Heat-inactivated probiotic *Lactobacillus curvatus* BGMK2-41 activates *Caenorhabditis elegans* host defense via p38 MAPK signaling pathway

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Introduction

The host-microbiota cross-talk represents an important factor contributing to innate immune response and host resistance during infection. It has been shown that probiotic lactobacilli exhibit ability to modulate the immune system to enhance pathogen elimination. Therefore, the aim of this study was to test the potential of heat-inactivated probiotic *Lactobacillus curvatus* BGMK2-41 to stimulate immune response and resistance of the *Caenorhabditis (C.) elegans* against pathogens. The molecular pathways initially triggered by pathogens are highly conserved in a large variety of organisms ranging from nematodes to mammals which justifies the use of *C. elegans* as a useful model system for innate immunity studies in terms of pathogen-host-microbiota interactions.

Results

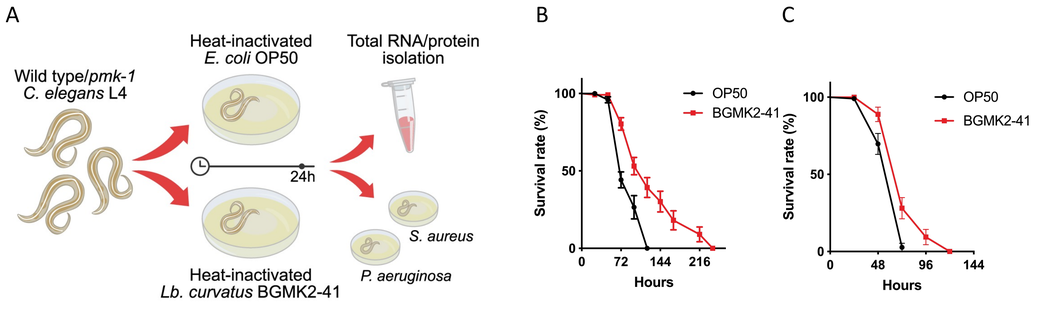

Figure 1. Heat-inactivated *Lactobacillus curvatus* BGMK2-41 prolongs survival of *C. elegans* exposed to pathogenic bacteria. As shown in Figure 1A we exposed L4 stage N2 wild-type worms to heat-inactivated BGMK2-41 or heat-inactivated *Escherichia coli* OP50 (control) for 24 h followed by the worms transfer to pathogenic bacteria. Animals previously exposed to BGMK2-41 exhibited enhanced survival upon infection both with *Staphylococcus aureus* ATCC25923 (Figure 1B) and *Pseudomonas aeruginosa* PA14 (Figure 1C) pathogenic strains in comparation to OP50 control.

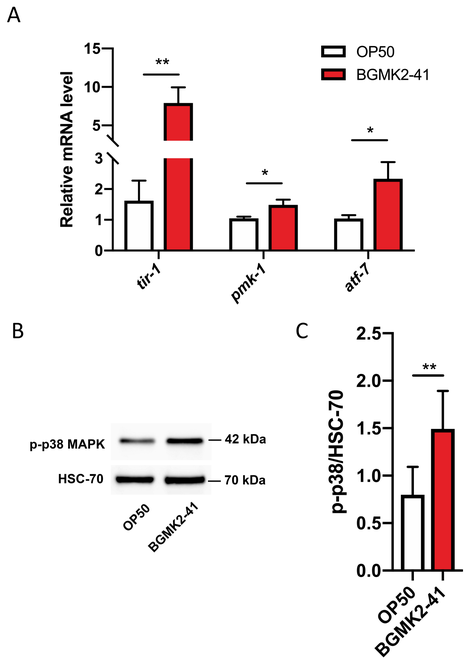

Figure 2. Heat-inactivated *Lactobacillus curvatus* BGMK2-41 activates p38 MAPK immune pathway in *C. elegans*. Transcription profiling revealed that all genes involved in p38 MAPK signaling (*tir-1*, *pmk-1*, *atf-7*) were significantly upregulated in worms treated with BGMK2-41 (Figure 2A). Next, we used western blot analysis to confirm the activation of p38 MAPK pathway by determining the level of p38 phosphorylation, which was upregulated in *C. elegans* treated with BGMK2-41 relative to OP50 control (Figure 2B, 2C).

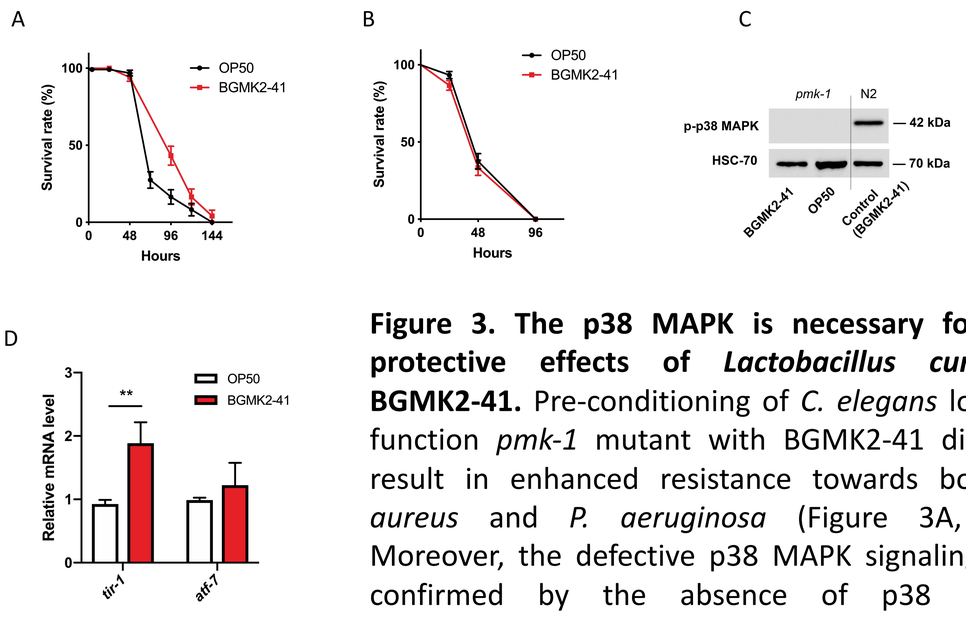

Figure 3. The p38 MAPK is necessary for the protective effects of *Lactobacillus curvatus* BGMK2-41. Pre-conditioning of *C. elegans* loss-of-function *pmk-1* mutant with BGMK2-41 did not result in enhanced resistance towards both *S. aureus* and *P. aeruginosa* (Figure 3A, 3B). Moreover, the defective p38 MAPK signaling was confirmed by the absence of p38 MAPK phosphorylation in *pmk-1* mutant and gene expression profile (Figure 3C, 3D).

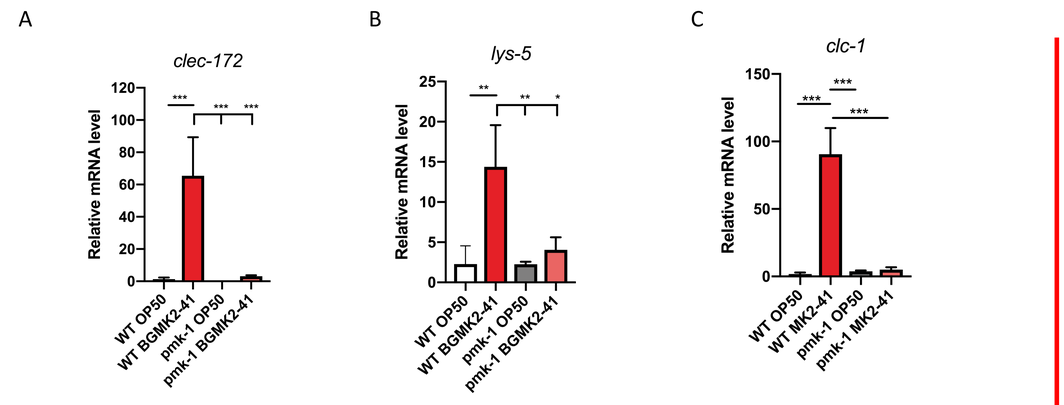

Figure 4. Heat-inactivated *Lactobacillus curvatus* BGMK2-41 controls the expression of antimicrobials and tight-junction protein via p38 MAPK. The BGMK2-41 treatment of N2 worms results in a major upregulation of C-type lectin (*clec-172*, Figure 4A), lysozyme (*lys-5*, Figure 4B) and claudin-like protein (*clc-1*, Figure 4C). Remarkably, a drastic reduction of mRNAs of these genes were detected in *pmk-1* mutant worms suggesting that BGMK2-41 upregulated these genes in p38 MAPK dependent manner.

Conclusion

The results of this study suggest that p38 MAPK-dependent immune regulation by BGMK2-41 is essential for probiotic-mediated *C. elegans* protection against gram-positive and -negative pathogenic bacteria and could be further explore for development of probiotics with potential to increase the resistance of the host towards pathogens.

Acknowledgements:

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/200042). The study was further supported by the Science Fund of the Republic of Serbia, Diaspora Collaboration Program grant (PLASH, 6426409) and by the Center for Leadership Development, Start Up for Science grant.