20

Spectral analysis of epidermal staphylococcus with hemolytic activity isolated with mucosa oral cavity in patients with periodontitis

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Received May 11, 2023
Revised July 08, 2023
Accepted October 30, 2023.

The combination scattering spectroscopy method investigated strains of the epidermal staphylococcus with hemolytic activity and without hemolytic activity. The main spectral differences of the studied groups are established. Complex analysis was used to estimate hemolytic activity using combined scattering (SR) spectroscopy and mathematical approaches.

Keywords: raman spectroscopy, mathematical methods of analysis, spectral analysis, staphylococcus, hemolytic activity.

DOI: 10.61011/EOS.2023.11.58037.5076-23

Introduction

An urgent task of modern clinical dentistry is the identification and timely treatment of inflammatory diseases of the oral cavity that are caused by bacterial pathogens. One of the potential participants in the pathological process in diseases of the oral cavity may be coagulase-negative staphylococci with a certain pathogenicity factor, which over the last century have been the most significant opportunistic pathogens in medical practice. In the literature there is a large number of works devoted to staphylococci, including using spectral research methods [1]. However, the role of this microorganism in the pathology of the oral cavity (including periodontitis) remains poorly understood, despite the presence of a significant number of pathogenicity factors, one of which includes their hemolytic activity.

Optical methods have found widespread use in biomedical problems [1–3].

The analysis of the literature [1–3] showed that at present there is no work on the use of optical methods to study the hemolytic activity of bacteria.

The goal of the work was to study *S.epidermidis* strains with hemolytic activity using Raman spectroscopy.

Materials and research techniques

12 strains of *S.epidermidis* isolated from clinical material from patients with chronic periodontitis (from periodontal pockets) were studied. Identification of all isolated strains was carried out using the MALDI-ToF mass spectrometry method on a MicroflexLT device (Bruker) using a standard sample preparation method and mass spectral libraries. Hemolytic activity was additionally determined in all the isolates, with 50% of the strains having pronounced hemolytic activity, which was detected when hanged under aerobic conditions on 5% sheep blood agar (HiMedia). To set up the experiment, an inoculum of 1.0 McFarland in physiological solution was obtained from daily cultures of staphylococcal strains, which corresponded to $3 \cdot 10^8$ CFU/ml. Non-inoculated physiological solution was used as a negative control.

The samples under study are split into 2 groups: I — samples without hemolytic activity (28 Raman scattering — Raman), II — samples with hemolytic activity (27 spectra).

As the main method for analyzing *S.epidermidisc* strains for hemolytic activity, the Raman spectroscopy method was used, described in detail in the works [4,5]. The laser power of 350 MW with an exposure duration of 60 s ensured the stability of the measurements and the results obtained. Raman scattering spectra were recorded using an optical probe placed above the subject under study at a distance of 7 mm. The spectra were normalized using the Extended multiplicative signal correction (EMSC) method. The spectra were smoothed using the Maximum Like Lihood Estimation Savitzky-Golay filter (MLE-SG) [6] method with the $\sigma = 4$ parameter.

To exclude the contribution of autofluorescence in the Raman spectrum, a modified method of subtracting the fluorescent component by polynomial approximation Improved Modified Multi-Polynomial Fitting (I-ModPoly) with a polynomial degree of 11 was used. In this work, the analysis of Raman spectra was carried out in the $450-2000\,\mathrm{cm}^{-1}$ range.

Results

Figure 1 shows the averaged Raman spectra of two groups of samples. Minor spectral differences appear on the lines 1265 cm^{-1} (Amide III), 1338 cm^{-1} (one of the characteristic bands *P.gingivalis*), 1414 cm^{-1} (C55C stretching in quinoid ring), 1556 cm^{-1} (Amide II). The most pronounced spectral differences appear on the line 1647 cm^{-1} (Amide I vibration (collagen-like proteins)), which can be interpreted as the hemolytic activity of bacteria. This is most likely due to the fact that as a result of the vital activity of bacteria, fatty acids are released [7].

To increase the information content of the obtained Raman spectra and further analysis using linear discriminant analysis, a nonlinear regression analysis of the spectra was performed, consisting of their decomposition into the sum of asymmetric Gaussian lines. The composition of spectral lines was determined based on automatic multi-iteration modeling of 55 Raman spectra and tested based on the results of literature analysis. When stimulating the profile of the spectral lines used as a template, the position of x_0 and the half width at half-maximum (HWHM) dx of a line were fixed. When simulating, only the line intensity was fit in the range from 0 to the local maximum of the spectrum near x_0. The HWHM was limited in the range from 1 to 13 cm^{-1}. This allowed achievement of high stability of the results when simulating the profile and taking into consideration all shifts of RS lines. The criterion variable was the amplitude of lines a, which is dependent on the independent regressors dx and x_0 that determine initial conditions of the analysis. The average corrected coefficient of determination for the initial spectrum in the range in the area of 450–2000 cm^{-1} for all 55 spectra was $R^2 = 0.9997$.

Figure 2 shows the results of a comparison of LDA (linear discriminant analysis) of two groups of samples; the analysis was carried out as per LD-1. Positive LD-1 values (from 0 to 10) predominantly characterize the Raman spectra of bacterial samples with hemolytic activity, and negative values (from −10 to 0) characterize the Raman spectra of bacteria without hemolytic activity. Discriminant function LD-1 describes 100% dispersion.

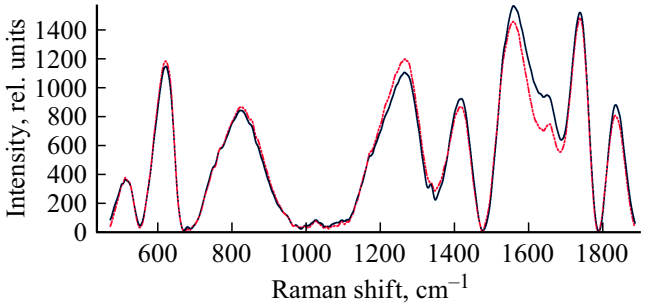

Figure 1. Averaged Raman spectra of the studied samples: broken line — spectra of samples without hemolytic activity (based on 28 Raman spectra), solid line — spectra of samples with hemolytic activity (based on 27 spectra).

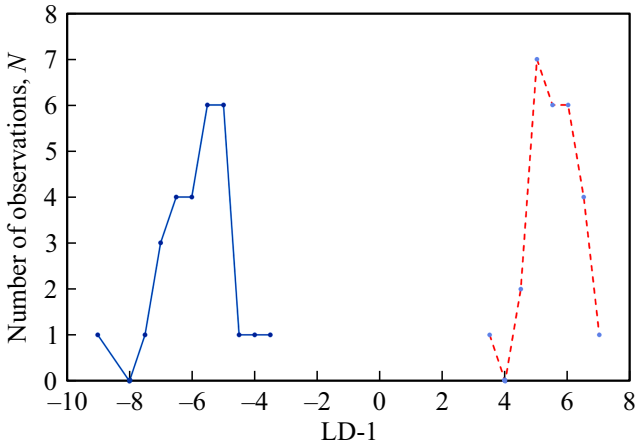

Figure 2. Graph of LD-1 — linear discriminant function values for bacterial samples with hemolytic activity (solid line) and without it (dashed line).

Conclusions

In this work, spectral differences between Staphylococcus epidermidis strains with hemolytic activity and without hemolytic activity have been established. Such studies were not carried out in previously published works by other authors. In the literature there were works related to the study of the spectral properties of different strains of bacteria without assessing their hemolytic activity (pathogenicity).

To assess the influence of hemolytic activity on changes in the Raman spectra, a nonlinear regression analysis of the Raman spectra was carried out, consisting of their expansion into the sum of asymmetric Gaussian lines, as well as discriminant analysis. As a result, a spectral difference was established between the two spectral groups, which is observed on the Raman line $\sim 1650\ cm^{-1}$ (Amide I vibration (collagen-like proteins)).

The results obtained can be further used as a rapid assessment of markers of the pathogenicity of staphylococcus and other opportunistic microorganisms and to identify their potential participation in the development of diseases of the mucous membranes of the oral cavity.

Ethics committee

The studies were performed according to Declaration of Helsinki, the protocol was approved by the Ethics Committee (extract from the protocol № 207 from a session of the Bioethics Committee of the Samara State Medical University dated 09.12.2020).

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by E.Potapova