UDC 579.2

O. Ilchenko

MARKER LIPIDS IDENTIFICATION FOR THE DETECTION OF RESISTANT AND ANTIBIOTICSENSITIVE *STREPTOCOCCUS PNEUMONIAE*

Department of Microbiology, Virology and Biotechnology, Odessa I. I. Mechnikov National University, Dvoryanska str. 2, 65082 Odessa, Ukraine

E-mail: aleks.delta2013@gmail.com

Streptococcus pneumoniae is an important pathogen that causes respiratory-tract infections, sepsis, meningitis and pneumonia. *S. pneumonia* commonly causes diseases in the youngest and oldest sections of the population and patients with immunodeficienciesin both more and less developed countries [1].

Autolysis is one of the virulence factors that is induced by specific enzyme LytA during stationary phase of growth. Autolysis occurs due to drug treatment or nutritional starvation and not shown in logarithmic phase. Autolysis facilitates spread of toxins in infected organism [2].

The aim of this study was to find differences in lipid profiles of drug resistant and non-resistant *Streptococcus pneumoniae* and a possible target for a new antibacterial compound 2A.

The experimental part of the work performed in Chemistry Department of Umeå University in Sweden in Swedish Metabolomics Center. Two strains of *S. pneumoniae* were used in this study: wild type (WT) T4 strain and strain 852. T4 strain is sensitive to the 2A and lyse in presence of 2A Strain 852, to the contrary, is resistant to 2A compound and does not lyse. Compound 2A is suggested as a new antibacterial agent that triggers the autolysis of pathogen Streptococcus pneumoniae. We hypothesized that the target metabolites will be present in the sensitive wild type but absent in the resistant mutant. To directly identify extracted lipids we used ultra-high performance liquid chromatography coupled to triple quadrupole time-of-flight tandem mass spectrometry (UHPLC–Qq-TOF-MS/MS).

The analysis of chromatograms (raw data – Fig. 2) was done in Profinder, Agilent. Two approaches were used: targeted and untargeted data analysis. Targeted analysis is a mode of operation when specific library is used to find certain groups of compounds based mainly on their known mass, retention time and adducts. This mode provides identification and quantification of compounds. The shortcoming of this method is that only compounds that exsist in the library can be found. Contrary, the untargeted type of analysis quantifies basically all metabolites in the sample but the identification of the compound is not provided. Therefore untargeted analysis is followed by identification of the compounds through available databases. The aim of the data analysis was to find the differences in metabolite levels for 4 experimental conditions.

Both targeted and untargeted analyses show the difference between mutant and wild type and the difference between antibiotic and solvent (Fig. 1).

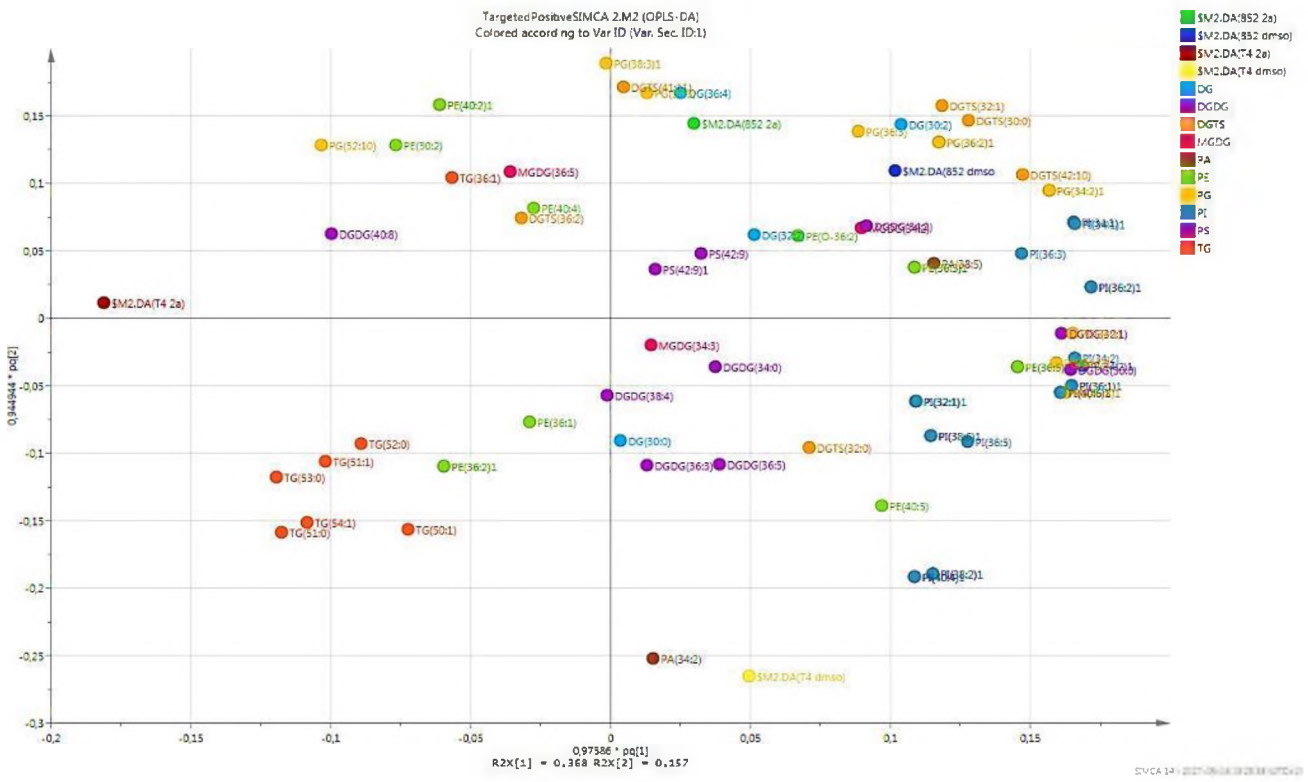

Fig. 1. OPLS-DA Loading plot for Targeted Lipidomics.

Triradylglycerols were increased in wild type. Diacylglyceryl-trimethylhomoserines were increased in mutant strain. Phosphatidylinositols were increased in absence of 2A in both strains. Table 1 shows differences between of presence and absence of significant metabolites in experimental conditions. Importantly, only a small group of metabolites was substantially increased in T4 strain grown in presence of 2A but not in control. These compounds are suggested as potential target for 2a binding.

Table 1

The presence of major groups of metabolites in *S. pneumoniae T4* and *S. pneumoniae 852* in the presence and absence of an antibiotic compound

Metabolites	***S. Pneumoniae 852* in presence of 2A**	***S. Pneumoniae T4***	***S. Pneumoniae T4* in presence of 2A**	***S. Pneumoniae T4***
Diacylglyceryl-trimethylhomoserine	+	+	-	-
Glycerophosphates	-	-	-	+
Triradylglycerols	-	-	+	+
Glycosyldiradylglycerols	+	-	+	-
Glycerophosphoethanolamines	+	+	+	+
Phosphatidylinositol	-	+	-	+
Monogalactosyldiacylglycerol	+	+	+	+

References

1. Prevention of Pneumococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 1997;46(RR-8):1-25.

2. *Peter Mellroth, Tatyana Sandalova, Alexey Kikhney, Francisco Vilaplana,* etc. Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of Streptococcus pneumoniae. mBio. 2014 Jan-Feb; 5(1): e01120-13.