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Small Regulatory RNAs as Key Players in *Yersinia* Adaptation: A Comparative Analysis of *Y. enterocolitica* and *Y. ruckeri* in Pathogenesis and Ecology

Microorganisms, including bacteria, face challenges adapting to changing environmental conditions. Bacteria use various mechanisms, including small regulatory RNAs (sRNAs), to quickly adjust their gene expression, when needed. This is a faster and less energy-intensive process than synthesizing regulatory proteins. Pathogenic bacteria, transitioning from the external environment to the host organism, must navigate changes in temperature, osmolarity, pH, nutrient availability, and interactions with host immune cells. Survival in this environment depends on quickly modulating gene expression to synthesize set of virulence and adaptation factors.

The close interaction between humans and animals presents a significant global public health challenge due to the risk of zoonotic disease transmission. Among the most prevalent zoonoses in the European Union are campylobacteriosis, salmonellosis, and yersiniosis, with *Yersinia enterocolitica* identified as the causative agent of yersiniosis. This disease predominantly affects vulnerable groups, such as children, the elderly, and immunocompromised individuals, causing symptoms like fever, diarrhea, and, in severe cases, extraintestinal complications. Transmission primarily occurs through contaminated food, especially pork, which serves as a major reservoir. *Y. enterocolitica* encompasses six biotypes, each exhibiting distinct levels of virulence. In contrast, *Yersinia ruckeri* is a significant pathogen in aquaculture, causing enteric redmouth disease (ERM) in salmonids such as trout and salmon, resulting in considerable economic losses. This bacterium is globally distributed, capable of surviving in water for over four months, and spreads through infected fish feces. ERM causes symptoms including hemorrhaging, organ inflammation, and mouth discoloration, with transmission facilitated by direct contact or biofilms in aquaculture systems. This project investigates these two bacterial species from complementary perspectives: *Y. enterocolitica* as a human pathogen responsible for zoonotic diseases and *Y. ruckeri* as a fish pathogen that threatens the aquaculture industry. By exploring their regulatory mechanisms, the project aims to enhance understanding of their adaptation and pathogenicity, addressing both public health and economic challenges.

The scientific goal of this project is to identify and characterize sRNAs in *Y. enterocolitica* and *Y. ruckeri*. Specific aims include using RNA sequencing (RNA-seq) to identify sRNAs, confirming their expression under different growth conditions, determining potential pathogenesis-linked targets, conducting detailed molecular characterization, and understanding their roles in *Y. enterocolitica* and *Y. ruckeri* physiology and virulence. The research will use clinical isolates with varying virulence levels.

Project implementation will enhance understanding of sRNA-mediated gene expression regulation in *Yersinia* species, particularly in influencing pathogenicity. These findings contribute to broader knowledge of sRNA functions, offering possibilities for combating bacterial infections amid rising antibiotic resistance. The project also aims to identify targets for selected sRNAs, potentially leading to new therapies.

In bioinformatics, identifying sRNAs in bacterial genomes typically relies on comparative genomics, limiting insights into *Yersinia* sRNAs, due to their unique characteristics and genetic variation. This project seeks to streamline the process, identifying molecules across strains from different biotypes representing distinct subspecies.

Understanding *Yersinia*'s adaptive responses to environmental cues requires comprehensive knowledge of bacterial gene expression across diverse settings. RNA-seq is a suitable tool, providing sensitivity and resolution for high-throughput analysis under various conditions. Results will enhance understanding of post-transcriptional mechanisms of gene regulation in *Y. enterocolitica*, *Y. ruckeri* and other pathogens, offering a broader perspective on pathogenesis dynamics. Identifying sRNAs targeting genes linked to the examined enteropathogen's virulence holds promise for gene silencing approaches, opening new avenues in fighting bacterial infections. Identified sRNAs or their synthetic derivatives may serve as a foundation for novel antibacterial therapies. The project addresses the urgent need for innovative approaches to treating bacterial infections amid escalating antibiotic resistance. Moreover, envisioning the identification of targets for selected sRNAs holds potential for tailored interventions, offering a selective advantage over drugs with broader impacts on the intestinal microbiota.