Inactivation of genes *lysR* and *mtfA* increases antagonistic activity of bacteria *Pseudomonas brassicacearum* S-1

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Key message. Genes *lysR* and *mtfA* localized in chromosome of bacteria *P. brassicacearum* S-1 were inactivated. The role of these genes in the expression of strain antagonistic activity against several bacterial and fungal pathogens was investigated. The technique of markerless mutagenesis of bacteria *P. brassicacearum* was optimized.

Keywords: mutagenesis, antimicrobial activity, *Pseudomonas brassicacearum*, sequencing

Modern methods of genetic engineering and advances in functional genomics enable to formulate biopreparations of novel generation to stimulate growth and development of agricultural crops rather than simply screen promising variants among natural isolates. Molecular-genetic and functional analysis of the genome allows to raise activity of biotechnologically valuable bacteria in the directed manner [1]. Aim of this study was molecular-genetic analysis of genes *lysR* and *mtfA* encoding synthesis of transcription factor LysR and metalloproteinase M90, respectively, and their role in expression of antagonistic activity of bacteria *P. brassicacearum* S-1.

Natural bacterial strain *P. brassicacearum* S-1 – antagonist of crop phytopathogens served as object of study. Transposon [2] and site-directed mutagenesis [3] methods were used to derive mutant strains of bacteria *P. brassicacearum*. Evaluation of antimicrobial activity was carried out using delayed antagonism technique.

Earlier we deciphered full nucleotide sequence of *P. brassicacearum* S-1 bacterial genome. Major metabolites and enzymes involved in expression of strain antagonistic activity were putatively revealed. Transposon mutagenesis was engaged to detect genes not belonging to the principal clusters but affecting strain activity. The analysis of 100 mutants enabled to sort out bacterial variants with improved parameters of antagonistic activity toward phytopathogenic bacteria *Pseudomonas syringae*, *Pectobacterium carotovorum* and fungi *Alternaria alternata*, *Fusarium oxysporum*, *Colletotrichum lupini*. Transposon miniTn5 insertion sites were determined in the selected variants as well as genes *lysR* and *mtfA* responsible for synthesis of the transcription factor LysR and metalloproteinase M90 were localized. The modified strains carried in chromosomes the mobile genetic element - transposon miniTn5, not securing stability of acquired characteristics since earlier we demonstrated translocation of miniTn5 across chromosome of strain *P. brassicacearum* S-1. Further on, markerless mutagenesis was engaged in relation to genes *lysR* and *mtfA*. As a result variants with sole mutations of genes *lysR* or *mtfA* and variant with dual mutation of both genes were derived. These variants preserved improved antagonistic properties in regard to the tested phytopathogens.

The study enabled to optimize the method of markerless mutagenesis of *P. brassicacearum* S-1 and to select bacterial variants with upgraded characteristics. The role of genes *lysR* and *mtfA* in expression of antagonistic activity in bacteria *P. brassicacearum* S-1 was originally ascertained.

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