Title: A mirnomic-wide analysis reveals a close relation between small non-coding RNAs (sncRNAs) and virulence-associated regions in *Yersinia pestis* and *Yersinia pseudotuberculosis* genomes

Authors: Souza, R.A.[1], Cerdeira, L.T. [2,3], Mitne-Neto, M.[2], Almeida, A.M.P.[4], Falcão, J.P.[1]

Institutions: [1] Brazilian Reference Center on *Yersinia* spp. other than *Yersinia pestis*, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo (Av. do Café, s/n, Ribeirão Preto, SP, Brazil), [2] Research and Development Department, Fleury Group (Av. General Valdomiro de Lima, 508, São Paulo, SP, Brazil), [3] Centro de Facilidades de Apoio à Pesquisa – Universidade de São Paulo (Av. Prof. Lineu Prestes, 1730, São Paulo, SP, Brazil), [4] Brazilian Reference Service on Plague, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz (Av. Professor Moraes Rego, s/n, Recife, PE, Brazil)

Abstract: Although *Yersinia pestis* and *Yersinia pseudotuberculosis* are genetically very similar, they exhibit very different patterns of pathogenicity. The drastic difference in pathogenicity may result from the acquisition of a few species-specific genes, but also from differences in various types of RNA that are not translated into protein but are involved in cell regulatory functions. The aim of this work was to identify small non-coding RNAs (sncRNAs) encoded by the chromosome and plasmids of *Y. pestis* P.EXU 171-2 and *Y. pseudotuberculosis* IP 32954 using a deep sequencing approach to perform a mirnomic-wide analysis. The strains were culture in parallel at 28ºC in brain heart infusion (BHI) broth supplemented with 2.5 mM of $CaCl_2$ to an OD_{620nm} of 0.65 (mid-exponential growth phase). RNA enriched for sncRNAs was extracted using the *mir*Vana™ miRNA Isolation kit. The quality of the isolated RNA was assessed using 15% denaturing polyacrylamide gel and its concentration was determined in Qubit® 2.0 Fluorometer. cDNA libraries were generated using the Ion total RNA-seq V2 and the Ion Xpress™ RNA-seq Barcode 1-16 kits. cDNAs templates were prepared with the Ion PGM™ Template OT2 400 kit. The samples were sequenced simultaneously in Ion Torrent Personal Genome Machine™ platform using the Ion PGM™ 400 Sequencing kit with the Ion 318™ Chip V2. By the end of the run, the generated reads were automatically processed for 3' and 5' adapter trimming and FASTQ files generation. The reads that passed the purity filtering and had a unique alignment were mapped to the *Y. pestis* CO92 chromosome (NC_003143) and plasmids (pCD1, NC_003131; pPCP1, NC_003132; pMT1, NC_003134) or *Y. pseudotuberculosis* IP32953 chromosome (NC_006155) and plasmids (pYV, NC_006153; pYptb, NC_006154) with the aid of the CLC Genomics Workbench 7.0 software. We found that the most sncRNAs of *Y. pestis* P.EXU 171-2 and *Y. pseudotuberculosis* IP 32954 are encoded by their chromosome. Additionally, our results showed that some of these sncRNAs are intertwined with virulence-associated regions in the *Y. pestis* CO92 and *Y. pseudotuberculosis* IP32953 chromosomes and are differently expressed between *Y. pestis* P.EXU 171-2 and *Y. pseudotuberculosis* IP 32954 strains. These results suggest evolutionary changes in post-transcriptional regulation of virulence in *Y. pestis* and *Y. pseudotuberculosis* that may be related to the drastic difference in pathogenicity of these two *Yersinia* species.

Keywords: *Y. pestis*, *Y. pseudotuberculosis*, deep sequencing approach, small non-coding RNAs (sncRNAs), mirnomic-wide analysis

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