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Research article

N-acetylcysteine (NAC) attenuates quorum sensing regulated phenotypes in *Pseudomonas aeruginosa* PAO1

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ABSTRACT

The expression of many virulence genes in bacteria is regulated by quorum sensing (QS), and the inhibition of this mechanism has been intensely investigated. *N*-acetylcysteine (NAC) has good antibacterial activity and is able to interfere with biofilm-related respiratory infections, but little is known whether this compound has an effect on bacterial QS communication. This work aimed to evaluate the potential of NAC as a QS inhibitor (QSI) in *Pseudomonas aeruginosa* PAO1 through *in silico* and *in vitro* analyses, as well as in combination with the antibiotic tobramycin. Initially, a molecular docking analysis was performed between the QS regulatory proteins, LasR and RhlR, of *P. aeruginosa* with NAC, 3-oxo-C12-HSL, C4-HSL, and furanone C30. The NAC sub-inhibitory concentration was determined by growth curves. Then, we performed *in vitro* tests using the QS reporter strains *P. aeruginosa lasB-gfp* and *rhlA-gfp*, as well as the expression of QS-related phenotypes. Finally, the synergistic effect of NAC with the antibiotic tobramycin was calculated by fractional inhibitory concentrations index (FIC_i) and investigated against bacterial growth, pigment production, and biofilm formation. In the molecular docking study, NAC bound to LasR and RhlR proteins in a similar manner to the AHL cognate, suggesting that it may be able to bind to QS receptor proteins *in vivo*. In the biosensor assay, the GFP signal was turned down in the presence of NAC at 1000, 500, 250, and 125 μM for *lasB-gfp* and *rhlA-gfp* ($p < 0.05$), suggesting a QS inhibitory effect. Pyocyanin and rhamnolipids decreased ($p < 0.05$) up to 34 and 37%, respectively, in the presence of NAC at 125 μM. Swarming and swimming motilities were inhibited ($p < 0.05$) by NAC at 250 to 10000 μM. Additionally, 2500 and 10000 μM of NAC reduced biofilm formation. NAC-tobramycin combination showed synergistic effect with FIC_i of 0.8, and the best combination was 2500–1.07 μM, inhibiting biofilm formation up to 60%, besides reducing pyocyanin and pyoverdine production. Confocal microscopy images revealed a stronger, dense, and compact biofilm of *P. aeruginosa* PAO1 control, while the biofilm treated with NAC-tobramycin became thinner and more dispersed. Overall, NAC at low concentrations showed promising anti-QS properties against *P. aeruginosa* PAO1, adding to its already known effect as an antibacterial and antibiofilm agent.

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the slide. These changes were greater in the combinations of NAC-tobramycin (Fig. 6D and E), since CLSM images showed an even less dense biofilm. The most synergistic combination, 2500–1.07 μM of NAC-tobramycin (Fig. 6E) also showed the dispersed and thinner biofilm with spaced adhered cells, proving the efficiency of the drug combination. NAC can increase the sensitivity of *P. aeruginosa* to tobramycin and is a potential agent to be co-administered with antibiotics.

Quorum sensing regulates the expression of many genes associated with biofilm formation, and biofilms are associated with reduced sensitivity to antibiotics [79]. Thus, QS interference can be explored as an alternative to reduce bacterial virulence and increase the efficacy of antibiotic treatment. The combination of QSIs with antibiotics was demonstrated by Mion et al. [79], combining lactonases with fluoroquinolones and efficiently decreasing the amount of antibiotics required to fight *P. aeruginosa* clinical isolates. The flavonoid quercetin exhibited synergism between tobramycin and amikacin, providing better anti-biofilm activity against *P. aeruginosa* strains compared to their MIC dose [50]. Combinations of gallic acid-ampicillin and hamamelitannin-erythromycin inhibited the growth, biofilm viability, and motilities of *Escherichia coli* better than individual antibiotics, being promising candidates for eradicating pathogenic *E. coli* in humans and animals, according to Hossain et al. [51].

Finally, it is important consider that the virulence factors, motility, and biofilm formation are controlled by many pathways and molecules, in addition to quorum sensing. Thus, it is important to identify the exact target and mechanism of inhibitory compounds to ensure the influence on QS pathways [80]. For instance, more robust analysis could be performed, such as molecular and genetic techniques, gene expression assays of target QS genes, molecular dynamic analysis, and *in vivo* assays with animal models. Besides, it is necessary to treat bacteria with a specific concentration range, since low concentrations may not show an anti-QS effect, whereas high concentrations may be toxic or influence bacterial growth [67]. Here, in some tests, we used concentrations well below the MIC, and higher concentrations may present even better results for inhibiting the tested phenotypes.

4. Conclusion

To our knowledge, this is the first study to show NAC as a potential QSI against *P. aeruginosa* PAO1. The *in silico* analyses suggest a possible inhibitory mechanism, since NAC interacts with LasR and RhlR proteins similarly to cognate AHLs and, sometimes, even better than the QSI furanone C30. Assays with biosensor strains and QS-related phenotypes demonstrated the *in vitro* inhibitory potential, besides swarming and swimming motilities were strongly inhibited at the same concentration of NAC that inhibited biofilm formation The combination of NAC-tobramycin seems to be an interesting way to reduce the drug dose and inhibit biofilm formation by *P. aeruginosa* PAO1, as visualized by confocal microscopy. We suggest further studies to evaluate the expression of QS target genes of *P. aeruginosa* PAO1 in the presence of NAC in different concentrations and in combination with tobramycin to elucidate the mechanism of action, as well as to perform *in vivo* assays with animal models and also test other strains of *P. aeruginosa*.

Author contribution statement

Emília Maria França Lima: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Felipe Alves de Almeida: Analyzed and interpreted the data; Wrote the paper.

Marcelo Palma Sircili: Contributed reagents, materials, analysis tools or data.

Vanessa Bueris: Contributed reagents, materials, analysis tools or data.

Uelinton Manoel Pinto: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

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