Data Article

Data set of in silico simulation for the production of clavulanic acid and cephamycin C by *Streptomyces clavuligerus* using a genome scale metabolic model

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**Abstract**

*Streptomyces clavuligerus* (*S. clavuligerus*) is a Gram-positive bacterium which produced clavulanic acid (CA) and cephamycin C (CephC). In this data article, a curated genome scale metabolic model of *S. clavuligerus* is presented. A total of eighteen objective functions were evaluated for a better representation of CA and CephC production by *S. clavuligerus*. The different objective functions were evaluated varying the weighting factors of CA and CephC between 0, 1 y 2, whereas for the case of biomass the weight factor was varied between 1 and 2. A robustness analysis, by mean of flux balance analysis, showed five different metabolic phenotypes of *S. clavuligerus* as a function of oxygen uptake: (I) and (II) biomass production, (III) biomass and CephC production, (IV) simultaneous production of biomass, CA and CephC and (V) production of biomass and CA. Data of shadow prices and reduced cost are also presented.

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https://doi.org/10.1016/j.dib.2019.103992
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1. Data

A total of twenty-four reactions were added for a better representation of the production of clavulanic acid (CA) and cephamycin C (CephC) by *Streptomyces clavuligerus* (see Table 1). An array of eighteen combinations of different objectives functions varying the weighting factor of the slack variables was evaluated (see Table 2). The objective function was the maximization of biomass, CA and CephC. In order to evaluate the functionally of the objective functions the weighting factor of biomass, CA and CephC were varied (see experimental design). Table 2 also shows the metabolic scenarios where CA and CephC are produced or not.

The objective function No. 6 was the only one that included a metabolic phenotype that produced CA and CephC, simultaneously. Table 3 shows the fluxes of biomass, CA and CephC under different oxygen uptake for all eighteen combinations of the objective function (see also supplementary material 1).

Fig. 1 shows five different metabolic phenotypes of *S. clavuligerus* as a function of oxygen uptake: (I) and (II) biomass production, (III) biomass and CephC production, (IV) simultaneous production of biomass, CA and CephC and (V) production of biomass and CA. See also supplementary material 2.

2. Experimental design, materials, and methods

2.1. Model

The genome scale metabolic model reported by Ramirez-Malule et al. (2018) was used as starting point [10]. The published model consists of 1510 reactions (1305/205 internal/exchange fluxes) and 1187 metabolites (982/205 internal/external metabolites). The model was curated manually according to KEGG pathway (https://www.genome.jp/kegg/) and enzyme database (https://www.enzyme-database.org/). The improved metabolic model encompassed 1534 reactions (1322/212 internal/exchange fluxes) and 1199 metabolites (987/212 internal/external metabolites). Cytoscape was used to visualize unconnected reactions in the metabolic network [11].
2.2. Flux balance analysis

Flux balance analysis (FBA) was used to determine metabolic states [12,13]. Loop law constrains was applied to all FBA simulation ensuring that infeasible loops were not allowed [14]. The production of biomass, CA and CephC was used as objective function.

| Reaction Comment | Reference |
|-------------------|-----------|
| lys_L[c] <= 15dap[c] + co2[c] | Intracellular reaction/Added [1] |
| xyl_D[c] <= xylu_D[c] | Intracellular reaction/Added [2] |
| tre[c] + h2o[c] <= 2 glc_D[c] | Intracellular reaction/Added [3] |
| atp[c] + Dall[c] <= adp[c] + all6p[c] | Intracellular reaction/Added [4] |
| galur[c] <= dtgt[c] | Intracellular reaction/Added [5] |
| tsul[c] + cn[c] <= so3[c] + tcynt[c] | Intracellular reaction/Added [6] |
| xil[c] + nadp[c] <= xylu_L[c] + nadph[c] + h[c] | Intracellular reaction/Added [7] |
| aces[c] + tsul[c] <= sucys[c] + ac[c] | Intracellular reaction/Added [8] |
| xylu_L[c] <= lyx_L[c] | Intracellular reaction/Added [9] |
| mndl[c] <= cyan[c] + bزال[c] | Intracellular reaction/Added [9] |
| digalur[c] + h2o[c] <= 2 galur[c] | Intracellular reaction/Added [9] |
| LalaDglu[c] <= LalaLglu[c] | Intracellular reaction/Removed |
| dtgt[e] <= dtgt[c] | Transport reaction/Added |
| Dal[e] <= Dal[c] | Transport reaction/Added |
| mndl[e] <= mndl[c] | Transport reaction/Added |
| cn[e] <= cn[c] | Transport reaction/Added |
| sucys[e] <= sucys[c] | Transport reaction/Added |
| digalur[e] <= digalur[c] | Transport reaction/Added |
| xil[e] <= xil[c] | Transport reaction/Added |
| dtgt[e] | Exchange reaction/Added |
| Dal[e] | Exchange reaction/Added |
| mndl[e] | Exchange reaction/Added |
| cn[e] | Exchange reaction/Added |
| sucys[e] | Exchange reaction/Added |
| digalur[e] | Exchange reaction/Added |
| xil[e] | Exchange reaction/Added |

Table 1

| No. | Reaction Comment | Reference |
|-----|-----------------|-----------|
| 1   | lys_L[c] <= 15dap[c] + co2[c] | Intracellular reaction/Added [1] |
| 2   | xyl_D[c] <= xylu_D[c] | Intracellular reaction/Added [2] |
| 3   | tre[c] + h2o[c] <= 2 glc_D[c] | Intracellular reaction/Added [3] |
| 4   | atp[c] + Dall[c] <= adp[c] + all6p[c] | Intracellular reaction/Added [4] |
| 5   | galur[c] <= dtgt[c] | Intracellular reaction/Added [5] |
| 6   | tsul[c] + cn[c] <= so3[c] + tcynt[c] | Intracellular reaction/Added [6] |
| 7   | xil[c] + nadp[c] <= xylu_L[c] + nadph[c] + h[c] | Intracellular reaction/Added [7] |
| 8   | aces[c] + tsul[c] <= sucys[c] + ac[c] | Intracellular reaction/Added [8] |
| 9   | xylu_L[c] <= lyx_L[c] | Intracellular reaction/Added [9] |
| 10  | mndl[c] <= cyan[c] + bزال[c] | Intracellular reaction/Added [9] |
| 11  | digalur[c] + h2o[c] <= 2 galur[c] | Intracellular reaction/Added [9] |
| 12  | LalaDglu[c] <= LalaLglu[c] | Intracellular reaction/Removed |

Table 2

| No. | Objective function | Weighting factors | Robustness analysis: oxygen |
|-----|--------------------|-------------------|----------------------------|
|     |                    | Biomass | Clavulanic acid | Cephamycin C | Biomass | Clavulanic acid | Cephamycin C |
| 1   |                    | 1       | 0               | 0           | YES     | NO               | NO           |
| 2   |                    | 1       | 0               | 1           | YES     | NO               | NO           |
| 3   |                    | 1       | 0               | 2           | YES     | NO               | YES          |
| 4   |                    | 1       | 1               | 0           | YES     | YES              | NO           |
| 5   |                    | 1       | 1               | 1           | YES     | YES              | NO           |
| 6   |                    | 1       | 1               | 2           | YES     | YES              | YES          |
| 7   |                    | 1       | 2               | 0           | YES     | YES              | NO           |
| 8   |                    | 1       | 2               | 1           | YES     | YES              | NO           |
| 9   |                    | 1       | 2               | 2           | YES     | YES              | NO           |
| 10  |                    | 2       | 0               | 0           | YES     | NO               | NO           |
| 11  |                    | 2       | 0               | 1           | YES     | NO               | NO           |
| 12  |                    | 2       | 0               | 2           | YES     | NO               | NO           |
| 13  |                    | 2       | 1               | 0           | YES     | YES              | NO           |
| 14  |                    | 2       | 1               | 1           | YES     | YES              | NO           |
| 15  |                    | 2       | 1               | 2           | YES     | YES              | NO           |
| 16  |                    | 2       | 2               | 0           | YES     | YES              | NO           |
| 17  |                    | 2       | 2               | 1           | YES     | NO               | NO           |
| 18  |                    | 2       | 2               | 2           | YES     | NO               | NO           |
| No. Objective function | Biomass (h⁻¹) | Clavulanic acid (mmol/gCDW*h) | Cephamicyn C (mmol/gCDW*h) |
|------------------------|--------------|------------------------------|---------------------------|
|                        | Oxygen uptake (mmol/gCDW*h) | 2.1 | 4.35 | 9.15 | 14.1 | 2.1 | 4.35 | 9.15 | 14.1 | 2.1 | 4.35 | 9.15 | 14.1 |
| 1                      |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2                      |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 3                      |               | 1,433 | 1,917 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 4                      |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 5                      |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6                      |               | 1,433 | 1,917 | 2,541 | 2,848 | 0.000 | 0.000 | 0.952 | 2.151 | 0.000 | 0.205 | 0.108 | 0.000 |
| 7                      |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 8                      |               | 1,433 | 1,974 | 2,581 | 2,848 | 0.000 | 0.196 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 9                      |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 10                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 11                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 12                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 13                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.970 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 14                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.707 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 15                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.000 | 0.707 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 16                     |               | 1,433 | 2,156 | 2,848 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 17                     |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |
| 18                     |               | 1,433 | 1,992 | 2,581 | 2,848 | 0.000 | 0.222 | 1.069 | 2.151 | 0.000 | 0.000 | 0.000 | 0.000 |

**Fig. 1.** Profile of biomass, CA and CephC while varying oxygen uptake for the objective function No. 6.
2.3. Optimization problem statement

Metabolic fluxes were quantified by means of a two-stage optimization approach, which is a combination of the maximization of the objective function and minimization of the overall flux [10,15,16]. The mathematical problem can be represented as follows:

Stage one

\[
\text{maximize } Z = \left( w_{\text{biomass}} v_{\text{biomass}} + w_{\text{CA}} v_{\text{CA intracellular}} + w_{\text{CephC}} v_{\text{CephC intracellular}} \right) \tag{1}
\]

subject to: \( S^* v = 0 \)

\( v_{lb} \leq v \leq v_{ub} \)

Stage two:

\[
\text{minimize } \sum v_i^2 \tag{2}
\]

subject to: \( S^* v = 0 \)

\( v_{\text{biomass}} = v_{\text{optbiomass}} \)

\( v_{\text{CA extracellular}} = v_{\text{optCA extracellular}} \)

\( v_{\text{CephC extracellular}} = v_{\text{optCephC extracellular}} \)

\( v_{lb} \leq v \leq v_{up} \)

where \( Z \) is the objective function, \( S \) is the stociorometric matrix and \( v \) is the flux vector. \( w_{\text{biomass}}, w_{\text{CA}} \) and \( w_{\text{CephC}} \) are the weighting factors for biomass, intracellular flux of CA and CephC, respectively. \( v_{\text{biomass}}, v_{\text{CA intracellular}} \) and \( v_{\text{CephC intracellular}} \) are the biomass flux, intracellular flux of CA and CephC, respectively. \( v_{\text{optbiomass}}, v_{\text{optCA extracellular}} \) and \( v_{\text{optCephC extracellular}} \) are the optimal values for biomass and extracellular flux of CA and CephC, respectively, that resulted from solving the problem stated at stage one.

The first stage optimization problem was solved using a Gurobi solver, with a feasibility tolerance of \( 10^{-6} \), while the second stage was solved using the MATLAB’s built-in fmincon solver, with a first order optimality and a maximum constraint violation within \( 10^{-6} \).

Different objective functions were evaluated varying the weighting factors of CA and CephC between 0, 1 y 2, whereas for the case of biomass the weight factor was varied between 1 and 2 (see Table 2).

2.4. Robustness analysis

A robustness analysis was carried out to evaluate the functionally of the objective function when the optimal flux of oxygen was varied [12,13]. The identification of possible gene knockout was made by
sensitivity analysis using the concept of reduced costs. The reduced cost values represent the variation of the objective functions with respect to the fluxes related to each reaction and they are represented according to the equation (3). Additionally, the shadow prices were determined following the equation (4) [13,17].

\[
Z = Z_0 + \rho_i v_i, \quad \rho_i = -\frac{\partial Z}{\partial v_i}
\]

\[
\pi_i = -\frac{\partial Z}{\partial b_i}
\]

Where, \(\rho_i\) is the reduced cost, \(Z_0\) is the optimal solution, \(v_i\) is an internal flux that is not in the basis solution, \(\pi_i\) is the shadow prices and \(b_i\) is the exchange fluxes.

2.5. Computational tools

COBRA Toolbox v.3.0 synchronized with Matlab® as programming environment, and the Gurobi optimizer 7.5.2 was used to solve all optimization problems [18].

Transparency document

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2019.103992.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.103992.

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