Tytuł pracy: "Genetyczne różnicowanie wybranych szczepów *Salmonella enterica* subsp. *enterica*"

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Abstract

Molecular based techniques have a strong impact on improving diagnostic and epidemiology of bacterial infections. In an attempt to choose the best method we need to know its ability to differentiate bacteria and what kind of differentiation we want to obtain. Therefore, we will use one method for rapid bacteria identification and prioritizing further research and another one for monitoring sources and spread of infection.

The aim of my PhD research was to develop a simple, molecular-based technique that will be able to distinguish serovars of *Salmonella enterica* subsp. *enterica*. Approaches presented in this study depend on the occurrence of two kinds of repeated sequences in genomes of bacteria - trinucleotide repeat sequences (TRSs) and variable number tandem repeats (VNTR). It was also the objective to assess the stability of genome in areas where VNTR occur as we recognized the lack of this sort of experiments in works describing the usage of VNTR.

The collection used in these studies consists of *Salmonella enterica* subsp. *enterica* serovars (n=169) that are the most prevalent serovars isolated from humans in Poland: *S.* Enteritidis (n=40), *S.* Typhimurium (n=38), *S.* Infantis (n=25), *S.* Virchow (n=28), *S.* Hadar (n=22), *S.* Newport (n=8) and *S.* Anatum (n=8). I performed TRS-based PCRs separately for each of the 20 primers designed and 3 rep-PCRs with frequently used primers BOX, REP, ERIC. Band patterns obtained with these methods were compared based on presence, layout and intensity of each fingerprint element. Furthermore, very important part of current research was an evaluation of reproducibility of rep-PCR based methods. In the end, CAC- and GTG-PCR were the most effective methods that were able to differentiate *Salmonella enterica* subsp. *enterica* strains in our collection.

Another method developed in our laboratory, named Sal-175 analysis, distinguishes strains of *Salmonella enterica* subsp. *enterica* based on a region with VNTR. *In silico* analysis showed two sequences within this region that are repeated and number of repeats differs in *Salmonella* serovars. By comparing the length of one or two bands in given strains, it is possible to differentiate the whole collection. Due to potential instability in regions containing VNTR it was also desirable to assess if Sal-175 region will change during several cycles of bacterial recultivation. I have compared it also with stabilities of other VNTR which are widely used in epidemiological investigation of *S.* Typhimurium (STTR) and *S.* Enteritidis (SENTR) strains. Results show the number of cultures which should not be crossed to keep the validity of VNTR-based methods.

Herein, I present that GTG-PCR, CAC-PCR and VNTR analysis of Sal-175 can be used for rapid and easy single-tube DNA-based assays for the discrimination of commonly isolated serovars of *Salmonella enterica* subsp. *enterica*. The determination of TRS fingerprints for unknown *Salmonella* strains could serve as a useful predictor for their serovar affinity. Although conventional serotyping should still be performed, a rapid screen with TRS-based PCR may greatly reduce the number of antisera used for determination of *Salmonella* serovars and may help prioritize further investigation of *Salmonella* strains. Furthermore, Sal-175 region described in this work can be included in MLVA used for epidemiological investigations.