Assessing Kill Switches as a Biocontainment Strategy in Environmental Conditions

Anna Hartig[1*], Wentao Dai[1], Ke Zhang[1], Austin Rottinghaus,[1] Tae Seok Moon,[1] Kimberly M. Parker[1]
[1]*Department of Energy, Environmental & Chemical Engineering, Washington University in St. Louis, St. Louis, United States*
*e-mail: a.hartig@wustl.edu

Abstract

Biocontainment strategies, including engineered kill switches, have been developed to prevent the persistence of genetically engineered microbes (GEMs) in environmental systems. However, the impacts of environmental conditions on the effectiveness of the kill switch must be understood prior to the open release of GEMs in application scenarios. Therefore, in this study, we evaluated the influence of environmental factors in surface waters on the inhibition of a CRISPR kill switch triggered by anhydrotetracycline (aTc) in a model microbe, *Escherichia coli* Nissle 1917. We first demonstrated that the kill switch efficiency is lower in surface water conditions compared to laboratory media, implying that GEMs will have a higher escape rate in environment systems. We attributed this low kill switch efficacy to environmental stressors in surface water such as pH, temperature, and nutrient levels. Under varied pH and temperature conditions, the kill switch was inhibited due to decreased uptake of the kill switch trigger. Furthermore, we found that nutrient deficient surface waters inhibited the kill switch, likely by affecting the cellular metabolism of the GEM. Overall, the environmental conditions found to reduce the kill switch efficiency in this study should be considered in both the design of kill switches and the development of regulatory standards for biocontainment.

Key words: kill switch, biocontainment, genetically engineered microbes, environment, biosafety