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Improving gentamicin B and gentamicin C1a production by engineering the glycosyltransferases that transfer primary metabolites into secondary metabolites biosynthesis

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ABSTRACT

Gentamicin B and gentamicin C1a are the direct precursor for Isepamicin and Etimicin synthesis, respectively. Although producing strains have been improved for many years, both gentamicin B titer and gentamicin C1a titer in the fermentation are still low. Because all gentamicin components are biosynthesized using UDP-N-acetyl-D-glucosamine (UDP-GlcNAc) and UDP-xylose as precursors, we tried to explore strategies for development of strains capable of directing greater fluxes of these precursors into production of gentamicins. The glycosyltransferases KanM1 and GenM2, which are responsible for UDP-GlcNAc and UDP-xylose transfer, respectively, were overexpressed in gentamicin B producing strain *Micromonospora echinospora* JK4. It was found that gentamicin B could be improved by up to 54% with improvement of KanM1 and GenM2 expression during appropriately glucose feeding. To prove this strategy is widely usable, the KanM1 and GenM2 were also overexpressed in gentamicin C1a producing strain, titers of gentamicin C1a improved by 45% when compared with titers of the starting strain. These results demonstrated overexpression the glycosyltransferases that transfer primary metabolites into secondary metabolites is workable for improvement of gentamicins production.

1. Introduction

Aminoglycoside antibiotics are potent antimicrobial agents that have been used clinically for decades. Most clinically valuable aminoglycosides are 2-deoxystreptamine-containing aminoglycosides (Park et al., 2012). Structurally, 2-deoxystreptamine-containing aminoglycosides contain a core aminocyclitol moiety, 2- deoxystreptamine (2-DOS), which is elaborated with one or more aminosugar units, form pseudodisaccharide or pseudotrisaccharide. In the biosynthetic pathway of 2-DOS-containing aminoglycosides, paromamine is the common pseudodisaccharide intermediate. The biosynthetic pathway of paromamine has been elucidated in different aminoglycoside biosynthetic pathways (Guo et al., 2014; Huang et al., 2015). A glycosyltransferase enzyme, KanM1, which has been identified in the kanamycin biosynthetic pathway from *Streptomyces kanamyceticus*, transfer UDP-GlcNAc to 2-DOS (Nepal et al., 2009), and homologues have also been found in gentamicin producers (Park et al., 2008). The necessary UDP-GlcNAc is likely from the primary metabolism. Following the addition of GlcNAc to DOS, the N-acetyl group is removed by deacetylase to give the intermediate paromamine.

Gentamicin belongs to the group of 2-DOS-containing aminoglycosides and is produced by fermentation of *Micromonospora echinospora*. Typically, it is composed of 2-DOS, and two tailoring sugars: purpurosamine, and garosamine. Variations in the substitution pattern of purpurosamine result in four major components: C1, C1a, C2, and C2a. Gentamicin B is a minor component from gentamicin biosynthetic pathway. The biosynthetic pathway for production of gentamicin has been elucidated (Fig. 1). Through expressed the deduced gene sets in a nonaminoglycoside producing strain *Streptomyces venezuela*, *gtm*B, *gtm*A and *gac*H were found responsible for the biosynthesis of 2-DOS moiety of gentamicin (Park et al., 2008). GtmG is a glycosyltransferase that adds UDP-GlcNAc to 2-DOS. GtmM is responsible for *N*-acetyl paromamine deacetylation. GtmE (GenM2) is another glycosyltransferase that attaches D-xylose to paromamine, formed the first pseudotrisaccharide intermediate in the biosynthetic pathway for the gentamicin (Park et al., 2008). Enzymes for oxidation and transamination at C-6′ (Guo et al., 2014; Gu et al., 2015) and C-3″(Huang et al., 2015), methyltransferation at C-6′(Kim et al., 2013; Li et al., 2013), C-4″ (Huang et al., 2015) and N-3″ (Huang et al., 2015; Ni et al., 2016b) have been also identified.

The clarified biosynthetic pathways for gentamicin and other aminoglycosides provide great opportunity for improvement gentamicin

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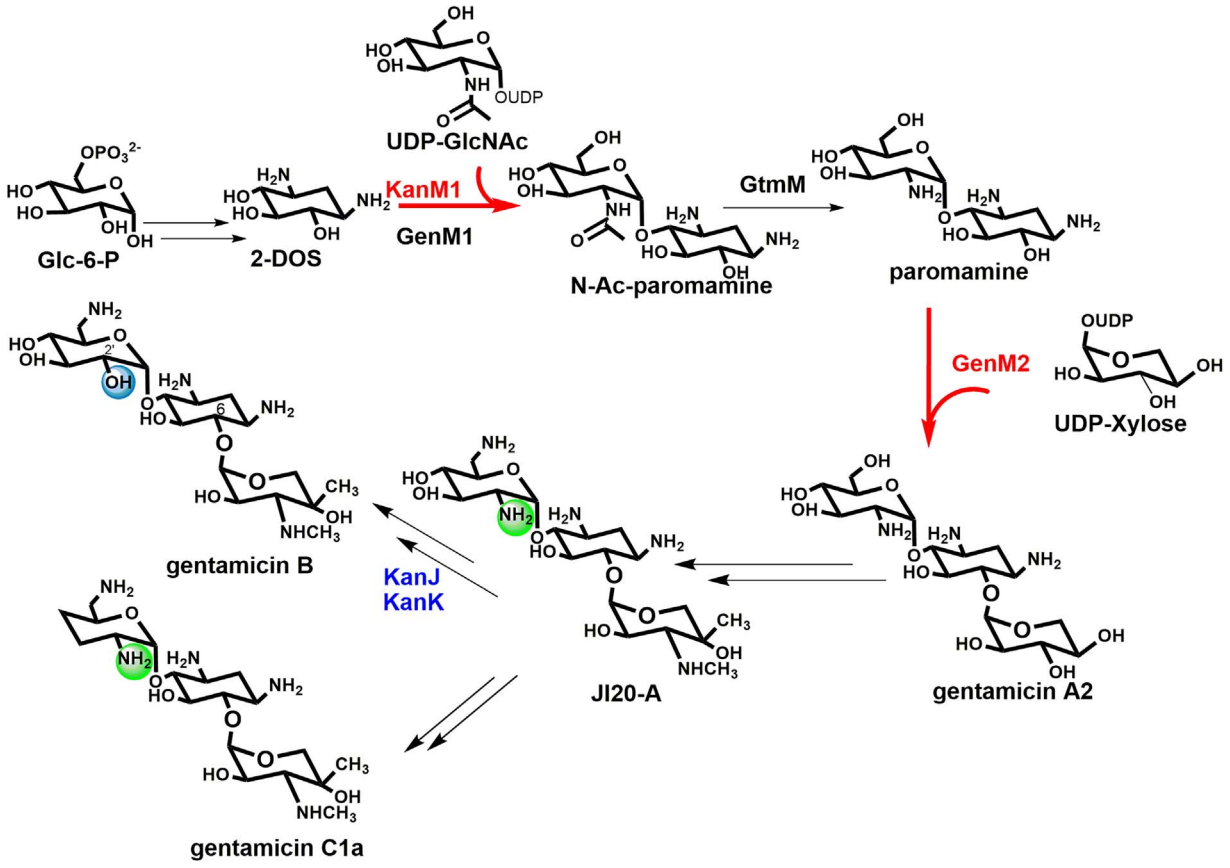

Fig. 1. Biosynthetic pathway for gentamicin B and gentamicin C1a.

production by modulating biosynthetic pathway. By assembling a new pathway using enzymes from *S. kanamyceticus* and *M. echinospora*, Gentamicin B producer was constructed previously (Ni et al., 2016a). Titers of 880 μg/ml gentamicin B were achieved in the engineered strain. In this work, to improve yields of the gentamicin B, two glycosyltransferases KanM1 and GenM2 were overexpressed in *M. echinospora* (Fig. 1). Because our previous experiments found that glucose feeding can improve gentamicin production (unpublished), this result indicated that precursor supply might be a rate-limiting step for gentamicin biosynthesis. So the glycosyltransferases that transfer UDP-

Table 1
Strains, plasmids used in this study.

Strains or plasmids	Relevant characteristic[a]	Reference or source
Strains		
E. coli TOP10	F$^-$ *mcr*AΔ(*mrr-hsd*RMS-*mcr*BC), φ80*lac*ZΔM15, Δ*lac*X74, *deo*R, *rec*A1, *ara*D139Δ(*ara-leu*)7697, *gal*U, *gal*K, *rps*L(Str^R), *end*A1, *nup*G	Invitrogen
E. coli ET12567/pUZ8002	Methylation defective, strain used in *E. coli-streptomyces* intergeneric conjugation	MacNeil et al. (1992)
S. kanamyceticus	Kanamycin producing strain	CGMCC4.1441
M. echinospora	Wild-type strain, gentamicin C1a, C2, C2a, and C1 producer	ATCC 15835
M. echinospora JK4	Overexpression *kan*J and *kan*K in *M. echinospora* with disrupted *gen*K and *gen*P	Ni et al. (2016a)
*M. echinospora*ΔK	*M. echinospora* with disrupted *gen*K	Li et al. (2013)
*M. echinospora*BM1	Heterologous expression of *kan*M1 in *M. echinospora* JK4	This study
M. echinospora BM2	Heterologous expression of *gen*M2 in *M. echinospora* JK4	This study
M. echinospora BM1M2	Heterologous expression of *kan*M1 and *gen*M2 in *M. echinospora* JK4	This study
M. echinospora CM1	Heterologous expression of *kan*M1 in *M. echinospora* ΔK	This study
M. echinospora CM2	Heterologous expression of *gen*M2 in *M. echinospora* ΔK	This study
M. echinospora CM1M2	Heterologous expression of *kan*M1 and *gen*M2 in *M. echinospora* ΔK	This study
Plasmids		
pEAP1	pSET152 carrying *erm*E, the apramycin resistance-conferring gene *aac*(3)IV was replaced by the ampicillin resistance-conferring gene *bla*, Amp^R, Erm^R	Ni et al. (2014)
pSPU341	pIJ2925 carrying *Spe*I enzyme cutting site, the P*hrd*B promoter, *to* terminator in the multiple cloning site, Amp^R	This study
pM1	pSPU341 carrying P*hrd*B-*kan*M1-*to*, Amp^R, Erm^R	This study
pSPUM1	pEAP1 carrying P*hrd*B-*kan*M1-*to*, used in generating *M. echinospora* BM1 and CM1, Amp^R, Erm^R	This study
pM2	pSPU341 carrying P*hrd*B-*gen*M2-*to*, Amp^R, Erm^R	This study
pSPUM2	pEAP1 carrying PSP44-*gen*M2-*to*, used in generating *M. echinospora* BM2 and CM2, Amp^R, Erm^R	This study
pM1M2	pSPU341 carryingPSP44-*gen*M2-P*hrd*B-*kan*M1-*to*, Amp^R, Erm^R	This study
pSPUM1M2	pEAP1 carrying PSP44-*gen*M2-P*hrd*B-*kan*M1-*to*, used in generating *M. echinospora* BM1M2 and CM1M2, Amp^R, Erm^R	This study

[a] Amp^R, ampicillin resistance; Erm^R,erythromycin resistance.