Text S1. The detailed descriptions of the biomass composition and FK506 biosynthesis by S. tsukubaensis

1. Biomass composition

The macromolecular composition of the cell was partly measured and partly estimated from literature data. Dry weight was measured by washing cells with 0.9% NaCl solution and drying them at 80 °C on a pre-weighted filter until a steady weight was achieved. Biomass components contain protein, RNA, DNA, lipids, small molecules, cell wall components (peptidoglycan, carbohydrate and teichoic acid). Total protein was determined using the Bradford method [1], which was estimated based on a standard curve of bovine serum albumin (25–200 μg/mL). The protein content of samples for our experiments was within the range 40–50% of dry cell mass. Pellet hydrolysates were used to measure amino acid composition. The amino acid composition of the protein fraction has also been determined by Agilent HPLC systems (Agilent 1200, USA) with Agilent Zorbax Eclipse column (4.6 × 150 mm). No differences were observed in composition within the dilution rate range of 0.1-0.5 h⁻¹. Total carbohydrate was measured by the phenol/sulfuric acid method [2]. Cellular composition was estimated based on a standard curve of glucose (10–200 μg/mL). The carbohydrate content of samples was within the range 10–15% of dry cell mass, thus 1250 μg/mL of freeze-dried biomass was used in order to acquire a carbohydrate concentration that would be within the mid-standard range. Total DNA was determined by the diphenylamine method using a hot perchloric acid extraction of freshly-harvested biomass [3]. Samples were estimated based on a calf-thymus DNA standard solutions (100–500 μg/mL). Total RNA content was determined using an KOH/UV-based procedure based on calf liver RNA standard solutions (100–500 μg/mL) [4]. Ash composed of inorganic compounds and ions, as well as intracellular metabolites, were not taken into account in the model. The biomass equation consists of biomass components and the growth-associated ATP consumption. Based on the data shown in Table S1.1, the biomass equation used in the model is:
0.446 protein + 0.163 RNA + 0.042 DNA + 0.027 phospholipid + 0.018 triacylglycerol + 0.030 small molecules + 0.137 peptidoglycan + 0.0082 teichoic acids + 0.055 carbohydrate + 47 ATP -> biomass + 47 ADP + 47 Pi

The stoichiometric coefficients have unit g/g DCW.

**Table S1.1:** Macromolecular composition (biomass formation) of *S. tsukubaensis*.

| Biomass component | Component (g/g DCW) |
|-------------------|---------------------|
| Protein           | 0.4460\(^a\)        |
| DNA               | 0.0420\(^a\)        |
| RNA               | 0.1630\(^a\)        |
| Phospholipid      | 0.0273\(^b\)        |
| Triacylglycerol   | 0.0182\(^b\)        |
| Small molecules   | 0.0300\(^b\)        |
| Peptidoglycan     | 0.1368\(^b\)        |
| Carbohydrate      | 0.0547\(^a\)        |
| Teichoic acid     | 0.0820\(^b\)        |

\(^a\)Calculated for an average macromolecular composition of *S. tsukubaensis* with starch as carbon sources and few yeast extract, peptone as nitrogen sources. Biomass composition was experimentally measured at the exponential growth phase of aerobic batch cultivation (specific growth rate: 0.0502 h\(^{-1}\)).

\(^b\)Data was summarized from [5,6].
The amino acid composition was determined by an Agilent HPLC systems at 354 nm.

Table S1.2: Protein composition of *S. tsukubaensis*.

| Amino acid type | Molecular weight (g/mol) | Composition (% g/g protein) | Composition (mmol/g) |
|-----------------|--------------------------|-----------------------------|----------------------|
| Alanine         | 71.09                    | 10.52                       | 1.508                |
| Arginine        | 156.20                   | 3.89                        | 0.256                |
| Asparagine      | 114.12                   | 5.08                        | 0.445                |
| Aspartate       | 115.10                   | 5.15                        | 0.448                |
| Cysteine        | 103.15                   | 2.02                        | 0.196                |
| Glutamate       | 129.13                   | 7.80                        | 0.612                |
| Glutamine       | 128.15                   | 4.41                        | 0.345                |
| Glycine         | 57.07                    | 7.66                        | 1.378                |
| Histidine       | 137.16                   | 2.66                        | 0.194                |
| Isoleucine      | 113.17                   | 6.82                        | 0.603                |
| Leucine         | 113.17                   | 3.84                        | 0.339                |
| Lysine          | 128.19                   | 2.22                        | 0.173                |
| Methionine      | 131.21                   | 3.84                        | 0.301                |
| Phenylalanine   | 147.19                   | 4.83                        | 0.328                |
| Proline         | 97.13                    | 8.80                        | 0.916                |
| Serine          | 87.09                    | 4.51                        | 0.518                |
| Threonine       | 101.12                   | 6.55                        | 0.658                |
| Tryptophan      | 186.23                   | 1.48                        | 0.097                |
| Tyrosine        | 163.19                   | 2.59                        | 0.159                |
| Valine          | 99.15                    | 5.33                        | 0.539                |

Energy requirement for polymerization (mmol ATP/g) = 40.04
The DNA composition was calculated based on the genome information of \textit{S. tsukubaensis}. GC content of \textit{S. tsukubaensis} is 71.5\%, which is then used to calculate the ratio of the nucleic acids in the DNA. The polymerization energy was assumed to be the same as in \textit{E. coli} \cite{7}.

\begin{table}
\centering
\caption{DNA composition of \textit{S. tsukubaensis}.}
\begin{tabular}{lcccc}
\hline 
DNTP type & Molecular weight (g/mol) & Composition \textit{(\%mol)} & Composition \textit{(mmol/g)} \\
\hline 
DAMP & 313.24 & 14.25 & 0.4611 \\
DCMP & 289.18 & 35.75 & 1.1567 \\
DTMP & 304.20 & 14.25 & 0.4611 \\
DGMP & 329.21 & 35.75 & 1.1567 \\
\hline 
\end{tabular}
\end{table}

Energy requirement for polymerization (mmol ATP/g) = 4.4

The RNA composition was determined from genomic data assuming the following composition: 5\% mRNA, 75\% rRNA and 20\% tRNA \cite{5}. The polymerization energy was assumed to be the same as \textit{E. coli} \cite{7}.

\begin{table}
\centering
\caption{RNA composition of \textit{S. tsukubaensis}.}
\begin{tabular}{lccccccc}
\hline 
\multirow{2}{*}{NTP type} & \multicolumn{3}{c}{mol/mol RNA} & \multirow{2}{*}{Molecular weight (g/mol)} & \multicolumn{2}{c}{Composition (mol/mol RNA)} & \multicolumn{2}{c}{Composition (mmol/g RNA)} \\
& mRNA & rRNA & tRNA & \multicolumn{2}{c}{(mol/mol RNA)} & \multicolumn{2}{c}{(mmol/g RNA)} \\
\hline 
AMP & 0.139 & 0.195 & 0.198 & 329.21 & 0.192 & 0.600 \\
GMP & 0.361 & 0.238 & 0.342 & 345.21 & 0.265 & 0.826 \\
CMP & 0.361 & 0.340 & 0.289 & 305.18 & 0.331 & 1.031 \\
UMP & 0.139 & 0.228 & 0.171 & 306.17 & 0.212 & 0.662 \\
\hline 
\end{tabular}
\end{table}

Energy requirement for polymerization (mmol ATP/g) = 1.25
The composition of phospholipids was assumed to be the same as *E. coli*. Biosynthesis of phospholipids and fatty acids components are included in the reaction set.

**Table S1.5**: Phospholipid composition of *S. tsukubaensis*.

| Phospholipids              | Molecular weight<sup>a</sup> (g/mol) | Composition<sup>b</sup> (g/g) | Composition (mmol/g) |
|----------------------------|--------------------------------------|-------------------------------|----------------------|
| Phosphatidylethanolamine   | 690.84                               | 0.75                          | 1.086                |
| Cardiolipin                | 1349.59                              | 0.07                          | 0.052                |
| Phosphatidylinositol       | 824.90                               | 0.18                          | 0.218                |

Energy requirement for polymerization (mmol ATP/g) = 2.63

<sup>a</sup> The molecular weight was calculated from chemical structure of the phospholipid by assuming an average molecular weight of different fatty acids.

<sup>b</sup> Three components in phospholipids were assumed to be the same with the compositions existing in *S. coelicolor* model [5].

**Table S1.6**: Fatty acid composition of *S. tsukubaensis* for synthesis of phospholipid.

| Fatty acids | Molecular weight<sup>a</sup> (g/mol) | Composition<sup>a</sup> (% (w/w)) | Composition (mmol/g) | Composition (mol/mol) |
|-------------|--------------------------------------|----------------------------------|----------------------|-----------------------|
| C14:0       | 227.37                               | 1.2                              | 0.05                 | 0.013                 |
| C15:0       | 241.40                               | 42.6                             | 1.77                 | 0.450                 |
| C16:0       | 255.43                               | 11.3                             | 0.44                 | 0.113                 |
| C17:0       | 269.45                               | 44.5                             | 1.65                 | 0.421                 |
| C18:1       | 267.44                               | 0.4                              | 0.01                 | 0.004                 |

<sup>a</sup> Data was taken from *S. coelicolor* composition [5].
Table S1.7: Triacylglycerol composition of *S. tsukubaensis*.

| Fatty acids       | Molecular weight (g/mol) | Composition^a^ (mol/mol) | Composition (mmol/g) |
|-------------------|--------------------------|--------------------------|----------------------|
| Glycerol-3-phosphate | 174.08                  | 1                        | 1.244                |
| C14:0             | 227.37                   | 0.040                    | 0.050                |
| C15:0             | 241.40                   | 1.349                    | 1.677                |
| C16:0             | 255.43                   | 0.338                    | 0.421                |
| C17:0             | 269.45                   | 1.262                    | 1.570                |
| C18:1             | 267.44                   | 0.011                    | 0.014                |

Energy requirement for polymerization (mmol ATP/g) = 1.24

^a^ Data was taken from *S. coelicolor* composition [5].
It was assumed that the selected small molecules were equally presented (w/w) in the pool.

Table S1.8: Small molecules pool composition of *S. tsukubaensis*.

| Molecules | Molecular weight (g/mol) | Pool of small molecules (g/g) | Composition (mmol/g) |
|-----------|--------------------------|-------------------------------|----------------------|
| NAD    | 664.438                  | 0.125                         | 0.188                |
| NADP   | 744.418                  | 0.125                         | 0.168                |
| COA    | 767.534                  | 0.125                         | 0.163                |
| ACP    | 10689                    | 0.125                         | 0.012                |
| MK     | 853.365                  | 0.125                         | 0.146                |
| THF    | 445.434                  | 0.125                         | 0.281                |
| FMN    | 456.348                  | 0.125                         | 0.274                |
| FAD    | 785.557                  | 0.125                         | 0.159                |
Table S1.9: Peptidoglycan composition of *S. tsukubaensis*.

| Peptidoglycan composition       | Molecular weight<sup>a</sup> (g/mol) | Ratio<sup>b</sup> (mol/mol) | Composition (mmol/g) |
|---------------------------------|--------------------------------------|-----------------------------|----------------------|
| N-acetylmuramic acid            | 275.26                               | 0.88                        | 1.0069               |
| N-acetylglucosamine             | 203.19                               | 1.05                        | 1.1969               |
| Alanine                         | 71.08                                | 1.67                        | 1.8999               |
| Diaminopimelic acid             | 154.07                               | 1.00                        | 1.1399               |
| D-glutamate                     | 129.12                               | 0.89                        | 1.0145               |
| Glycine                         | 39.04                                | 0.85                        | 0.9727               |

Energy requirement for polymerization (mmol ATP/g) = 5.026

<sup>a</sup> Glycine and diaminopimelic acid were required for peptidoglycan polymerization with molecule of water. Other components were required without molecule of water.

<sup>b</sup> Data was taken from average molecular composition of peptidoglycan from *S. coelicolor* [5].
Table S1.10: Carbohydrate composition of *S. tsukubaensis*.

| Carbohydrate composition   | Molecular weight (g/mol) | Ratio\(^a\) (mol/mol) | Composition (mmol/g) |
|---------------------------|--------------------------|------------------------|----------------------|
| UDP-N-acetylglucosamine   | 203.19                   | 1                      | 1.897                |
| UDP-D-galactose           | 162.14                   | 2                      | 3.794                |

\(^a\) Data was taken from *S. coelicolor* composition [5].
| Teichoic acids composition     | Molecular weight (g/mol)<sup>a</sup> | Ratio<sup>b</sup> (mol/mol) | Composition (mmol/g) |
|-------------------------------|--------------------------------------|----------------------------|---------------------|
| Polyglycerophosphate chain    | 1848.71                              | 1                          | 0.5177              |
| Lysine                        | 128.17                               | 0.25                       | 0.1294              |
| N-acetylglucosamine           | 203.19                               | 0.25                       | 0.1294              |

Energy requirement for polymerization (mmol ATP/g) = 0.129

<sup>a</sup>The molecular weight of polyglycerophosphate chain was calculated using \((C_3H_7O_5P)_{12}\) as molecular formula [5].

<sup>b</sup>Data was taken from *S. coelicolor* composition [5].
2. FK506 biosynthesis

Biosynthesis of FK506 macrolide is composed of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS), similar with FK520 and rapamycin. Generally, FkbA, FkbB and FkbC modules consist of three common elements, including ketoacylsynthase, acyltransferase (AT) and acyl carrier protein (ACP), which are responsible for chain elongation. Some modules also contain dehydratase (DH), enoylreductase (ER) and ketoreductase (KR), which catalyze the reduction reactions. After using a chorismate-derived 4,5-dihydroxycyclohex-1-enecarboxylic acid (DHCHC) as the starter unit, the PKSs assemble ten two-carbon extensions into chains [8]. The ten extender units contain two malonyl-CoA, five methylmalonyl-CoA, two methoxymalonyl-ACP and an unusual allylmalonyl-CoA. After the condensation, a lysine-derived pipicolate catalyzed by FkbL, incorporates into the resulting polyketide intermediate with an amide bond [9]. This reaction is catalyzed by the FkbP peptide synthetase, which cyclizes the 23-membered core. For the last step, post-PKS processing reactions are catalyzed by a specific 31-O-methyl transferase FkbM and a P450 C9 hydroxylase FkbD.

For ten extender precursors, the biosynthetic pathways are different. Malonyl-CoA can be transformed from acetyl-CoA by acetyl-CoA carboxylase, similar with fatty acid biosynthesis pathway. For methylmalonyl-CoA synthesis, there are four metabolic pathways according to presented literatures. First, propionyl-CoA can be catalyzed to methylmalonyl-CoA by propionyl-CoA carboxylase [10,11]. Second, conversion of succinyl-CoA to methylmalonyl-CoA is catalyzed by two steps, encoded by methylmalonyl-CoA mutase and epimerase, respectively [12,13]. Next, isobutyryl-CoA which is originated from valine catabolism need several steps of oxidation (3-methyl-2-oxobutanoate dehydrogenase, butyryl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase, methylmalonate-semialdehyde dehydrogenase, aldehyde dehydrogenase) [14]. Lastly, the exogenous methylmalonate can be transported into cells via dicarboxylate carrier protein and malonyl/methylmalonyl-CoA synthetase pathway [15]. Of course, the last step is also applied to malonyl-CoA due to the shared enzyme.
Besides, the PKSs in FK506 biosynthesis incorporate two unusual extender units named methoxymalonyl-ACP and allylmalonyl-CoA. The methoxymalonyl-ACP biosynthesis utilizes glycolytic pathway intermediate 1,3-biphosphoglycerate as the starter. An unusual ACP protein FkbJ is loaded onto glyceroyl by FkbH, generating glyceroyl-ACP. Subsequently, two oxidases, FkbK (oxidizes 3-hydroxyacyl-CoA compounds to 3-oxoacyl-CoA compounds, mediated by NAD\(^+\)) and FkbI (oxidizes saturated acyl-CoA compounds to 2,3-enoyl-CoA compounds, mediated by FAD), and an O-methyltransferase FkbG modify the glyceroyl-ACP to methoxymalonyl-ACP [16]. The \textit{tcsABCD} identified from the FK506 biosynthetic gene cluster have been demonstrated to catalyze the allylmalonyl-CoA synthesis [17,18]. In detail, the propionyl-acylated TcsB catalyzes the condensation of the propionyl-CoA with malonate loaded on TcsA, generating 3-oxopentanoyl-ACP, and subsequently is converted to 2-pentenyl-ACP by KR and DH of fatty acid synthase. Then, the 2-pentenyl-ACP is further reduced by TcsC (mediated by NADPH) following a carboxylation step. Last step is a dehydrogenation by TcsD (mediated by FAD), generating the allylmalonyl-ACP, which is subsequently converted to allylmalonyl-CoA, a unique extender unit for the FK506 side chain. Similar with the allylmalonyl-CoA, the propylmalonyl-CoA is biosynthesized by TcsA, TcsB and TcsC, but without TcsD [17,18]. The TcsC also catalyzes crotonyl-CoA to ethylmalonyl-CoA by a similar way [18]. The propylmalonyl-CoA and ethylmalonyl-CoA can be incorporated into the initial polyketide chain to form 37,38-dihydro-FK506 and FK520, respectively.

Detailed reactions can be found in Additional file 3.
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