The spread of antibiotic-resistant pathogens is becoming an extremely serious clinical and public health problem worldwide. Currently, there is a worrying increase in the resistance to extended-spectrum cephalosporins which are commonly used for the treatment of infections caused by *Enterobacteriacea* mainly from the genera *Escherichia, Klebsiella, Enterobacter, Proteus* and *Salmonella*. Strains producing extended spectrum beta-lactamases (ESBLs) most often belong to multi-resistant strains characterized by a fast spread rate in the environment, causing e.g serious hospital epidemics. Currently, research is underway to clarify the mechanisms and ways of transferring bacterial resistance to the new generation of beta-lactam antibiotics. The main way of dissemination of ESBLs is horizontal gene transfer (HGT). The main factors of HGT are plasmids, transposable and conjugative elements, gene tranfer agents and bacteriophages. Conjugation mechanisms of HGT are known and have long been studied. However, the role of wide-host range bacteriophages (such as P1) that can lysogenize cells in the form of plasmids have been overlooked and nearly entirely omitted in the most studies. Also, recent reports suggest that the involvement of P1-like phages in the spread of resistance to extended spectrum beta-lactam-antibiotics is significant. Many studies show that plasmids derived from P1 bacteriophage are found in bacteria such as *Salmonella enterica, Escherichia coli, Klebsiella pneumoniae* that produce extended spectrum beta-lactamase (ESBL). No full conjugation system-encoding genes were identified in plasmids that have the P1 replicon and partition system, which indicates that these plasmids could have entered the bacterial cell through phage infection. Therefore, it is important to know the adaptation mechanisms of phages such as P1 to infection of many different hosts, which significantly contributes to the transfer of antibiotic resistance genes. The high incidence of P1 phage and its relatives carrying antibiotic resistance genes in e.g. *E. coli, Klebsiella pneumoniae* or *Salmonella* isolates indicates that the role of these phages in transmitting antibiotic resistance has been underestimated.

Bacteriophage P1 selected by us for the study was the first transducing bacteriophage discovered. For decades, it has been used as a model in the study of basic genetic processes and as a tool in biotechnology. However, only recent metagenomic analysis of human and animal microbiota revealed the significant role of phages and plasmids derived from P1 in HGT, especially in the transfer of ESBL and MBL genes. Despite the widespread use of bacteriophage P1, a number of aspects of its biology remains unknown. Our preliminary results show that the complexity of the P1 lysis system may significantly contribute to the adaptation of P1 to lyse cells of wide range of hosts, so that the progeny phages could be released. In the canonical model of lysis, this process requires three proteins: endolysin - an enzyme that digest cell wall, holin - a protein that creates "holes" in the cytoplasmic membrane that allows the endolysin to penetrate the membrane and to reach its substrate (cell wall), and antiholin - lysis time regulating protein. It turns out that the effective lysis of P1 infected cells in a programmed time, in addition to previously identified genes encoding endolysin, holin, and antiholin, depends on at least two additional genes for putative holins and an additional gene that potentially could encode antiholin. Our preliminary results show that the complexity of P1 lysis system may significantly contribute to the adaptation P1 and related phages to a wide range of hosts, making it an important factor in HGT. Therefore, a thorough understanding of the functions of individual P1 lytic genes, their functions and relations between them and their role in lysis of cells of different P1 hosts is of fundamental importance for expanding knowledge on efficient HGT mechanisms involving temperate bacteriophages of wide host-range. This is the aim of the proposed project. In addition to P1 mutants in lytic genes that have been constructed new ones will be obtained. The lysis efficiency of various mutants will be tested in cells of various P1 hosts known to carry ESBL and MBL genes. The functions of individual lytic proteins will also be determined in isolated systems upon introduction to cells plasmids containing their cloned genes.

The obtained results will bring us closer to understanding the mechanisms of the wide spread of clinical strains of bacteria carrying P1 derivatives with genes encoding resistance to new generation of beta-lactam antibiotics.