Genetic basis of bacterial responses to antimicrobial blue light: implications for safety and resistance development

mgr Beata Agnieszka Kruszewska-Naczk

Antimicrobial resistance (AMR) represents one of the biggest challenges affecting healthcare, agriculture, and industry. Most infections are nowadays caused by pathogens resistant to first-line antibiotics. Additionally, an increasing number of strains are developing resistance to last-resort antibiotics and are exhibiting tolerance to disinfectants, significantly limiting available treatment options.

One of the most promising antibiotic alternatives to combat these infections is antimicrobial Blue Light (aBL). This strategy is based on the presence of endogenous photosensitizers in bacteria, which are activated by light of a specific wavelength in the blue spectrum. In the presence of oxygen, activated photosensitizers generate reactive oxygen species (ROS), damaging cellular structures and eventually leading to bacterial cell death. The detailed mechanism of aBL action remains poorly elucidated, which impacts the rare application of this method in routine practice. The gap in understanding the genetic basis of bacterial protection against aBL limits the development of safe treatment protocols that minimize the risk of resistance or other adaptations to aBL.

This study aims to identify genes involved in the bacterial response to aBL, to evaluate the risk of aBL resistance developing, and to assess the implications for the safety of this method. This doctoral dissertation is based on five publications. The first is a review of the current state of knowledge and identification of its research gaps. The remaining four research articles address the main and specific aims of this study.

The Keio collection of single-gene *Escherichia coli* BW25113 mutants was screened to identify mutants hypersensitive to aBL in comparison to the wild-type strain. Sixty-four mutants were selected, and the functions of deleted genes were identified. These genes are involved in essential cellular processes such as DNA repair, energy production, metabolism regulation, and stress response. To confirm the role of the identified genes in the bacterial response to aBL, complementation of the mutations was performed for several mutants. Complementation of selected knockout mutants restored wild-type aBL susceptibility or rendered mutants even less susceptible to aBL, confirming the protective role of the identified genes against aBL. The expression levels of the selected genes following aBL exposure were additionally evaluated in the wild-type *E. coli* strain, with a comparative analysis of the effects of two wavelengths, 409 nm and 415 nm. The gene *ihfB* was selected as the reference gene due to its stable expression post-aBL exposure. Quantitative PCR (qPCR) analysis revealed significantly elevated expression levels in irradiated samples relative to non-irradiated controls for five of the seventeen genes examined: *dacA, fabH, rbfA, umuD*, and *yihE*, across both wavelengths. Additionally, the genes *purA* and *rfaC* demonstrated increased expression exclusively at 409 nm. Analysis results indicate that not all aBL protective gene products are engaged in the aBL response simultaneously, and this depends on irradiation parameters such as aBL wavelength and dose. Next, all mutants were exposed to aBL-generated single stressors, and their growth defects were assessed to evaluate the role of protective genes in response to a specific stress condition. Most of the mutants were sensitive to hydroxyl radicals (•OH) and oxygen radicals (O^{2-}), suggesting that most of the identified genes protect bacteria against these aBL-generated stressors. Notably, no correlation was observed between sensitivity to aBL and to aBL-generated single stressors. This emphasizes that the antibacterial mode of aBL's action is the effect of the combined action of stressors rather than the effect of a single one.

The last publications present findings regarding the safety implications of aBL application in the food industry, utilizing *E. coli* as a representative foodborne pathogen. A phenotypically stable tolerance to heat and aBL has developed. Importantly, the antibiotic susceptibility of both tolerant populations remained unaffected. The potential for cross-stress tolerance development between these two treatments was thoroughly evaluated. The analysis demonstrated that bacterial populations tolerant to aBL exhibited increased heat tolerance, whereas the susceptibility of heat-tolerant populations to aBL did not significantly change. Additionally, short-term and long-term heat preexposure diminished the aBL susceptibility of the tested strain. Eleven genes potentially involved in cross-stress adaptation were identified, and a potential mechanism was proposed.

All sixty-four identified genes could potentially contribute to resistance development. However, no specific genes have been identified whose knockouts confer definitive resistance to aBL, supporting its safety as an alternative to antibiotics. Nevertheless, ongoing monitoring of all sixty-four aBL-protective genes is essential to detect any signs of adaptation. Additionally, potential risks should be carefully assessed when developing suitable aBL treatment protocols.