microorganisms

MDPI

Article

Improved Plasmid-Based Inducible and Constitutive Gene Expression in *Corynebacterium glutamicum*

Nadja A. Henke, Irene Krahn and Volker F. Wendisch *

Genetics of Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, 33615 Bielefeld, Germany; n.henke@uni-bielefeld.de (N.A.H.); irene@cebitec.uni-bielefeld.de (I.K.)
* Correspondence: volker.wendisch@uni-bielefeld.de; Tel.: +49-521-106-5611

Abstract: *Corynebacterium glutamicum* has been safely used in white biotechnology for the last 60 years and the portfolio of new pathways and products is increasing rapidly. Hence, expression vectors play a central role in discovering endogenous gene functions and in establishing heterologous gene expression. In this work, new expression vectors were designed based on two strategies: (i) a library screening of constitutive native and synthetic promoters and (ii) an increase of the plasmid copy number. Both strategies were combined and resulted in a very strong expression and overproduction of the fluorescence protein GfpUV. As a second test case, the improved vector for constitutive expression was used to overexpress the endogenous xylulokinase gene *xylB* in a synthetic operon with xylose isomerase gene *xylA* from *Xanthomonas campestris*. The xylose isomerase activity in crude extracts was increased by about three-fold as compared to that of the parental vector. In terms of application, the improved vector for constitutive *xylA* and *xylB* expression was used for production of the *N*-methylated amino acid sarcosine from monomethylamine, acetate, and xylose. As a consequence, the volumetric productivity of sarcosine production was 50% higher as compared to that of the strain carrying the parental vector.

Keywords: expression vector; *Corynebacterium glutamicum*; overexpression; promoter; origin of replication

Citation: Henke, N.A.; Krahn, I.; Wendisch, V.F. Improved Plasmid-Based Inducible and Constitutive Gene Expression in *Corynebacterium glutamicum*. **2021**, *9*, 204. https://doi.org/10.3390/microorganisms9010204

Received: 27 December 2020
Accepted: 18 January 2021
Published: 19 January 2021

1. Introduction

C. glutamicum was discovered in the 1960s as a natural L-glutamate producer [1]. Since then, both its genetic toolbox [2] and its number of heterologous pathways [3,4] have been extended. On the one side, production of value-added compounds such as amino acids [5,6], organic acids [7,8], and terpenoids [9,10] has been established. Recently, the production of sarcosine (*N*-methylglycine) was enabled by overexpression of the imine reductase DpkA from *Pseudomonas putida* in a glyoxylate-overproducing *C. glutamicum* strain by providing monomethylamine as the methyl-donor [11]. On the other side, several approaches were followed in order to establish a flexible feedstock concept that allows *C. glutamicum* production strains to grow/produce on the basis of a variety of non-food competitive substrates such as industrial or agricultural/aquatic side streams [12]. The access to glycerol, the stoichiometric byproduct of biodiesel production, was enabled and applied to various production strains [13,14]. Moreover, recent attempts have aimed to establish the methylotrophy in *C. glutamicum* for methanol utilization [15]. Besides the sugar polymers starch [16] and cellulose [17], the pentose sugars xylose and arabinose [18,19] that derive from hemicellulose can be used as alternative substrates for a variety of high-value products including the fragrance compound patchoulol [20] and the potential antipsychotic compound sarcosine [11].

Many of these production strain-engineering efforts rely on gene expression vectors, which represent a powerful tool not only for metabolic engineering, but also for in depth