Poster Presentation 2

P2-36

Tolerance Induction of Polyhexamethylene Biguanide on *Purpureocillium lilacinum* Strains

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Purpose: Recently, we have found a fungus to contaminate the product containing *polyhexamethylene biguanide (PHMB)*, and this isolate was identified as *Purpureocillium lilacinum* based on morphology and phylogeny. The aim of this study is the elucidation of a resistant mechanism against *PHMB* on *P. lilacinum*.

Methods: At first, we induced the resistant strains against *PHMB* from the type strain of *P. lilacinum* by repeated cultivation in a medium containing high concentrations of *PHMB*. Then we analyzed the DNA sequences by Illumina sequencing in order to explore the presence of genetic mutations in the induced strains. Further, we made the *P. lilacinum* uracil auxotrophic strain, and the pyrG gene as a selection marker tried to knock out the mutant gene at the induced strain by CRISPR-Cas9 genome editing technique.

Results: Initially, we got the resistance induced strains from type strain cultured in medium containing high concentrations of *PHMB*. The induced strain growth rate on *PHMB* medium was accelerated and the MIC value was also increased. According to analyze the DNA sequences data we found a nonsynonymous point mutation in the mutant gene. Furthermore, the mutant gene has successfully knocked out from the induced strain by the novel CRISPR-Cas9 gene transformation method. The growth rate test and MIC in *PHMB* condition show that, when mutant gene does not exist, both of the values were dramatically decreased.

Conclusions: Knocking out the mutant gene on the induced strain, the resistance on the *PHMB* was extraordinary reduced. This gene is one of the important resistant factors in *P. lilacinum*.