} indicates cystine monoxide (–SO–S–) [19,29]. This finding could be interpreted as evidence of extensive destruction of cysteine in these archeological keratin fibers. Disulfide bonds are the main chemical cross-linkage maintaining the keratin fiber's secondary structure [30,31]. Cleavage of a large proportion of the disulfide bonds between its molecular chains causes a high degree of keratin degradation.

The growth of microorganisms on an archeological textile may cause the weakening or loosening of its structure, odor emission, pH changes, aesthetic changes, staining and discolorations on the surface, the production of slimy substances, decreases in strength, and finally complete deterioration [32,33]. Many bacteria that are present in these kinds of organic substrata grow using very low concentrations of nutrients and allow the development of micro-niches for the growth of other microorganisms. As a consequence, the biodeterioration of archeological materials can cause significant economic losses and the destruction of priceless cultural artefacts. Colonization and the development of biofilms by fungi and bacteria significantly modify the material. Microorganisms which form biofilm on textiles also degrade the cellulose and proteins, which are one of the main components of natural fibers such as cotton or wool [32]. Proper storage in special containers, under suitable and stable conditions in terms of light (<50 lux), temperature (18–20 °C), and relative humidity (<55%), may be an effective preservation strategy [34].

4. Conclusions

This study compared the virulence attributes of *P. aeruginosa* isolated from pre-Columbian textiles to those of a clinical strain. Both examined environmental strains showed virulence-related features typical for clinical isolates, i.e., strong ability to form biofilms on glass and biomaterial, and the production of hemolysin. The strain KP842565 was a poor pyocyanin producer, while strain KP842564 exhibited moderate pyocyanin production and extensive proteolytic activity. The fact that the tested environmental strains exhibited virulence-related attributes indicates a potential threat to people working in museums and conservators. Another consideration highlighted by the present study which should be included in the risk assessment of the tested strains is their ability to efficiently colonize archeological textiles, which suggests that they can survive and develop even without additional sources of nutrients in museum conditions.

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