Concomitant knockout of target and transporter genes in filamentous fungi by genome co-editing

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March 16, 2022

Abstract

In most countries, genetically modified microorganisms are not approved for use for fermentation in the food industry. Therefore, random mutagenesis and subsequent screening are performed to improve the productivities of valuable metabolites and enzymes as well as other specific functions in an industrial microbial strain. In addition, targetted gene knockout is performed by genetic recombination using its enzyme genes as selectable markers to maintain self-cloning status. However, random mutagenesis has a drawback as it does not guarantee improvement of the targeted function. Conversely, self-cloning is rarely used to breed an industrial microbial strain. This is probably because a self-cloning strain is similar to a genetically modified strain, although exogenous genes are not introduced. In this article, I discuss the usefulness of genome editing technology as a substitute for conventional techniques to breed filamentous fungal strains. This article particularly focusses on “genome co-editing,” a genome editing technology used for knocking out two genes concomitantly, as reported in Magnaporthe grisea and Aspergillus oryzae. Especially, when genome co-editing is applied to a target gene and a membrane transporter gene that aid the entry of toxic compounds into cells, the resulting clone can be categorized as an autotrophic and non-genetically modified clone. Such a clone should easily apply to industrial fermentation without being restricted by a genetically modified status. Genome co-editing will also be used to construct mutant strains with multiple target gene knockouts by eliminating multiple membrane transporter genes. This could substantially improve the productivities of valuable metabolites and enzymes in a stepwise manner. Thus, genome co-editing is considered a potentially powerful method to knock out single or multiple target genes that can contribute to the breeding of filamentous fungal strains in the food industry.

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