Kinetic model of metabolic network for xiamenmycin biosynthetic optimisation

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Abstract: Xiamenmycins, a series of prenylated benzopyran compounds with anti-fibrotic bioactivities, were isolated from a mangrove-derived Streptomyces xiamenensis. To fulfil the requirements of pharmaceutical investigations, a high production of xiamenmycin is needed. In this study, the authors present a kinetic metabolic model to evaluate fluxes in an engineered Streptomyces lividans with xiamenmycin-oriented genetic modification based on generic enzymatic rate equations and stability constraints. Lyapunov function was used for a viability optimisation. From their kinetic model, the flux distributions for the engineered S. lividans fed on glucose and glycerol as carbon sources were calculated. They found that if the bacterium can utilise glucose simultaneously with glycerol, xiamenmycin production can be enhanced by 40% theoretically, while maintaining the same growth rate. Glycerol may increase the flux for phosphoenolpyruvate synthesis without interfering citric acid cycle. They therefore believe this study demonstrates a possible new direction for bioengineering of S. lividans.

1 Introduction

Xiamenmycins, a series of prenylated benzopyran compounds, were isolated from a mangrove-derived Streptomyces xiamenensis [1–3]. Streptomyces are well-known microbial cell factory for bioactive secondary metabolites based on various biosynthetic pathways [4]. The natural products with benzopyran skeleton have been demonstrated to have considerable bioactivities, including anti-fungi [5], anti-herbivore [6], and anti-inflammatory, which have been widely used as traditional medicine [7]. It is recently reported that xiamenmycin A can significantly attenuate hypertrophic scar formation and suppress local inflammation, which may serve as a potential medical candidate against excessive fibrotic disease [1–3]. Since fibrotic diseases, such as idiopathic pulmonary fibrosis, liver cirrhosis, systemic sclerosis, progressive kidney disease, and cardiovascular fibrosis, are some of serious threats to public health [8, 9]. Thus, identifying bioactive molecules that can reduce fibrosis and improving the production for pharmaceutical investigation have become an urgent topic.

It is well known that the production level of most secondary metabolites is always too low to satisfy the industrial production [10]. To fulfil the requirements of pharmaceutical investigations, a large-scale culture and high production for secondary metabolites are now top of the priorities in the research. However, a bottleneck in bioengineering of secondary metabolites is lack of in silico model which can guide the enhancement of production. In this paper, we present a kinetic model for benzopyran production in Streptomyces.

Some in silico works on metabolic network modelling based on flux balance analysis (FBA) have been applied to the bacteria aiming for strain improvement with desired properties [10–12]. Although FBA can be a good method to obtain the optimal fluxes for biomass production, the production of secondary metabolites does not fall into the objective as it does not coincide with rapid growth of Streptomyces. We use a new kinetic metabolic modelling approach in the present paper [10, 13].

Kinetic modelling of metabolic network is a potential useful tool to simulate the biological processes in vivo and predict the flux in various circumstances. Most importantly, it tests the time-dependent dynamics of metabolites against the steady solutions obtained in the modelling. Traditionally, kinetic modelling is hindered by the numerous unknown enzymatic parameters. In this paper, we have employed two methods to overcome the main obstacles. We first use generic enzymatic rate equation which standardises and reduces the parameters needed to construct a model. We then further imposed on the metabolic network a stability requirement by introducing regulations on the reaction parameters. The metabolic network is thus regulated in such a way that it adjusts to a set of parameters that guarantee its stability.

We are able to successfully obtain a set of parameters for genetically engineered Streptomyces lividans with glucose feed that are not only stable against metabolic fluctuations but show a reasonable amount of xiamenmycin. To further verify the in silico solution, we also simulated the strain growing on a combination of glucose and glycerol media. We found that xiamenmycin production rate is significantly higher in the combination, which is consistent with experiments.

The links between primary and secondary metabolisms in the biosynthesis can be explored. Phosphoenolpyruvate (PEP) is the key intermediate supporting xiamenmycin biosynthesis because the flux increases of PEP synthesis from 2-phosphoglycerate (2-PG) associates with the output flux of xiamenmycin. Adding glycerol as an extra carbon source is predicted to enhance xiamenmycin production. Calculations show glycerol may provide another carbon source for PEP without interfering TCA cycle. The framework established in this paper sets the foundation for more difficult tasks in the research. It can provide a blue print and ways to evaluate engineered S. lividans with xiamenmycin-oriented genetic modification aiming to enhance xiamenmycin production.
2 Results and discussion

2.1 Construction of bioreaction network

Streptomyces is known for producing novel secondary metabolites and for highly effective processing of bioactive compounds. Xiamenmycin is one of the leading anti-fibrotic compounds isolated from S. xiamensis [14, 15]. The biosynthetic pathway has been elucidated encoded by a gene cluster named Xim A−E [16]. It was found that the biosynthetic pathway is closely related to central metabolism and starts with the formation of 4-hydroxynonic acid (4HB) by XimC [16]. The linkage of the geranyl side chain (GPP) to the benzene nucleus is catalysed by Xim B, then a possible epoxide intermediate is generated and cyclised by Xim D and Xim E. The forming of the subsequent amide bond connected the threonine (Thr) moiety with the geranyl side chain (GPP) to the benzene nucleus is catalysed by Xim B, then a possible epoxide intermediate is generated and cyclised by Xim D and Xim E. The forming of the subsequent amide bond connected the threonine (Thr) moiety with the

2.2 In silico simulation of xiamenmycin production

Our purpose is to understand the bioprocess of secondary metabolites and provide guidance for optimising the production of xiamenmycin biosynthesis including the flux distribution.

Table 1

| ID | Equation | Flux | ID | Equation | Flux |
|----|----------|------|----|----------|------|
| 1  | 2-PG + 3-PG → GDP + 3-PG | 0.09137 | 43 | 4-HB + 4HB → ATP + 2 ADP | 0.14 |
| 2  | 2-PG + PEP → GDP + 3-PG | 0.09137 | 44 | ATP + G6P → GDP + 3-PG | 0.14 |
| 3  | PEP + CO2 + OAA + Pi → GDP + 3-PG | 0.14 | 45 | ATP + G6P → GDP + 3-PG | 0.14 |
| 4  | OAA + NADH + Mal + NAD → GDP + 3-PG | 0.14 | 46 | ATP + G6P → GDP + 3-PG | 0.14 |
| 5  | Pyr + CoA + NAD → Ac-Coa + CoA + ATP | 0.09137 | 47 | ATP + G6P → GDP + 3-PG | 0.14 |
| 6  | Ac-Coa + OAA → Cit + Coa | 0.09137 | 48 | ATP + G6P → GDP + 3-PG | 0.14 |
| 7  | Cit-accon → Cit | 0.09137 | 49 | ATP + G6P → GDP + 3-PG | 0.14 |
| 8  | Cit-accon → Cit + Coa | 0.09137 | 50 | ATP + G6P → GDP + 3-PG | 0.14 |
| 9  | 2 ox-Phosphate + Cit → Succ-Coa + ATP | 0.09137 | 51 | ATP + G6P → GDP + 3-PG | 0.14 |
| 10 | succ + ATP + Coa + Coa + Pi + ADP → ATP + ADP + ATP | 0.09137 | 52 | ATP + G6P → GDP + 3-PG | 0.14 |

Continued
| ID | Metabolite                                      | Abbreviation | Biomass Glucose | Biomass Glucose + Glycerol |
|----|------------------------------------------------|--------------|----------------|---------------------------|
| 1  | nicotinamide adenine dinucleotide              | NAD          | 0.8            | 0.8                       |
| 2  | nicotinamide adenine dinucleotide phosphate    | NADP         | 0.2            | 0.2                       |
| 3  | adenosine triphosphate                         | ATP          | 2.5            | 2.5                       |
| 4  | serine                                         | Ser          | 0.03           | 0.03                      |
| 5  | 3-phosphoglycerate                              | 3-PG         | 0              | 0                         |
| 6  | 2-phosphoglycerate                              | 2-PG         | 0              | 0                         |
| 7  | phosphoenolpyruvate                             | PEP          | 0.05           | 0.05                      |
| 8  | oxaloacetate                                   | OAA          | 0.2            | 0.2                       |
| 9  | malate                                         | Mal          | 0              | 0                         |
| 10 | acetyl-CoA                                      | Ac-CoA       | 0.2            | 0.2                       |
| 11 | pyruvate                                       | Pyr          | 0.2            | 0.2                       |
| 12 | coenzyme A                                     | CoA          | 0.23           | 0.23                      |
| 13 | citrate                                        | Cit          | 0              | 0                         |
| 14 | cis-aconitate                                   | cis-acon     | 0              | 0                         |
| 15 | alpha-ketoglutarate                            | a-KG         | 0.07           | 0.07                      |
| 16 | succinic acid                                   | Succ          | 0.03           | 0.03                      |
| 17 | succinate                                      | Succ          | 0.03           | 0.03                      |
| 18 | fumarate                                       | Fum          | 0              | 0                         |
| 19 | flavin adenine dinucleotide                    | FAD          | 0              | 0                         |
| 20 | glucose 6-phosphate                            | G-6-P        | 0              | 0                         |
| 21 | fructose 6-phosphate                           | F-6-P        | 0.07           | 0.07                      |
| 22 | fructose 1,6-biphosphate                       | FBP          | 0              | 0                         |
| 23 | dihydroxyacetone phosphate                     | DHAP         | 0              | 0                         |
| 24 | glyceraldehyde 3-phosphate                     | TP           | 0.01           | 0.01                      |
| 25 | 1,3-bisphosphoglycerate                        | 1,3-BPG      | 0              | 0                         |
| 26 | 6-phosphogluconate                             | 6-PG         | 0              | 0                         |
| 27 | ribose 5-phosphate                             | R-5-P        | 0.05           | 0.05                      |
| 28 | ribulose 5-phosphate                           | Ru-5-P       | 0              | 0                         |
| 29 | xylulose 5-phosphate                           | Xu-5-P       | 0              | 0                         |
| 30 | sedoheptulose 7-phosphate                      | S-7-P        | 0              | 0                         |
| 31 | erythrose-4-phosphate                          | E-4-P        | 0.02           | 0.02                      |
| 32 | glutamate                                      | Glu          | 0.07           | 0.07                      |
| 33 | ubiquinone                                     | Q            | 0              | 0                         |
| 34 | cytochrome-c (oxidised)                        | Cyt-Cox      | 0              | 0                         |
| 35 | nicotinamide adenine dinucleotide (reduced)    | NADH         | 0.8            | 0.8                       |
| 36 | nicotinamide adenine dinucleotide phosphate (reduced) | NADPH   | 0.2            | 0.2                       |
| 37 | adenosine diphosphate                          | ADP          | 2.5            | 2.5                       |
| 38 | flavin adenine dinucleotide (reduced)          | FADH2        | 0              | 0                         |
| 39 | ubiquinol                                      | OH2          | 0              | 0                         |
| 40 | cytochrome-c (reduced)                         | Cyt-Cred     | 0              | 0                         |
| 41 | carbon dioxide                                 | CO2          | 0              | 0                         |
| 42 | ammonium                                       | NH4          | 0.06           | 0.1                       |
| 43 | phosphate                                      | Pi           | 0.19996        | 0.4                       |
| 44 | membrane-bound proton                          | H            | 0              | 0                         |
| 45 | D-glycerol-3-phosphate                         | glycerol-3P  | 0              | 0                         |
| 46 | glycerol                                       | glycerol     | 0              | 0                         |
| 47 | 3-deoxy-D-arabino-heptulosonic-7-phosphate      | DAHP         | 0              | 0                         |
| 48 | 3-dehydroquinate                               | DHQ          | 0              | 0                         |
| 49 | 3-dehydroshikimate                             | DHS          | 0              | 0                         |
| 50 | shikimate                                       | shikimate    | 0              | 0                         |
| 51 | shikimate 3-phosphate                          | shikimate-3P | 0              | 0                         |
| 52 | 5-enolpyruvyl-shikimate 3-phosphate             | EPSP         | 0              | 0                         |
| 53 | chorismate                                     | chorismate   | 0              | 0                         |
| 54 | 4-Hydroxybenzoic acid                         | 4HB          | 0              | 0                         |
| 55 | 3-octaprenyl-4-hydroxybenzoic acid             | G-4HB        | 0              | 0                         |
| 56 | xiamenmycin B                                 | XiaB         | 0              | 0                         |
| 57 | xiamenmycin A                                 | XiaA         | 0              | 0.05                      |
| 58 | geranyl pyrophosphate                          | GPP          | 0              | 0                         |
| 59 | L-threonine                                    | Thr          | 0              | 0                         |
| 60 | adenosine monophosphate                        | AMP          | 0              | 0                         |
| 61 | 1-deoxy-D-xylulose-5-phosphate                 | DXP          | 0              | 0                         |
| 62 | 2-C-methyl-D-erythritol 4-phosphate             | MEP          | 0              | 0                         |
| 63 | 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol | CDP-ME   | 0              | 0                         |
| 64 | 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol | CDP-MEP | 0              | 0                         |
| 65 | 2-C-methyl-D-erythritol 2,4-cyclophospho        | ME-CPP       | 0              | 0                         |
| 66 | 1-Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate  | HMBPP        | 0              | 0                         |
| 67 | isopentenyl diphosphate                        | IPP          | 0              | 0                         |
| 68 | dimethylallyl diphosphate                      | DMAPP        | 0              | 0                         |
| 69 | cytidine-triphosphate                          | CTP          | 0              | 0                         |
| 70 | cytidine-5'-monophosphate                      | CMP          | 0              | 0                         |
| 71 | cytidine-diphosphate                           | CDP          | 0              | 0                         |
| 72 | oxidised ferredoxin                            | ox-FD        | 0              | 0                         |
| 73 | reduced ferredoxin                             | re-FD        | 0              | 0                         |
| 74 | L-aspartate                                    | Asp          | 0              | 0                         |
| 75 | L-aspartate 4-phosphate                        | Asp-4P       | 0              | 0                         |
| 76 | L-aspartate-semi-aldehyde                      | Asp-SA       | 0              | 0                         |
| 77 | L-homoserine                                   | HSER         | 0              | 0                         |
| 78 | O-phospho-L-homoserine                         | PSER         | 0              | 0                         |
| 79 | glucose                                        | Glc          | 0              | 0                         |
| 80 | acetate                                        | Acetate      | 0              | 0                         |
identification of the key reactions. From our kinetic simulation, the flux distributions feed on various carbon sources were predicted (Table 1) and the carbon efficiency was used as a criterion for evaluating the production. By setting the value of biomass as a fixed number, we simulated the metabolic behaviour under the same growth rate. The predicted steady-state flux distribution using glucose as preferential carbon source was shown in Fig. 1 when growing on GYM (glucose 4 g/l, yeast extract 4 g/l, malt extract 10 g/l, and pH 7.2–7.4) medium.

Glycerol is one of the carbon sources for Streptomyces metabolism which can support the fastest growth rate for S. lividans [17]. We investigated the glycerol plus media for improvement of xiamenmycin production in silico since S. xiamenesis also contained the glpCABX genes for glycerol utilisation. Thus, glycerol may serve as another carbon source when we try to improve the compositions of medium. We simulated the flux distribution when growing on mixture carbon sources (Fig. 2 and Table 1). Theoretically, the maximum efficiency of xiamenmycin production is 20% on GYM media (glucose), 27% on GYM (glucose) plus glycerol media as total. The efficiency is 28.8% on glucose in the combined media. While maintaining the same growth rate, adding glycerol as an extra carbon source is predicted to enhance xiamenmycin production by 40%. As shown in Fig. 3, PEP is the key intermediate supporting xiamenmycin biosynthesis because the flux increases of PEP synthesis from 2-PG (reaction 2) associates with the output flux of xiamenmycin (reaction 50). Calculations show glycerol may provide another carbon source for PEP without interfering TCA cycle through glycerol degradation pathway to enter the glycolysis by seven steps, which is glycerol \(\rightarrow\) glycerol-3P \(\rightarrow\) DHAP \(\rightarrow\) TP \(\rightarrow\) 1,3-bPG \(\rightarrow\) 3-PG \(\rightarrow\) 2-PG \(\rightarrow\) PEP.

### 2.3 Discussion

In the metabolic engineering aspect, increase of productivity has been investigated experimentally and theoretically. There are still many problems need to be solved. One of the difficulties of experimental design is to find possible bottlenecks of metabolic network [18]. There are a lot of possible metabolic feedback loops that we can modify by genetic strategies for metabolic engineering, including increasing the precursor supply, overexpressing or increasing the efficiency of bottleneck enzyme, altering the regulation of gene expression, reducing flux toward unwanted products or competing pathway, and reconstructing entire pathways in a heterologous host [18]. To predict the production and flux under various circumstances and provide a

![Fig. 1](image)  
**Fig. 1** Main metabolic pathways and fluxes for production of xiamenmycin using glucose as carbon source. (A total of eight pathways are presented for xiamenmycin biosynthesis from glucose, containing 82 reactions and 86 metabolites. Prediction of fluxes is given for each reactions. Abbreviations of metabolites are listed in Table 2.)
rational guidance for experiments, kinetic modelling of metabolic network is a straightforward solution for us. However, previously due to the complexity of the biological systems and the diverse of enzymatic rate equation, it is impossible to manually adjust the parameters of kinetic model. Our kinetic model of large-scale metabolic network based on a generic enzymatic rate equation \[19\]. In the generic form, kinetic parameters are reduced to a manageable level \[20\]. The generic rate equation is symmetrical in both directions of reversible reaction and formally exact under the quasi-steady-state condition. We used the carbon metabolism of \textit{Methylobacterium extorquens} AM1 as a model study \[13\].

Even with reduced parameters, since there are complex regulations and non-linear function, the steady solutions for the kinetic model found by trial and error is not possible for large-scale network. Previously, there was no standard method for systematical adjustments. We demonstrated that a dynamical network may be stabilised with a simple forward form of regulation constructed on Lyapunov function derived from stochastic \[21\].

### 3 Materials and methods

#### 3.1 Generic enzymatic rate equation

Though the large number of parameters in our kinetic model make it difficult to determine all the parameters experimentally, the full knowledge of mechanistic reaction rates is not always necessary in order to correctly characterise the behaviour of the organism. This is because physiologically metabolite concentrations are usually restricted to a rather narrow subspace of the whole range \[13\]. Moreover, enzymes catalyse most biological reactions. Therefore, we are able to construct the kinetic model based on generic enzymatic rate equation with a minimum set of parameters, see supplementary material I.

A chemical reaction can be written in the general form of (1)

\[
A_1 + A_2 + \cdots + A_m \rightleftharpoons P_1 + P_2 + \cdots + P_n
\]

Each \(A_i\) or \(P_j\) can be the same substrate as the previous \(A_i\) or they can be a different metabolite. In this way, the stoichiometry is specified. Implicit in the above is an enzyme which appears unbound on both sides of the reactions \[13, 21, 22\].

A generic enzymatic rate equation can be written in the general form of (2) (see (2))

\[
f_1(V_F, V_B) \prod_{i=1}^{m} \frac{[A_i]}{K_i} + f_2(V_F, V_B) \prod_{j=1}^{n} \frac{[P_j]}{K_j} = 1
\]

To summarise, we use generic rate equation for our calculations. This general form of enzymatic rate equation requires a minimum set of parameters, that is, the maximal forward and backward reaction velocities \((V_F\) and \(V_B))\), and the Michaelis–Menten-like parameters \((K_i)\).

#### 3.2 Metabolic optimisation by network Lyapunov function

We introduce a mathematic method to stabilise a dynamic network with a simple and straightforward form of regulation constructed based on network Lyapunov function.
Consider a metabolic network with $N$ metabolites whose dynamics can be described by (6)

$$\frac{dx}{dt} = Su - b = f(x, V)$$

with metabolite concentrations $x$ and parameter set $V$. $S$ is the stoichiometric matrix and $b$ is a vector containing inputs and outputs to the system as well as maintenance energy requirements, generally named as boundary [23]. We used the precursor requirements in *M. extorquens* AM1 adjusted to our growth rate of *Streptomyces* [23].

We found that the regulatory dynamics, as given by (7) and (8)

$$W(x, V)\frac{dV}{dt} = -\nabla_{\phi} \phi(x, V)$$

$$\phi(x, V) = -\nabla_{\phi} \phi(x, V) \cdot f(x, V)$$

stabilises the original network (6). Here $W(x, V)$ is a positive-definite modulation matrix and $\phi(x, V)$ is a Lyapunov function for the metabolites dynamical network [21, 22]. The steady-state solutions are then presented in this paper. In our previous publication, (7) was used to obtain suitable parameters $V_I$ and $V_b$ [21, 22]. This equation is assumed to govern a dynamical system whose Lyapunov function takes the form of $\psi = \phi$, in which $S$ scales inversely with diffusion matrix $D$. In our calculation, we assumed a simple diagonal form of $D$ and $S$, by assuming smaller metabolite compounds having bigger $D$, inversely linked to the number of carbon chain. We used $V_I = 0.4$, $V_b = 0.4$ as initial values. In this range, different initial values result in slightly output fluxes of slightly different carbon efficiencies. In (7), we set $W = I$. Using these input values, (7) is solved by MATLAB software package ode23t for $t = 0:100,000 s$. The output files are (Metabolites,f, Reactions_f) for calculation represented in Table 1. More information was provided in supplementary material II. $V_I$ and $V_b$ for reactions were optimised by calculations using (7) and (8), as explained more in supplementary material II. Glucose and glutamate were used as the nutrition in GYM media. Glycerol was added as another carbon source afterwards as a comparison. Biomass is defined as flux exchanges between the metabolites considered in the model and the rest not included in our model, which can be estimated according to the growth rate. The values of biomasses for *Streptomyces* have been increased by ten times, compared with *M. extorquens* AM1, due to different growth rate. Xiamennymycin A was used as our target output compound, whose biomass was set to be $-0.05$ accordingly.

### Fig. 3

Changes of selected fluxes using glucose and glycerol as mixture carbon source

### 4 Conclusions

The primary focus of metabolic engineering is on enhancement of productions. On the basis of an optimised kinetic metabolic modelling, we demonstrate in silico that a significant improvement on the production of xiamennymycin can be obtained when adding glycerol feed as an additional carbon source. The flux distributions of genetically engineered *S. lividans* were calculated. In addition, our modelling offers a practical approach to deal with unavailable kinetic parameters for metabolic network modelling.

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