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N-terminal engineering of overlapping genes in the nitrile hydratase gene cluster improved its activity

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Highlights

- A fusion tag strategy was applied to tune the expression of activator.
- A silent mutation strategy was applied to improve the expression of β-subunit.
- The expression levels of three ORFs were balanced and the final activity improved.

Abstract

Nitrile hydratase which catalyzes the hydration of nitriles to the corresponding amides is operon-encoded. However, when heterologously expressed, genes in the same operon are usually not equally expressed, and the ratio needs to be fine-tuned. A gene cluster of three genes (corresponding to α-subunit, β-subunit and activator) encoding the nitrile hydratase was cloned from *Aurantimonas manganoxydans* ATCC BAA-1229 and expressed in *Escherichia coli*. However, difficulty was encountered in heterologous expression of the activator and the expression level of β-subunit was lower than that of α-subunit, which together resulted in low catalytic efficiency. To improve the expression of activator, a set of SKIK tags were fused to the N-terminus of the activator. To elevate the expression level of β-subunit, a silent mutation strategy was applied in the overlapping sequence with α-subunit around its translation initial region. Finally, the expression of β-subunit and activator were improved and the maximum activity of NHase1229 was doubled, reaching 160 U/mL towards 3-cyanopyridine. These results indicate that N-terminal engineering is an efficient strategy for optimizing the expression of multiple genes in operons.

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