



Delivering functional metagenomic libraries into pathogenic bacteria by genetically reprogrammed bacteriophage particles

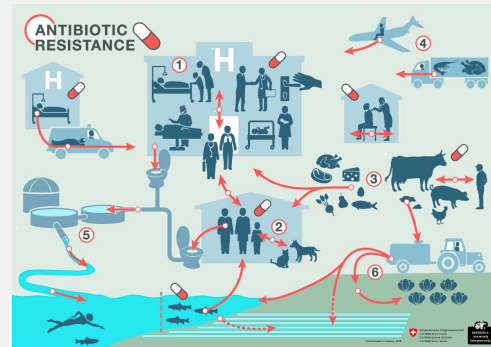
Erika Nagrand^{1*} Mónika Számel^{1,2} Ildikó Ilona Lantos¹ Dorina Kovács¹ Chryso Christodoulou¹ Viktória Seregi^{1,3} Gábor Apjok^{1,2} Tamás Stirling¹

András Asbóth¹ Bálint Márk Várhelyi¹ Tóbiás Sári^{1,2} Bálint Eszenyi¹ Orsolya Méhi¹ Mihály Koncz¹ Fanni Vidovics¹ Petra Szili^{1,4} Pramod K. Jangir^{1,2,5} Róbert Herczeg⁶ Bence Gálik^{6,7} Urbán Péter⁶ Attila Gyenesei^{6,7} Gábor Draskovits¹ Ákos Nyerges¹ Gergely Fekete¹ László Bodai⁸ Nóra Zsindely⁸ Béla Dénes⁹ Ido Yosef¹⁰ Udi Qimron¹⁰ Balázs Papp^{1,11} Csaba Pál¹ Bálint Kintses^{1,3,8}

¹ National Laboratory of Biotechnology, Synthetic and System Biology Unit, Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network (ELKH), 6726 Szeged, Hungary. ² Doctoral School of Biology, University of Szeged, 6726 Szeged, Hungary. ³ HCEMM-BRC Translational Microbiology Research Group, Szeged, Hungary. ⁴ Doctoral School of Multidisciplinary Medical Sciences, 6726 Szeged, Hungary. ⁵ Current address: Department of Zoology, University of Oxford, Oxford, OX1 3PS, UK. ⁶ Szentagotai Research Centre, Genomics and Bioinformatics Core Facility, University of Pécs, Ifjúság útja 20, Pécs, H-7624, Hungary. ⁷ Department of Clinical Molecular Biology, Medical University of Białystok, ul. Jana Kilińskiego 1, 15-089 Białystok, Poland. ⁸ Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, 6726, Szeged, Hungary. ⁹ Veterinary Diagnostic Directorate, National Food Chain Safety Office, 1143, Budapest, Hungary. ¹⁰ Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel. ¹¹ HCEMM-BRC Metabolic Systems Biology Lab, Szeged, Hungary

Introduction

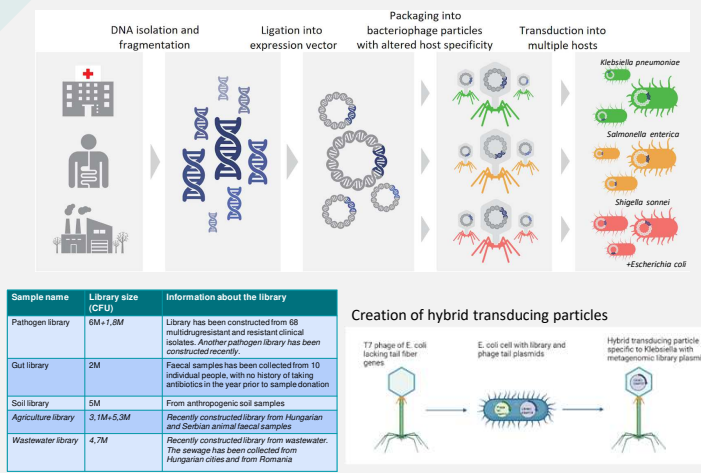
Functional metagenomics is a key tool to identify antibiotic resistance genes (ARGs) in the environment and the routes where these ARGs transfer. However, functional metagenomic studies usually use a single expression host, most commonly a laboratory strain of *Escherichia coli*. This limitation results in a lower coverage of resistomes as certain genes cannot be expressed in this strain. To find a solution this problem, our aim was to extend the host range of functional metagenomics by developing a new method called DEEPMINE. This method utilizes genetically reprogrammed bacteriophage particles, with altered host specificity that is achieved by exchanging the phage tails between different bacteriophages. In our study these particles were used to introduce metagenomic libraries into two pathogenic hosts beside *E. coli* and *Klebsiella pneumoniae*. After introducing the metagenomic libraries into different hosts, we screened against recently developed and old antibiotics. The results identified that performing functional metagenomics in more hosts is more relevant to predict which ARGs will be dominant in the environment and in the clinic. Therefore, our next aim is to construct more libraries from different environments to cover most of the antibiotic resistance hotspots. Furthermore, this method also gives an opportunity to detect antibiotic resistance genes against recently developed antibiotics.



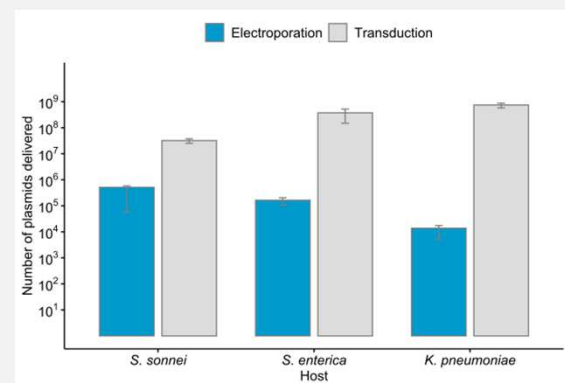
Areas which are the most exposed to antibiotics can be antibiotic resistance hotspots in the environment. These environments are the best source for functional metagenomic library construction.

<https://www.bmc.com/doi/10.1186/s13059-018-1500-0>

Methodology of DEEPMINE



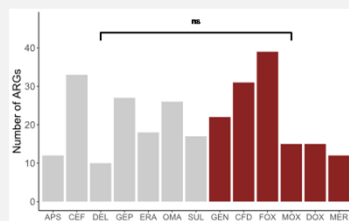
Transduction is significantly more efficient for delivering functional metagenomic libraries into different hosts



Distribution of detected ARGs in different host species, some genes cannot be detected with a single host



Detecting antibiotic resistance genes against recently developed and old antibiotics



No significant difference in number of ARGs detected against new and old antibiotics.

Conclusions and Future plans

- DEEPMINE gives a new possibility to deliver big metagenomic libraries into different host species
- Transduction is significantly better than electroporation for delivering libraries to different hosts
- Some ARGs in the environment cannot be detected by using only one host
- With this method we could identify ARGs from the environment and the routes where these ARGs transfer
- Screening these libraries against recently developed and old antibiotics revealed there is no significant difference between these antibiotics in the number of detected ARGs.
- Recently we created new metagenomic libraries: Agriculture, Wastewater and a new pathogen library. With these libraries we cover most of the antibiotic resistance hotspots in the environment.

Future plans:

- Transduce these libraries into different host species
- Screen these libraries against new antibiotics, besides beta-lactamase inhibitor and beta-lactam antibiotic combinations



<http://group.szbk.u-szeged.hu/sysbiol/kintses-balint-lab-index.html>
nagrand.erika@brc.hu

