Journal of Biotechnology 230 (2016) 3–10

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Effects of modulation of pentose-phosphate pathway on biosynthesis of ansamitocins in *Actinosynnema pretiosum*

Yuxiang Fan[a], Fengxian Hu[a], Liujing Wei[a], Linquan Bai[b], Qiang Hua[a,c,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China
[b] State Key Laboratory of Microbial Metabolism and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[c] Shanghai Collaborative Innovation Center for Biomanufacturing Technology, 130 Meilong Road, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 2 May 2016
Accepted 6 May 2016
Available online 9 May 2016

Keywords:
Actinosynnema pretiosum
Ansamitocins
Pentose phosphate pathway
Phosphoglucomutase
Metabolic engineering

ABSTRACT

Ansamitocins, produced by *Actinosynnema pretiosum*, are a group of maytansinoid antibiotics that block the assembly of tubulin into functional microtubules. The precursors of ansamitocin biosynthesis are generally derived from the Embden-Meyerhof-Parnas (EMP) pathway and the tricarboxylic acid cycle. In this study, central carbon flux distributions were analyzed by ^{13}C-based flux analysis to reveal the contribution of individual central carbon metabolism pathways. To direct more carbon flux into ansamitocin biosynthesis, pentose phosphate (PP) pathway only and the combination of PP pathway and Entner-Doudoroff (ED) pathway were weakened, respectively. Ansamitocin P-3 (AP-3) productions by both kinds of pathways weakened mutant strains were significantly enhanced in chemically defined medium. In order to draw metabolic flux to the biosynthesis of ansamitocins more efficiently, heterologous phosphoglucomutase was subsequently overexpressed based on a mutant strain with combinational regulation of PP pathway and ED pathway. More fluxes were successfully directed into the UDP-glucose synthetic pathway and the AP-3 production was further improved in this case, reaching approximately 185 mg/L in fermentation medium. It was demonstrated that eliminating the bypass pathways and favoring the precursor synthetic pathway could effectively improve ansamitocin production by *A. pretiosum*, suggesting a promising role of metabolic strategy in improving secondary metabolite production.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Ansamitocins, extraordinary potential antitumor agents that block the assembly of tubulin into functional microtubules, are synthesized by the *Actinosynnema pretiosum* spp. *auranticum* ATCC 31565 (Cassady et al., 2004; Higashide et al., 1977). Among these agents, Ansamitocin P-3 (AP-3) is used as a toxic "warhead" in immunotoxin conjugants, as its high affinity to the conjugants suppresses the dynamic instability of microtubules (Liu et al., 1996). Recently, ansamitocins conjugated with antibodies have been proposed as cytotoxic agents. Kadcyla, an FDA-approved antibody drug for breast cancer, is formed from a derivative of ansamitocin P-3 (AP-3) called DM-1 (Amiri-Kordestani et al., 2014).

The biosynthesis of ansamitocins is a complicated process, in which UDP-glucose (UDPG) converts to the important intermediate 3-amino-5-hydroxybenzoic acid (AHBA) via the aminoshikimate pathway (Kang et al., 2012). AHBA is subsequently synthesized to proansamitocin by polyketide synthase (PKS). The carbon framework is an assemblage of three types of precursors formed by central carbon metabolism (Fan et al., 2014; Yu et al., 2002). Finally, AP-3 is synthesized through a series of post-PKS modification steps (Spiteller et al., 2003). This complex biosynthetic pathway requires complicated regulation and abundant precursors. The original starter unit for ansamitocin biosynthesis, UDPG, is converted from glucose-6-phosphate (G6P) during glycolysis. The reaction is catalyzed by phosphoglucomutase (Pgm) and UTP-G1P uridylyltransferase (also called UDP-glucose pyrophosphorylase, Ugp) (Fan et al., 2016). The extension chains of PKS (methoxymalonyl-ACP, malonyl-CoA and methylmalonyl-CoA) are synthesized from glycerate-1,3-diphosphate (G-1,3-P), acetyl CoA and succinyl CoA, respectively, which are sourced from glycolysis and the tricarboxylic acid (TCA) cycle (Fan et al., 2014; Kang et al., 2012; Zhao et al., 2010). Flux through glycolysis is considered an important mediator of ansamitocin biosynthesis.

Despite their importance, ansamitocins are of limited applicability because of their low production yields. Although the yields

* Corresponding author at: State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China.
E-mail address: qhua@ecust.edu.cn (Q. Hua).

http://dx.doi.org/10.1016/j.jbiotec.2016.05.010
0168-1656/© 2016 Elsevier B.V. All rights reserved.

Table 1
Microorganisms and plasmids.

Strains or Plasmids	Descriptions	Sources
E. coli		
DH10B	F⁻, *end*A1, *rec*A1, *gal*E15, *gal*K16, *nup*G, *rps*L, Δ*lac*X74, Φ80*lac*ZΔM15, *ara*D139Δ(*ara*, *leu*)7697, *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC), λ-	Invitrogen
ET12567/pUZ8002	F⁻ *dam*-13:Tn9*dcm*-6 *hsdM hsdR zjj*-202:Tn10 *recF*143 *galK*2 *galT*22 *ara*-14 *lacY*1 *xyl*-5 *leuB*6 *thi*-1 *tonA*31 *rpsL*136 *his*G4 *tsx*-78 *mtl*-1 *glnV*44 pUZ8002 (derivatives of pUB307)	Kieser et al. (1992)
Actinosynnema pretiosum ssp. *auranticum*		
ATCC 31565	Wild-type producer of ansamitocins	Hatano et al. (1982)
FYX001	Derivative of ATCC 31565, *asm0003* gene disruption mutant with apramycin resistance	This study
FYX004	Derivative of ATCC 31565, *asm5683* gene disruption mutant with apramycin resistance	This study
FYX007	Derivative of ATCC 31565, *asm5189* gene disruption mutant with apramycin resistance	This study
FYX033	Derivative of ATCC 31565, *amir6327* gene and apramycin resistance replaced *asm5189* gene mutant	This study
Actinosynnema mirum		
DSM 43827	Wild-type producer of ansamitocins, genome sequenced	Land et al. (2009)
Plasmids		
pBluescript II SK⁺	*bla, lacZ orif1*	Stratagene
pIB139	*oriT, attP, int, aac(3)IV PermE**	Wilkinson et al. (2002)
pJTU1278	*tsrbla*(*neo*) *ori*(pIJ101) *oriTlacZa*	He et al. (2010)
pJTU1289	*tsrbla*(*neo*) *ori*(pIJ101)	He et al. (2010)
pECU0003	pJTU1278 containing 4.5 kb *apr*-disrupted *asm0003* fragment	This study
pECU0024	pJTU1289 containing 6.3 kb *apr*-disrupted *asm5189* fragment	This study
pECU0035	pJTU1278 containing 5.0 kb *apr*-disrupted *asm5189* fragment	This study
pECU0117[a]	pJTU1278 containing 6.6 kb *amir6327*-disrupted *asm5189* fragment	This study

[a] The *amir6327* overexpression cassette contained individual *PermE** promoter.

can be enhanced by modifying the genes involved in ansamitocin biosynthetic clusters (Bandi et al., 2006; Ng et al., 2009) and by optimizing the culture conditions of ansamitocin producers (Fan et al., 2014; Jia and Zhong, 2011; Lin et al., 2011, 2010), they remain insufficiently low.

Metabolic engineering is increasingly used to elucidate intracellular metabolic flux distributions and to guide metabolite production, especially in actinomyces (Li et al., 2014; Olano et al., 2008; Ryu et al., 2006; Zabala et al., 2013). High yields of secondary metabolites require an adequate supply of biosynthesis precursor, which is generally provided by catabolism of carbon substrates. Tracing the carbon flux through the central carbon metabolism can assist in mapping the metabolic network and understanding the status of the precursor supplement. For instance, the primary metabolic pathway in the actinomycete *Nonomuraea* sp. ATCC 39727 was identified by ^{13}C-based metabolic engineering. Glucose was found to be predominantly metabolized by the Entner-Doudoroff (ED) pathway, and phosphate limitation shifted the flux of the central carbon metabolism, thereby increasing the productivity of A40926 (Gunnarsson et al., 2004a,b). ^{13}C metabolic flux analysis also revealed the rearrangement of the central carbon flux distribution in *Streptomyces coelicolor* with a disrupted phosphofructokinase gene *pfkA2*, which led to antibiotic overproduction by the strain (Borodina et al., 2008). Meanwhile, deletion of the glucose-6-phosphate dehydrogenase (G6PD, Zwf) genes *zwf1* or *zwf2* in *S. coelicolor* shifted the carbon flux to actinorhodin (Ryu et al., 2006). The Embden-Meyerhof-Parnas (EMP) and pentose phosphate (PP) pathways, usually considered as the sources of precursors, play an important role in antibiotic biosynthesis.

The present study investigates the flux ratio of central carbon metabolism in the EMP, PP and ED pathways of *A. pretiosum*. Multiple genetic manipulations were carried out to reduce the superfluous flux of the central carbon metabolism, and to redirect the flux into the relevant precursor supplement pathway. The AP-3 production was enhanced by alleviating the PP pathway. Simultaneously, the flux toward the original starter unit of ansamitocin biosynthesis was increased by overexpressing phosphoglucomutase. The results of this study provide necessary information for further understanding the contribution of central carbon metabolism to ansamitocin biosynthesis.

2. Materials and methods

2.1. Microorganism, plasmids and cultivation

The microorganism and plasmids used in this study are shown in Table 1. *Escherichia coli* DH10B and ET12567/pUZ8002 were used as the host for conservation of the constructed plasmids and conjugation between *E. coli* and *A. pretiosum*. The genome of *Actinosynnema mirum* was used as the genetic template for heterologous overexpression.

Luria-Bertani medium was used for *E. coli* incubation. One milliliter of stock cell suspension was incubated into 250 mL Erlenmeyer flask containing 50 mL seed medium for 48 h. 0.5 mL milliliter of culture was subsequently inoculated into another fresh seed medium for 24 h. 0.5 mL culture was then inoculated into fermentation medium or M6 chemically defined medium after three time abstersion of sterile water to remove residual nutrient.

Download English Version:

https://daneshyari.com/en/article/22645

Download Persian Version:

https://daneshyari.com/article/22645

Daneshyari.com