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CRISPR–Mediated Genome Editing and Gene Repression in *Scheffersomyces stipitis*

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***Scheffersomyces stipitis*, renowned for its native xylose-utilizing capacity, has recently demonstrated its potential in producing health-promoting shikimate pathway derivatives. However, its broader application is hampered by the low transformation efficiency and the lack of genetic engineering tools to enable sophisticated genomic manipulations. *S. stipitis* employs the predominant non-homologous end joining (NHEJ) mechanism for repairing DNA double-strand breaks (DSB), which is less desired due to its incompetence in achieving precise genome editing. Using CRISPR technology, here a *ku70*Δ*ku80*Δ deficient strain in which homologous recombination (HR)-based genome editing appeared dominant for the first time in *S. stipitis* is constructed. To build all essential tools for efficiently manipulating this highly promising nonconventional microbial host, the gene knockdown tool is also established, and repression efficiency is improved by incorporating a transcriptional repressor Mxi1 into the CRISPR-dCas9 platform. All these results are obtained with the improved transformation efficiency, which is 191-fold higher than that obtained with the traditional parameters used in yeast transformation. This work paves the way for advancing a new microbial chassis and provides a guideline for developing efficient CRISPR tools in other nonconventional yeasts.**

1. Introduction

Scheffersomyces stipitis is recognized as an important yeast species in the field of biorenewables due to its desired capacity for utilizing xylose,[1] the second most abundant sugar in lignocellulosic biomass. While its previous applications were mainly demonstrated as a repository for isolating genes involved in xylose assimilation and transport, its potential as a better-suited microbial host than *Saccharomyces cerevisiae* for producing compounds derived from the shikimate pathway was recently proposed.[2] The much more active pentose phosphate pathway associated with the native xylose assimilating ability in *S. stipitis* renders a higher availability of the precursor erythrose 4-phosphate (E4P), which was identified as the rate-limiting precursor of the shikimate pathway in *S. cerevisiae*.[3] Considering that in plants the downstream products of the shikimate pathway include many kinds of flavonoids and alkaloids with important pharmaceutical and nutraceutical proper-