# PRODUCTION OF ENDOTOXINS BY *DESULFOVIBRIO DESULFURICANS* CELLS GROWING ON THE Ti6Al4V ALLOY

**BEATA CWALINA[1]*, WERONIKA DEC[2], MARZENA JAWORSKA-KIK[3]**

[1] ENVIRONMENTAL BIOTECHNOLOGY DEPARTMENT, SILESIAN UNIVERSITY OF TECHNOLOGY, POLAND
[2] INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY, BRANCH PSZCZYNA, POLAND
[3] DEPARTMENT OF BIOPHARMACY, MEDICAL UNIVERSITY OF SILESIA, POLAND
*E-MAIL: BEATA.CWALINA@POLSL.PL

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## Introduction

The sulphate-reducing bacteria of the species *Desulfovibrio desulfuricans* colonize the human digestive system, being an element of the physiological microflora of the intestine [1-4]. However, like most intestinal bacteria, they become pathogens in specific conditions. The endotoxins secreted by these bacteria into the environment are among the threats [5]. Various strains of the *D. desulfuricans* species show the ability to colonize titanium alloys, creating biofilms on them [6,7]. Ti6Al4V alloy is one of the most widely used in implantology. Therefore, there is a direct risk to human health, resulting from the possibility of colonization of the titanium implant in the human body by the tested bacteria. The aim of this work was to check the endotoxins production capacity of three selected strains of *D. desulfuricans* bacteria grown on Ti6Al4V alloy surface.

## Materials and Methods

The experiments have been conducted to investigate the endotoxins production capacity of three selected strains of *D. desulfuricans* bacteria: the standard DSM strain (isolated from soil) and two wild strains DV/A and DV/B (isolated from human intestine [4]). The bacteria have formed biofilms on an alloy of titanium with aluminium and vanadium (Ti6Al4V) under various conditions of the surrounding medium. In the experiments, the Ti6Al4V alloy samples with a ground surface were used. The research was carried out during the cultivation of bacteria in four different environments imitating human body fluids, which were: artificial saliva, artificial saliva simulating inflammation, as well as Tyrode and Ringer fluids. The samples were incubated for 28 days under the adopted conditions. Endotoxin (lipopolysaccharide; LPS) concentrations in bacterial cells were determined using Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit.

## Results and Discussion

The obtained results of determination the LPS concentration in *D. desulfuricans* cells forming biofilm on samples of the tested titanium alloy are presented in FIG. 1 in the form of graphs, after subtracting the background value. The blank was sterile distilled water. The tests were performed in triplicate. The obtained results were averaged and recalculated, giving the results as the number of endotoxin units (eu) in a volume of 1 ml of biofilm suspension scraped from a single titanium alloy sample [eu/ml] ± average deviation. The ability of each of the three tested strains to grow, create a biofilm and produce endotoxins in all the tested culture conditions was found. Thus, the results confirm the natural presence of LPS in the cells of the *D.desulfuricans* bacteria. It was found that the differentiation of LPS production by three strains of *D. desulfuricans* bacteria creating biofilms on the alloy samples in solutions simulating body fluids was insignificant. Slight differences were only observed for the DV/B and DSM strains that formed biofilms in the presence of Tyrode's fluid. Besides, the DV/B strain showed the least stability in the production of endotoxins.

## Conclusion

The results showed no statistically significant differences in the concentrations of endotoxins produced by bacterial strains that formed biofilms on the surface of the Ti6Al4V samples in the presence of the tested culture fluids.

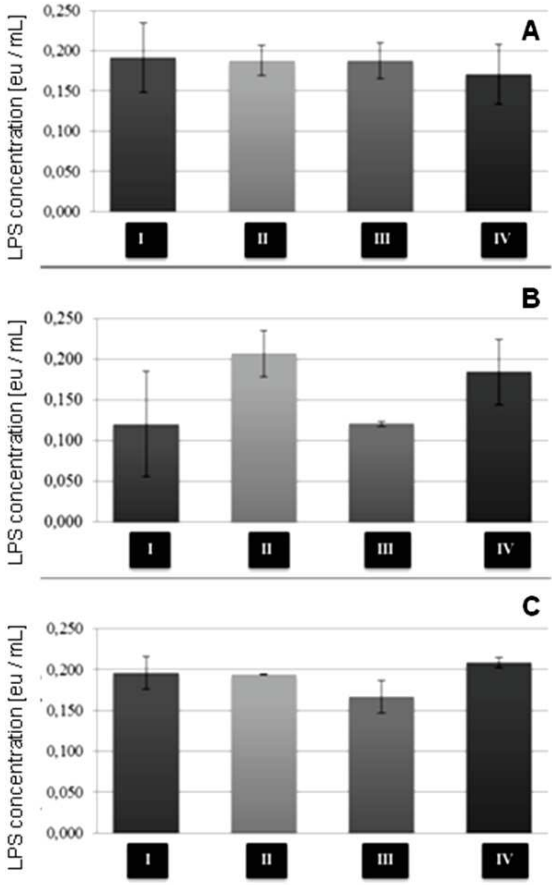


FIG. 1. Endotoxins (LPS) concentration in biofilms formed on the surface of the Ti6Al4V alloy samples by strains: A – DV/A, B – DV/B, C – DSM in various culture fluids: I – artificial saliva, II – artificial saliva simulating inflammation, III – Tyrode's fluid, IV - Ringer's solution.

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