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CO-OVEREXPRESSION OF GENES *RFE1*, *GND1*, AND *RIB6* ENHANCES RIBOFLAVIN PRODUCTION IN YEAST *CANDIDA FAMATA*

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Riboflavin (vitamin B_2) is an essential compound for the nutrition of living organisms, serving as a precursor of coenzymes flavin mononucleotide and flavin adenine dinucleotide, which are involved in numerous enzymatic reactions mainly in oxidative metabolism. The yeast Candida famata is a natural riboflavin-producing species, which are able to oversynthesis riboflavin under conditions of iron starvation. The aim of this study was to construct recombinant C. famata strains with increased riboflavin production by the simultaneous overexpression of the three genes: RFE1, GND1, and RIB6, which encode riboflavin excretase, 6-phosphogluconate dehydrogenase and 3,4-dihydroxy-2-butanone-4-phosphate synthase, respectively. The expression of various combinations of two genes, as well as the co-expression of all three genes, resulted in increased riboflavin production in C. famata VKM Y-9 in different media. The strains engineered for the co-overexpression of all three genes exhibited up to a 3.3-fold increase in riboflavin production by the fifth day of cultivation in cheese whey, compared to the parental strain.

Key words: *Candida famata, riboflavin, vitamin B_2, cheese whey, yeast.*

КОЕКСПРЕСІЯ ГЕНІВ *RFE1*, *GND1* ТА *RIB6* ПІДВИЩУЄ ПРОДУКЦІЮ РИБОФЛАВІНУ У ДРІЖДЖІВ *CANDIDA FAMATA*

Рибофлавін (вітамін B2) є невід'ємним компонентом живих організмів, слугуючи попередником коферментів флавінмононуклеотиду та флавінаденіндинуклеотиду, що беруть участь у численних ферментативних реакціях, переважно в окисному метаболізмі. Дріжджі *Candida famata* здатні до надсинтезу рибофлавіну за умов дефіциту заліза. Метою даної роботи було конструювання рекомбінантних штамів *C. famata* з підвищеною продукцією рибофлавіну шляхом одночасної надекспресії трьох генів: *RFE1*, *GND1* та *RIB6*, що кодують рибофлавін-екскретазу, 6-фосфоглюконатдегідрогеназу та 3,4-дігідрокси-2-бутанон-4-фосфатсинтазу, відповідно. Експресія комбінацій двох генів, а також коекспресія трьох генів приводила до зростання продукції рибофлавіну у *C. famata* VKM Y-9 при культивуванні на різних середовищах. Штами, з посиленою коекспресією трьох генів, характеризувалися збільшенням продукції рибофлавіну в 3,3 раза порівняно з батьківським штамом на п'ятий день культивування в середовищі з молочною сироваткою.

Ключові слова: *Candida famata,* рибофлавін, вітамін B2, молочна сироватка, дріжджі.

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