On the possibility of using the BactoSCREEN time-of-flight mass spectrometer to determine the resistance of microorganisms in agriculture

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Abstract. This article discusses the possibilities of using the BactoSCREEN mass spectrometer to determine the resistance of microorganisms in phytopathology. Examples and possibilities of using this mass spectrometer are considered. The scientific novelty of the work lies in considering the possibility of using the BactoSCREEN time-of-flight mass spectrometer in resistanceology, identifying the main features of this device - the speed of operation and the possibility of updating the database, as well as economic efficiency. As a result, this device is seen as a good alternative to traditional research methods.

1. Introduction

Currently, mass spectrography is used in various fields of science - from clinical medicine to forensic science. It is time to consider the use of time-of-flight mass spectrometers in phytopathology and resistanceology, especially for such an important science as agriculture. On the basis of a time-of-flight mass spectrometer (LaserToF LT2 Plus, manufactured by Scientific Analysis Instruments, Great Britain) NPF Litekh, the first domestic system for mass spectrometric identification of microorganisms and study of their sensitivity to antibiotics, the BactoSCREEN microbiological analyzer, was created. The identification system is based on an algorithm that allows you to quickly and accurately identify microorganisms by their unique protein composition. Identification in BactoSCREEN is based on ribosomal proteins, which are unique to any microorganism. Bioinformation software allows for reliable and accurate species identification of microorganisms by comparing the obtained mass spectra of bacteria with the BactoSCREEN database, which contains information on more than 2300 types of microorganisms of all types: bacteria, mycobacteria, filamentous and yeast fungi. The foregoing allows us to understand that the use of this device for the needs of resistanceology will be one of the best applications.

2. Features of the device of the BactoSCREEN time-of-flight mass spectrometer

The BactoSCREEN system uses an additional mechanism for confirming identification by protein mass spectra using genomic sequence data available in such world public repositories as GenBank, PATRIC. In this case, the mass spectra obtained during the operation of the device are no longer compared with the spectra from the BactoSCREEN database, but with the protein spectra, which are reconstructed using a special algorithm based on the genomic sequence of the identified microorganism available in the repository. BactoSCREEN software allows for cluster and correlation
analysis, which makes it possible to differentiate closely related organisms in more detail. BactoSCREEN software has dedicated sections for performing antibiotic susceptibility studies on bacterial cell death. In addition, there is the possibility of identifying resistant strains based on the technology of detecting specific products of biodegradation of antibiotics (detecting beta-lactamase activity).

2.1. Features of the methods used on the BactoSCREEN mass spectrometer and analogues.

The resistance technique combines MALDI-TOF microbial identification on the BactoSCREEN instrument with an assay that includes micro-droplet growth directly on the target (DOT-MGA), which was developed by German scientists. Small drops of the bacterial suspension with the antibiotic under investigation are placed on a MALDI matrix, incubated for the required time, then the drops are removed using an absorbent liquid, while the bacterial cells remain on the target. This eliminates the steps of removing cells from their culture and transferring them to the target, which reduces the time required for analysis. The target cells are then analyzed using a standard approach to identify microorganisms. A positive result means that the bacteria have increased their number in the composition of the antibiotic, negative - that they are susceptible to this antibiotic. The result can be obtained on the same day (after 4-5 hours instead of 10-20 hours), and this allows researchers to quickly switch to targeted antibiotics. MALDI targets usually contain 96 points, each of which can accommodate a drop of suspension, multiplex analysis is possible. The BactoSCREEN platform identifies microorganisms by comparing their protein profiles with those contained in its own database. Compared to traditional biochemical methods, this platform offers advantages in speed, cost and accuracy. MALDI BactoSCREEN is already becoming a common platform in clinical microbiology laboratories. Approaches using MALDI-TOF are ready to compete with DNA analysis. The search for a specific resistance gene is, in fact, the identification of one specific resistance mechanism. Many MALDI-based approaches also target specific mechanisms, such as the detection of the pKpQIL_p019 protein, which is often present on the same plasmid as the bkaKPC gene encoding the carbapenemase enzyme. However, BactoSCREEN also offers analyzes with more comprehensive coverage. CE-IVD whales have already been developed for this and similar platforms, which can detect hydrolysis products produced by bacteria when they destroy carbapenem, that is, determine antibiotic resistance, regardless of its mechanisms. Bruker also offers a similar kit for the determination of cephalosporin resistance. Based on the foregoing, the proposed analysis on a mass spectrometer makes it possible to carry out practically the same traditional universal tests for antibiotic susceptibility, but at a new level and at a higher speed. It is noted that susceptibility tests are usually carried out with a large number (up to 18) antibiotics, since there are not many antibiotics that may be of interest to certain bacteria. This means that the new technology will test susceptibility to a full range of antibiotics for two to three bacteria in a single pass. Researchers from the German city of Münster have developed and used a similar approach to identify resistant strains of Pseudomonas. In phytopathology, this type of bacteria has about 50 patovars-strains and is a strong phytopathogen that causes significant damage to agriculture and forestry. The work of these scientists shows that the method is suitable for testing many isolates. Bacterial suspensions were also prepared, antibiotic solution was added, placed on MALDI targets, incubated with control for three to four hours, and then analyzed on a MALDI mass spectrometer. The analysis made it possible to identify microorganisms resistant to this antibiotic with a sensitivity of 92% and a specificity of 100%. Researchers are currently working to improve the method and standardize the procedure and supplies.

2.2 Mass spectrometric assessment of antibiotic resistance

Approaches to assessing antibiotic resistance on mass spectrometers, in particular BactoSCREEN, can be roughly divided into 2 categories: 1) determining the presence of resistance to specific drugs without disclosing the mechanism of resistance, 2) determining resistance and identifying its mechanisms. The main approach of the 1st group is to determine the viability of microorganisms in the presence of an antibacterial drug. Within the framework of this approach, there are methods based
on the identification of constantly present specific differences in the mass spectra of resistant strains, which differ markedly from the spectra of sensitive microorganisms. Resistant microorganisms continue to function in the presence of the drug, consuming labeled nutrients from the nutrient medium (for example, deuterated amino acids, which have a specific signal in mass spectrometry). Antibiotic-sensitive microorganisms are inhibited and do not accumulate labels. The difference in their mass spectra allows us to draw a conclusion about the resistance. This methodological approach was named "sIlac-technology" (marking of stable isotopes with amino acids in cell culture). The effectiveness of sIlac has been demonstrated in experiments with S. aureus strains. They were incubated in a medium with standard and deuterated lysine with and without oxacillin. As a result, "massive" spectra (containing peaks with "massive" isotopes) characteristic of resistant strains and general spectra of susceptible strains were identified. Similar results were obtained with bacteria of the Pseudomonas family. Bacterial cells were cultured in media with standard deuterated lysine, adding various antibiotics (meropenem, ciprofloxacin). Mass spectrometry showed confirmed differences between resistant and susceptible strains. Within the framework of the first approach, there are methods based on the identification of constantly present specific differences in the mass spectra of resistant strains, which differ markedly from the spectra of sensitive microorganisms. In cluster analysis, the division of the spectra into groups clearly correlates with the presence of resistance. Clustering is usually performed based on the presence of peaks characteristic of antibiotic-resistant strains. It is logical to call such peaks "resistance peaks".

Resistance peaks are just one of the variants of markers for the spectra of resistant microorganisms. Due to the action of damaging factors in the bacterial cell, metabolism changes rapidly, which manifests itself in the form of a "stress reaction". The stress response caused by antibiotics triggers the rearrangement of many important structures of the microbial cell, from DNA repair enzymes to surface proteins. It has been proven that the stress response to antibiotics can manifest itself in different ways in sensitive and resistant bacteria. From the above, it can be concluded that the protein profile in such cases changes unevenly. This provision is the main theoretical basis for the development of methods for determining microbial resistance using a mass spectrometric study of the stress response on a BactoScreen time-of-flight mass spectrometer. Bruker mbT asTra technology is a practical implementation of antibiotic-induced stress response assessment on a BactoSCREEN TOF mass spectrometer. It involves assessing antibiotic susceptibility by quantitatively monitoring cell growth in the presence and absence of an antibiotic. Already after the first hour of incubation, MALDI revealed statistically significant differences between the mass spectra of Klebsiella (K. pneumoniae and K. oxytoca) growing in media with and without an antibiotic. It is important that the majority of the studied strains showed characteristic changes in mass spectra when using different concentrations of antibiotics. This gives hope for the development of quantitative criteria for evaluating stability using the BactoSCREEN mass spectrometer. Let us now consider another approach to studying the resistance of microorganisms using time-of-flight mass spectrometers. First, you should remember about its mechanisms. There are five traditional ways of the emergence of resistance: 1) violation of the permeability of the external structures of the microbial cell, 2) active removal of the antibiotic from the microbial cell (outflow), 3) modification of the target, 4) inactivation of the antibiotic, 5) formation of a metabolic "shunt". Mass spectrometry allows direct identification of the components of microorganisms responsible for the loss of sensitivity to antibiotics and, therefore, for the development of resistance. In other words, this technology can be used in such a way as to aid in the identification of specific proteins. Various examples confirm the possibility of this approach.

Experiments have been carried out in which it was proved that using a combination of two-dimensional electrophoresis and MALDI-TOF it is possible to analyze structures responsible for the transport of antibiotics across the cell membranes of gram-negative bacteria, such as porins and scavenging systems. Proteins responsible for resistance to tetracycline and ampicillin were determined using two-dimensional electrophoresis and MALDI-TOF. Later, peaks were identified for this, which are indicators of OmpK36-porin, which is responsible for the penetration of carbapenems into the bacterial cell. In this case, the absence or change in the mass spectrum of the peak of this protein...
serves as a marker of resistance, since resistance is acquired by modifying the porin structure. One of the mechanisms of resistance to agents affecting protein synthesis (for example, to aminoglycosides) is the modification of ribosome subunits by methylation of ribosomal RNA (rRNA). The principal possibility of detecting the methylation phenomenon using mass spectrometry was shown in the work of S. Douthwaite. A very laborious technique included several stages. First, from the biomass of the tested strain, an extract was obtained containing the material basis for the target modification - methyltransferase, which ferments the rRNA methylation process. In parallel with this, RNA transcripts were synthesized on an artificial DNA template, which are parts of rRNA in which methylation is usually carried out. At the second stage, the extract with methyltransferase and the resulting RNA transcripts were incubated to methylate the latter. Then the RNA treated with methyltransferase was fermented with RNases, the resulting oligonucleotides were purified and their mass spectromograms were obtained by the MALDI-TOF method using a matrix based on 3-hydroxypicolinic acid. The presence of modifications was checked by the appearance of characteristic peaks, indicating the presence of methylated nucleotides at the points that are standard for the appearance of resistance. For example, nucleotides located at positions 2056-2063 in rRNA are a standard target for erm-methyltransferases. Methylation of this region gives a diagnostic peak in the mass spectrum of oligonucleotides, which ultimately can serve as a criterion for the presence of resistance to erythromycin by modifying the target. Unfortunately, the complexity and high cost of this technique does not allow using it for solving problems of everyday microbiological and agrotechnical practice. This method only proves the fundamental ability of BastoSCREEN MALDI-TOF to detect modifications of the target matrix and has the potential for methodological simplification. In strains resistant to the action of polymyxins, including colistin, there is a specific modification of the main target of this class of antibiotics - the lipopolysaccharide of the outer membrane. Therefore, the detection of modified lipopolysaccharide is indicative of polymyxin resistance. Scientists have described in detail the methodology for searching for modifications in the structures of lipopolysaccharides. For the mass spectrometric study, an important lipopolysaccharide component, lipid A, was isolated from test bacteria (A. baumannii). It was purified, enclosed in a special matrix and analyzed with BactoSCREEN MALDI-TOF. Lipid A isolated from colistin-resistant strains showed characteristic changes in the mass spectrum, indicating altered regions of phosphoethanolamine and galactosamine. The modifications have been validated and further characterized using other mass spectrometric analysis options. The method turned out to be reliable and sensitive: in most studies, there was a coincidence of the results of MS analysis and the data obtained by molecular genetic (sequencing) and phenotypic methods. The use of this approach is possible when working directly with a biological sample. For example, after the detection of a microorganism in a plant part and primitive sample preparation, including the addition of an antibiotic, mass spectrometric registration of the drug or its hydrolysis products is carried out using a BactoSCREEN MALDI-TOF device. The introduction of the method of mass spectrometric assessment of enzyme-dependent destruction of antibiotics into practical microbiology was carried out by Bruker, which launched a module for determining the activity of b-lactamase mbT sT ar-bl, compatible with BactoSCREEN MALDI-TOF. The main advantage of the module is the presence of the mbT compass sT ar-bl module software package, which allows to automatically analyze the obtained mass spectra and draw conclusions about the lactamase activity of the investigated phytopathogen or other pathogen. Mass spectrometric technologies for assessing bacterial resistance to antibiotics are highly effective, but have significant drawbacks. The first is methodological complexity, which makes it impossible to replicate some methods in conventional laboratories. The second and main drawback is the lack of standardized and validated criteria for assessing resistance, similar to the control points of the European Committee for Antibiotic Susceptibility Testing (eucasT) and the US Clinical and Laboratory Standards Institute (CLSI).
3. Conclusion.

Analysis of scientific information on the application of mass-spectrometric studies on BactoSCREEN MALDI-TOF devices allows us to draw several important conclusions. The rapid development of mass-spectrometric technologies and their introduction into microbiology led to dramatic changes in microbiological diagnostics, which radically change the principles of work of microbiology laboratories. The introduction of mass spectrometry is global in nature - in Europe alone, more than a thousand MALDI-TOF systems are specialized in microbiological diagnostics. The main achievement of mass spectrometry technologies is the fast and reliable identification of most species, which is produced not only from pure cultures, but also from microbial biological material. The prospects of the BactoSCREEN MALDI-TOF system in agriculture are associated with the development and implementation into practice of methods for intraspecific determination of clinically and epidemiologically significant strains and assessment of their properties, including virulence, pathogenicity and resistance. The main problems of microbiological mass spectrometry are associated with the difficulties in identifying microorganisms in mixed cultures (including biological material) and with the lack of standard criteria for assessing microbial resistance. Rapid progress in the development of applied mass spectrometry technologies gives hope that these problems will be solved within the next few years.

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