Methylocrotrophic yeast *Komagataella phaffii* as Neoleukin producer

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Methylocrotrophic yeast *Komagataella phaffii* (also known as *Pichia pastoris*) is widely applied in biotechnology for recombinant protein production. *K. phaffii* particularly proved to be a successful host system for the synthesis of immunomodulators such as interferons [1].

In this study, we engineered *K. phaffii* strains capable of producing the immunomodulatory protein Neoleukin (Neo-2/15). Neo-2/15 is an interleukin-2 mimetic, designed by *in silico* methods [2]. In preclinical studies on murine cancer models, Neo-2/15 showed superior therapeutic effect to interleukin-2 with reduced toxicity.

In this work, we show that *K. phaffii* can successfully synthesize and secrete Neo-2/15. We have obtained a number of *K. phaffii* strains, including Mut$^S$ and Mut$^+$, with different Neo-2/15 expression cassettes integrated into the genome, carrying up to five copies of Neo-2/15 gene. In fact, the higher number of Neo-2/15 gene copies in *K. phaffii* genome allowed a higher protein yield.

In this study, we further developed a split marker approach [3] for yeast transformation using two DNA fragments, comprising of the expression cassette and the overlapping fragments of the marker gene. This allowed us to generate Mut$^S$ strains with two copies using pPICZαB vector, which is not originally intended for Mut$^S$ strain generation.

As a result, we demonstrated that *K. phaffii* is a perspective producer of Neo-2/15, providing wide opportunities to increase the production of this therapeutic protein.

REFERENCES

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