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

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Emerging evidence suggests that the high phenotypic plasticity of one of the world's 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—play a central role in gene regulation in these microorganisms. In *P. infestans,* these processes are mediated by 33 histone acetyltransferases (HATs) and 11 histone deacetylases (HDACs). 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.

Based on the above, 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. The research was based on a comparative analysis between the virulent (vr) MP977 and avirulent (Avr) MP946 isolates of *P. infestans* against the potato (*Solanum tuberosum* L.) 'Sarpo Mira', genotype with the *R3a* resistance gene, to identify changes that may affect the pathogen's virulence. The experiments included the saprophytic phase (*in vitro*) and the microorganism's parasitic phase (*in planta*). To mimic nitrosative stress, specific reactive nitrogen species (RNS) modulators were applied to the pathogen culture.

Firstly, a significant increase in the formation of NO and its derivative, peroxynitrite, was documented in *P. infestans*' structures during both the sporulation phase and *in planta* growth. It has been shown that similarly to *in planta* conditions, pharmacologically induced nitrosative stress results in 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 *HAT* genes, *i.e.*, *PifHAM1* and *PifHAC3*, which may catalyze the formation of H4K16ac and H3K56ac, respectively. The RNS-mediated changes in histone architecture in the form of enriched H3K56ac and H4K16ac mark accumulation in the promoter regions of the molecular markers of the pathogen's biotrophic phase (*i.e.*, *Avr3a* and *Hmp1*) and other critical pathogenicity-related genes (*CesA1*, *CesA2*, *CesA3*, *sPLD-like1*) up-regulated their expression.

Subsequent *in silico* characterization and identification of RNS-responsive nuclear HDACs in *P. infestans* revealed that PifHDAC3 potentially catalyzes H3K56ac deacetylation and shows the highest level of transcript accumulation in response to NO. Notably, PifHDAC3 showed high abundance under nitrosative environments (*in vitro* and *in planta*); however, RNS did not provoke S-nitrosation and inhibition of recombinant PifHDAC3.

As PifHDAC3 was associated with the host colonization by *P. infestans*, the final stage of the study evaluated whether NO and the subsequent shift in the redox environment could affect the HDAC's recruitment to chromatin. Thus, chromatin immunoprecipitation sequencing (ChIP-seq) profiling provided insight into the key pathways regulated by PifHDAC3 in Avr/vr *P. infestans* exposed to a nitrosative environment, and revealed PifHDAC3-targeted genes involved, including those related to the pathogen's offensive strategies in a genotype-dependent manner. Notably, the NO availability led to the displacement of PifHDAC3 from the *Avr3a* promoter, and the loss of repressive chromatin structure enabled the transcriptional activation of *Avr3a*.

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.

Key words: nitric oxide, reactive nitrogen species, nitrosative stress, epigenetic modifications, histone (de)acetylation, *Phytophthora infestans,* late blight.