The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary

Emerging evidence suggests that the high plasticity of one of the most destructive phytopathogens, *Phytophthora infestans* (Mont.) de Bary, is driven by epigenetic mechanisms that enable its rapid adaptation to internal signals and environmental stressors, including the host-plant. Notably, *Phytophthora* lacks 5-methylcytosine DNA modifications, suggesting that reversible histone modifications, particularly acetylation and deacetylation playing a central role in gene regulation in these microorganisms. Recent studies have shown that a potent signaling molecule, nitric oxide (NO), beyond its diverse regulatory roles, may also function as an epigenetic modulator of gene expression in both animals and plants. Although NO role in microbial epigenetics remains underexplored, it may accumulate in pathogen structures during critical developmental transitions and under stress.

The primary aim of the research was to determine whether and to what extent NO and the following nitrosative stress to which *P. infestans* is exposed during its lifecycle affect the histone (de)acetylation patterns, thereby modulating gene expression to enhance adaptability and/or pathogenicity. Comparative analysis was conducted on both virulent and avirulent *P. infestans* isolates during both *in vitro* and *in planta* growth. Obtained results showed increased NO and peroxynitrite ($ONOO^-$) during both the sporulation phase and host colonization. Pharmacologically induced nitrosative stress induced significant changes in the global acetylation of histones H3 and H4. The observed hyperacetylation of histone H3 lysine 56 (H3K56ac) and histone H4 lysine 16 (H4K16ac) correlated with the induction of the expression of histone acetyltransferases genes (HATs), including PifHAC3 and PifHAM1, and with the enrichment of active acetylation marks at promoters of biotrophy- and pathogenicity-related genes, such as *Avr3a*, *Hmp1*, *CesA1*–3, and *sPLD-like1*. Subsequent analysis identified PifHDAC3 as the most RNS-responsive nuclear histone deacetylases (HDAC), potentially involved in H3K56ac deacetylation. Although its abundance increased under nitrosative conditions, recombinant PifHDAC3 was not directly S-nitrosated or inhibited by RNS. Finally, Chromatin immunoprecipitation sequencing (ChIP-seq) analysis revealed that NO availability altered PifHDAC3 recruitment to chromatin, including its displacement from the Avr3a promoter, thereby relieving transcriptional repression.

Summarizing, the dynamic interplay between RNS and HATs/HDACs is vital in influencing the expression of diverse *P. infestans* genes and documents NO as an essential epigenetic signal in the pathogen biology. By altering the histone (de)acetylation status, NO/RNS trigger the transcriptional reprogramming of genes related to metabolic, developmental, and offensive strategies, which may promote high adaptability to new (micro)environments. Thus, NO signaling and nitrosative stress play a crucial role in the operation of *P. infestans*' under environmental pressure.