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BIOFILM FORMATION OF *ACHROMOBACTER XYLOSOXIDANS* ON CONTACT LENS

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Achromobacter spp. may contaminate lenses, lens cases, and contact lens solutions and cause ocular infections. The aim of this study was to investigate the possibility of isolated strain of *Achromobacter xylosoxidans* to form biofilm on the surface of soft contact lenses (CL), to quantify the production of the formed biofilm, and compare it with the reference strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae*). Bacterial strain isolated from one contact lens case was identified as *A. xylosoxidans* using Vitek2 Automated System. Biofilm forming capacity of isolated strain of *A. xylosoxidans* and reference strains of *P. aeruginosa*, *S. aureus*, and *H. influenzae* on soft CL were analyzed by commonly used microtitre plate method. Our results showed that isolated strain of *A. xylosoxidans* was capable to form biofilm on the surface of soft contact lens. *A. xylosoxidans* was strong biofilm producer while all examined reference strains were moderate biofilm producers. *A. xylosoxidans* appears to be superior biofilm producer on soft CL compared to reference strains.

Keywords: *Achromobacter xylosoxidans*, biofilm, contact lens

Introduction

Biofilms are composed of bacterial cells attached to biotic or abiotic surfaces and encased in a complex, self-secreted polymeric matrix. Bacteria in a biofilm differ from planktonic cells, in having an increased resistance to antimicrobials and the host immune defence [1].

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The presence of bacterial biofilms has been demonstrated on many medical devices such as urinary catheters, intrauterine devices, ventricular assist devices, neurosurgical ventricular shunts, prosthetic heart valves, intravascular catheters, coronary stents, prosthetic joint, cochlear implants, intraocular and contact lenses (CL) [1, 2]. Since CL are increasingly being used, they became one of the main predisposing risk factors for infectious keratitis [3, 4]. Integrity of the epithelial surface of cornea is compromised by the interactions of lens and ocular surface, which increases the ability of microbes to adhere and cause an infection in healthy host [5]. Also, the presence of biofilm on the contact lens may increase potential pathogenicity of bacteria, by prolonging their retention time at the ocular surface [6]. It is documented that biofilm present on lenses or lens cases shows enhanced resistance to the antimicrobial activities of contact lens solutions [7].

Pseudomonas aeruginosa is documented as the most common causative agent of infections related to contact lens wear, and *Serratia marcescens*, *Staphylococcus aureus*, *Acanthamoeba* spp., and *Fusarium* spp. as following major causative pathogens [8]. The ability of *P. aeruginosa*, *S. marcescens*, and *S. aureus* to form biofilms on CL is well documented in the literature [2, 7].

Achromobacter xylosoxidans is a closely related bacterium to *Pseudomonas* spp. – aerobic, non-glucose-fermenting, Gram-negative rod [9]. Thus, its role as an important pathogen may be underestimated, but recent studies showed that *Achromobacter* spp. may contaminate lenses, lens cases, and contact lens solutions and subsequently cause corneal infiltrative events (CIEs) [8, 10–12]. Based on a literature, there are 12 reported cases of *A. xylosoxidans* keratitis [12]. Infections due to *A. xylosoxidans*, as a water pathogen, are associated with humidifiers and disinfectants in hospitals, but contaminated hands can also spread the bacterium to the CL, lens solution, and lens case [8, 12, 13]. Also, a study using scanning electron microscopy (SEM) showed that *Achromobacter* spp. were able to form biofilms on the surface of CL from patients with microbial keratitis [8]. To the best of our knowledge, there is no data describing the occurrence of *A. xylosoxidans* as a causative agent of contact lens-associated keratitis as well as a contaminant of contact lens cases in Serbia.

The aim of this study was to investigate the possibility of isolated strain of *A. xylosoxidans* from contact lens case to form biofilm on the surface of soft contact lens, to quantify the production of the formed biofilm, and compare it with the reference strains (*P. aeruginosa*, *S. aureus*, and *Haemophilus influenzae*).

Materials and Methods

Bacterial strain

Samples from 71 contact lens cases used by asymptomatic contact lens wearers were streaked onto Columbia agar plate (Torlak, Serbia) containing 5% sheep blood and MacConkey agar plate (HiMedia, India). After incubation at 37 °C for 24 h, the colonies were identified by automated Vitek2 System (bioMérieux, France) using GN card. The strain from one contact lens case was identified as *A. xylosoxidans*.

Biofilm assay

In order to detect, quantify, and compare the production of biofilm on soft CL (Air Optix, Alcon, USA) by isolated strain of *A. xylosoxidans*, reference strains *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *H. influenzae* ATCC 49766 were also analyzed. Bacterial suspensions of tested strains were prepared in sterile saline and adjusted to the density of 0.5 McFarland standard.

CL were placed in separate wells of 24-well microtitre plates (Sarstedt) with 1,800 μL of tryptic soy broth (bioMérieux) supplemented with additional 1% glucose. Two hundred microliters of previously prepared bacterial suspension of analyzed strains were added to each well. Negative control for each plate represented only the medium with and without CL. After 24 h of incubation at 35 °C in aerobic conditions, plates with CL were decanted and rinsed gently three times with 2,000 μL of sterile phosphate-buffered saline (pH 7.2) in order to remove planktonic bacteria. After air drying, plates with CL were fixed with 2,000 μL methanol per well for 20 min, dried and stained with 2,000 μL per well of 2% crystal violet (bioMérieux) for 15 min. Unbounded dye was rinsed with water. After air drying, CL were transferred into new 24-well microtitre plates and dye bound to biofilm formed on CL was released with 2,000 μL of 96% ethanol per well for 20 min at room temperature, with gentle tapping. Extracted dye was transferred to 96-well microtitre plates (150 μL per well) and optical density (OD) was measured at 570 nm using a microtitre plate reader (ICN Flow Titertek Multiscan Plus, Germany). The results were calculated according to Stepanović et al. [14]. Each assay was repeated three times on three consecutive days. OD value of negative control (CL cultivated in medium without bacteria) was subtracted from measured OD values of all tested strains and mean OD values

from three experiments were calculated. To calculate the category of biofilm production, the cut-off optical density (ODc) was determined as three standard deviations above the mean OD of the negative control. According to the obtained results all tested strains were divided into four groups: OD ≤ ODc – category 0 (no biofilm producer); ODc < OD ≤ 2×ODc – category 1 (weak biofilm producer, +); 2×ODc < OD ≤ 4×ODc – category 2 (moderate biofilm producer, ++); 4×ODc < OD – category 3 (strong biofilm producer, +++).

Results

Bacterial strain isolated from one of 71 contact lens cases was identified as *A. xylosoxidans*. We examined if isolated strain of *A. xylosoxidans* was capable to produce biofilm on soft CL. Quantification of biofilm production on soft CL by isolated strain of *A. xylosoxidans* compared with reference strains is presented in Table I and Figure 1.

Discussion

We investigated the possibility of isolated strain of *A. xylosoxidans* from contact lens case to form biofilm on the surface of soft CL. We found that the examined strain was able to produce biofilm on contact lens.

Kilvington et al. [11] showed the ability of *Achromobacter* spp. to survive within dry storage cases and assumed it was related to protection gained from biofilm formation.

Study using culture-independent methods found that *Achromobacter* spp. was predominant bacterium in patients with contact lens-related corneal disease, with occurrence of 76% of lens cases. This outcome was in part explained by the

Table I. Category of biofilm production by isolated and reference strains

Strains	Category
Achromobacter xylosoxidans	+++
Pseudomonas aeruginosa ATCC 27853	++
Staphylococcus aureus ATCC 29213	++
Haemophilus influenzae ATCC 49766	++
Sterile lens	0

Note: +++, category 3 – strong biofilm producer; ++, category 2 – moderate biofilm producer; 0, category 0 – no biofilm producer.

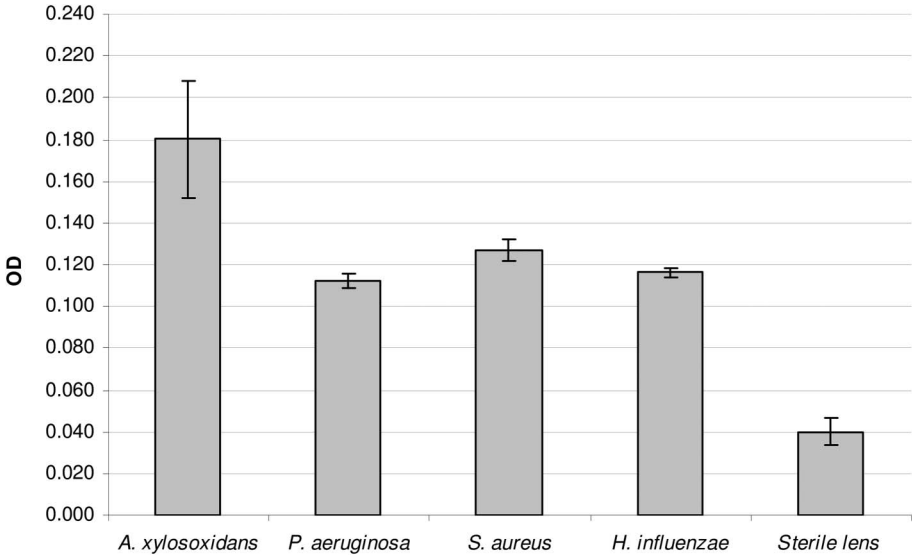

Figure 1. Biofilm production of analyzed strains on soft contact lenses. OD, optical density

ability of *Achromobacter* to form biofilms on the surface of CL, which was confirmed by SEM analysis [8].

Some studies showed that *Achromobacter* spp. can form biofilm on surfaces other than CL, such as central venous catheters and urinary catheters [15, 16]. The presence of biofilms in sputum from cystic fibrosis (CF) patients infected with *A. xylosoxidans* was revealed in one study. This study showed that bacterial cells in *A. xylosoxidans* biofilms are located very close to each other, unlike biofilms of *P. aeruginosa*, where rich extracellular matrix separates bacterial cells [17].

Our study showed that isolated *A. xylosoxidans* strain was strong biofilm producer, while reference strains were moderate biofilm producers. Trancassini et al. [18] showed the ability of *A. xylosoxidans* isolated from samples of CF and non-CF patients to form biofilms on abiotic surfaces. According to this study, the majority of isolated strains (57.9%) were strong biofilm producers, while 26.3% strains were moderate, and only 12.3% strains were weak biofilm producers [18].

According to Reddy et al. [13] penetrating keratoplasty and topical steroid use are the main risk factors in *A. xylosoxidans* ocular infections. However, recent study where 8 out of 28 patients with *A. xylosoxidans* keratitis were contact lens wearers, implicates that contact lens wear may also be an important risk factor [19]. Three case reports of keratitis and one case report of conjunctivitis caused by *A. xylosoxidans* in healthy contact lens wearers were previously described,

pointing out that this organism should be considered as potential pathogen in contact lens-related ocular infections [10, 12, 20].

A. xylosoxidans is often confused with other Gram-negative rods, which is the reason why *A. xylosoxidans* ocular infections are probably underreported [13]. Although *A. xylosoxidans* is very similar to *Pseudomonas*, it differs from *Pseudomonas* in being less virulent and having different antibiotic resistance profiles and clinical presentation [8, 19].

Since there are difficulties in identifying the causative organism in contact lens-related ocular infections, American Academy of Ophthalmology [21] recommended empiric administration of antibiotics for those infections with no causative organism identified. Their recommendations are fluoroquinolones or cefazolin/tobramycin combination therapy. A study using culture-independent methods, mentioned above, also explained the prevalence of *Achromobacter* over *Pseudomonas* by the resistance of this organism to empirical antibiotics used in treatment of corneal infection [8].

A. xylosoxidans should be considered as a potential pathogen in patients showing Gram-negative bacilli on smear examination who have compromised ocular status and slowly progressive disease [13].

The international ISO 14729 and FDA 510(k) standards for adequate antimicrobial efficacy of contact lens multipurpose solutions are based only on tests which determine the efficacy against reference strains of planktonic bacteria and fungi (*P. aeuruginosa*, *S. aureus*, *S. marcescens*, *Fusarium solani*, and *Candida albicans*) [22]. Antimicrobial activity against such strains does not guarantee efficacy against clinical isolates. In one study, *Achromobacter* spp. was isolated in the majority (61%) of contact lens cases of contact lens wearers with CIEs, that all used the same multipurpose disinfecting solution (MPDS). High levels of contamination were explained by the resistance of isolated strains to the biocidal components of the used MPDS [11].

A study showing that bacterial biofilms formed on contact lens surfaces are more resistant to contact lens solutions than planktonic cells, pointed out the importance of incorporating tests for activity against biofilms and not only against planktonic cells [7]. Chang et al. [23] found that *A. xylosoxidans* biofilms were more resistant to the two common ophthalmic preservatives than biofilms of *P. aeruginosa*, and suggested the incorporation of *A. xylosoxidans* biofilm in antimicrobial efficacy tests which would improve safety of multiuse ophthalmic products.

In our study, we have shown that isolated strain of *A. xylosoxidans* was capable to form strong biofilm on the surface of soft CL, and that it appears to be superior biofilm producer relative to reference strains.

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Conflict of Interest

The authors declare no conflict of interest.

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