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*. 