Supplementary Material

Investigation of the Relation between Temperature and M13 Bacteriophage Production via ATP Expenditure

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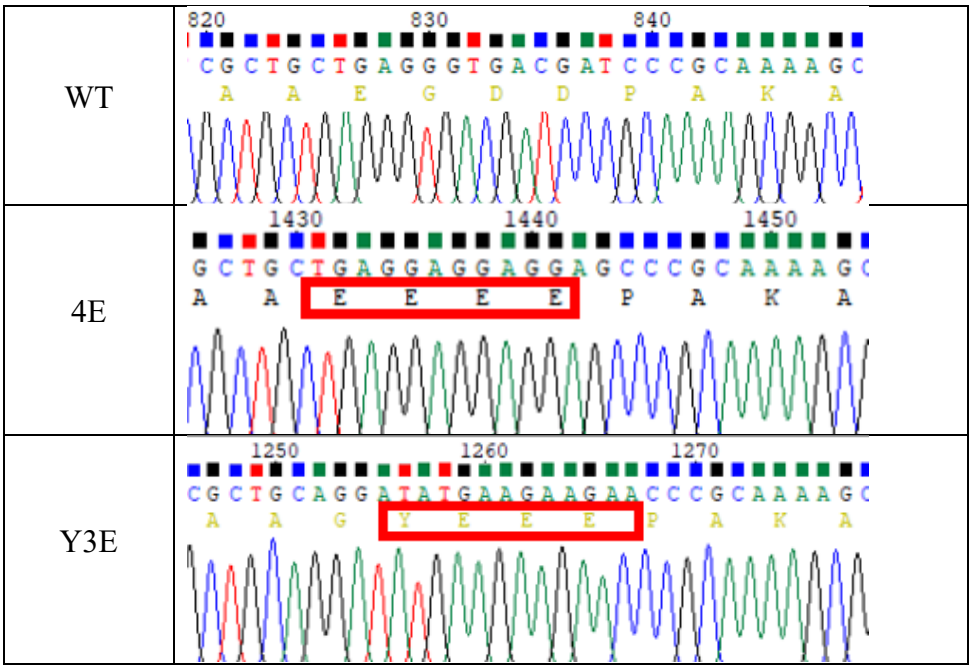


Figure S1. Comparison of the major coat protein p8 genetic sequence at the N terminus in genetically engineered M13 phages.

M13 phage can express a specific peptide or protein at the N terminus of the major coat protein p8 *via* genetic engineering, which provides various properties to the phage surface [1]. For genetic modification of the major coat protein, the desired peptide sequence is inserted using the inverse PCR cloning method. For this study, we introduced 4E (four glutamates; EEEE) and Y3E (one tyrosine, three glutamates; YEEE) peptides to the major coat protein p8 of wild type (WT) M13 phages. For 4E genetic modification, the peptide insertion provided a larger negative surface charge to the phage, whereas Y3E modification enabled cross-linking with other Y3E modified phages through di-tyrosine cross-linking as well as an increase in surficial negative charge.

Table S1. Factors of M13 phage production affected by temperature alteration.

Culture Temperature	25℃	30℃	37℃
Specific Growth Rates (1/h)	0.201 (for 0−16 h)	0.283 (for 0−12 h)	0.085 (for 0−16 h)
Mass Fraction of all ribosomal proteins	0.04	0.06	0.03
Ribosomal Efficiency (aa/rib/s)	9.53	10.57	6.55

The specific growth rates of XL1-Blue *E. coli* fermented with phage at different culture temperatures were calculated using Equation 3, excluding 37 ℃. The calculated specific growth rate at 30 ℃ was ~6-fold the value of 37 ℃ and ~3-fold the value of 25 ℃. Faster growth rate is advantageous for the translation of ribosomal proteins, which is expressed through the values of ribosomal protein mass fraction and ribosomal efficiency. Mass fraction of all cellular ribosomal proteins and ribosomal efficiency were calculated using Equation S1 and Equation S2, respectively. 30 ℃ culture temperature produced the largest mass fraction, which was ~1.5-fold the value of 25 ℃ and ~2-fold the value of 37 ℃. Ribosomal efficiency was also largest at 30 ℃, followed by 25 ℃ and 37 ℃.

Equation (S1). Calculation of the mass fraction of all ribosomal cellular proteins.

The coarse-grained model was applied to the measurement of mass fractions of all ribosomal cellular proteins and ribosomal efficiency using the specific growth rates of phage infected *E. coli* [2]. This model was developed and modified from a previously existing model [3-5], which focuses on four different factors: the production rate of ribosomal proteins, the production and gradation of non-ribosomal proteins (NRPs), the production and utilization rate of energy from adenosine triphosphate (ATP), and the steady-state specific growth rate of the cell. The modified coarse-grained model mainly focuses on the ATP, ribosome, and NRP of the *E. coli*. However, this model ignores certain factors and has several limitations: (i) Various processes in the *E. coli* (glucose transport through cell membranes, RNA transcription, ribosome protein translation) are not addressed in detail and are implied in the calculation equations; (ii) ATP produced from fermentation metabolism is ignored and only ATP from cell respiration is considered; (iii) Energy efficiency for biomass is difficult to measure, but is calculated using a simple equation that excludes several important factors.

The following equation obtained from the coarse-grained model was used to identify the relationship between the specific growth rate and mass fraction of all ribosomal cellular proteins in phage infected *E. coli* [2]. The equation calculates the mass fraction using the concentrations and molecular weights of ribosomes, ATP, and NRPs:

$$\phi(\lambda) = \phi_0 + \frac{\lambda}{k'_p \times f_p^{\infty}} \text{ , where } \phi_0 = \left(\frac{\gamma}{k'_p \times f_p^{\infty}}\right) \quad \text{(S1)}$$

$\phi(\lambda)$ represents the mass fraction of all the ribosomal cellular proteins at a specific growth rate, λ represents the specific growth rate of the cells, f_p^{∞} represents the maximum fraction of ribosomes translating NRPs (= 0.7), k'_p represents the rate of NRP elongation per ribosome (=20 aa/s) [6], and γ represents the NRP degradation rate (= 0.1 NRP per total NRP per hour) [7].

Equation (S2). Calculation of the ribosomal efficiency based on specific growth rates.

Ribosomal efficiency can be defined as the net peptide elongation rate. The following equation was obtained from the coarse-grained model and was used to identify the peptide elongation rate per ribosome to calculate ribosomal efficiency at a specific growth rate:

$$K_{per}=N_r \frac{\lambda}{\phi(\lambda)} \quad \text{(S2)}$$

K_{per} represents the peptide elongation rate (ribosomal efficiency) and N_r represents the respective number of amino acid residues per ribosome [2].

Equation (S3). Calculation of the average estimated residual energy for M13 phage production.

$$\text{Average } Residual\ Envergy_{Estimated}\ (\%) = \sum_{t=\beta}^{16} \frac{OD_{600\ E.\ coli\ w/o\ phage,t} - OD_{600\ E.coli\ w/phage,t}}{OD_{600\ E.coli\ w/o\ phage}} \times 100\ (\beta = 3, 6, 9, 12, 16) \quad \text{(S3)}$$

$ATP_{Phage\ Production}$ represents the percentage of ATP converted to phage proteins of a batch at a specific culture temperature, t represents β hours of fermentation, $OD_{600\ E.\ coli\ w/o\ phage,\ t}$ represents the OD_{600} value at t produced without phage, and $OD_{600\ E.\ coli\ w/phage,\ t}$ represents the OD_{600} value at t produced with phage.

Equation (S4). Calculation of ATP expenditure for *E. coli* cell doubling without phage infection.

$$ATP_{Doubling\ (E.\ coli\ w/o\ phage),\ t}\ (\%) = 100 - ATP_{Residual\ (E.\ coli\ w/o\ phage),\ t} \quad \text{(S4)}$$

$ATP_{Doubling\ (E.\ coli\ w/o\ phage),\ t}$ represents the intracellular ATP expenditure percentage of *E. coli* produced without phage for cell doubling at t hours of fermentation and $ATP_{Residual\ (E.\ coli\ w/o\ phage),\ t}$ represents the residual intracellular ATP percentage of *E. coli* produced without phage

at *t* hours of fermentation that was not used for cell doubling.

Equation (S5). Calculation of ATP expenditure for *E. coli* cell doubling with phage infection.

$$ATP_{Doubling\ (E.\ coli\ w/phage),\ t}\ (\%) = ATP_{Doubling\ (E.\ coli\ w/o\ phage),\ t} \times \frac{OD_{600\ (E.\ coli\ w/phage),\ t}}{OD_{600\ (E.coli\ w/o\ phage),\ t}} \quad \text{(S5)}$$

$ATP_{Doubling\ (E.\ coli\ w/phage),\ t}$ represents the intracellular ATP expenditure percentage of *E. coli* produced with phage for cell doubling at *t* hours of fermentation, $OD_{600\ (E.coli\ w/phage),\ t}$ represents the OD_{600} of *E. coli* produced with phage at *t* hours of fermentation, and $OD_{600\ (E.coli\ w/o\ phage),\ t}$ represents the OD_{600} of *E. coli* produced without phage at *t* hours of fermentation.

Equation (S6). Calculation of total ATP expenditure of *E. coli* with phage.

$$ATP_{Total\ Expenditure\ (E.\ coli\ w/phage),\ t}\ (\%) = 100 - ATP_{Residual\ (E.\ coli\ w/phage),\ t} \quad \text{(S6)}$$

$ATP_{Total\ Expenditure\ (E.\ coli\ w/phage),\ t}$ represents the total intracellular ATP expenditure percentage of *E. coli* fermented with phage at *t* hours of fermentation and $ATP_{Residual\ (E.\ coli\ w/phage),\ t}$ represents the residual intracellular ATP percentage of *E. coli* fermented with phage at *t* hours of fermentation that was not used for cell doubling.

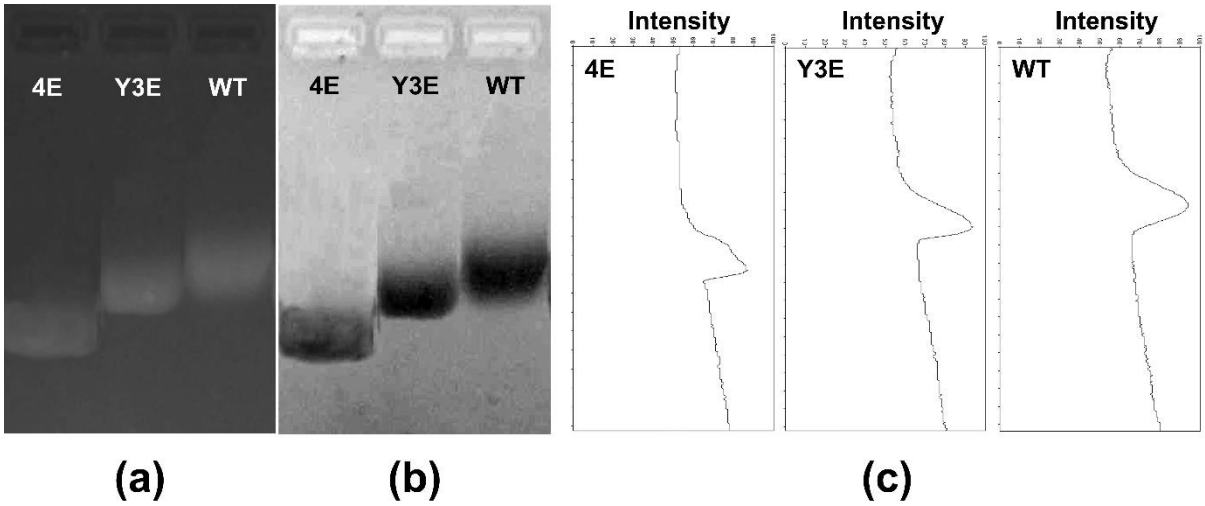
Equation (S7). Calculation of experimental ATP expenditure for phage production.

$$ATP_{phage\ production}\ (\%) = ATP_{Total\ Expenditure\ (E.\ coli\ w/phage),\ t} - ATP_{Doubling\ (E.\ coli\ w/phage),\ t} \quad \text{(S7)}$$

$ATP_{Total\ Expenditure\ (E.\ coli\ w/phage),\ t}$ represents the total intracellular ATP expenditure percentage of *E. coli* fermented with phage at *t* hours of fermentation and $ATP_{Doubling\ (E.\ coli\ w/phage),\ t}$ represents the intracellular ATP expenditure percentage of *E. coli* produced with phage for cell doubling

at *t* hours of fermentation.

Figure S2. Gel electrophoresis results of M13 phage purity.



Purity of WT and genetically engineered M13 phage (4E, Y3E) were confirmed by qualitative gel electrophoresis analysis. 20 μL of a 0.5 mg/mL phage stock solution (WT, 4E, and Y3E) was loaded into a 1.8 % agarose gel and electrophoresed at 1.4 V/cm for 8 h. After gel electrophoresis, the gel was immersed in 0.2 M NaOH solution for 30 min, stained with SYBR Green 1 (Invitrogen, USA), and qualitatively analyzed by observing the DNA band thickness and intensity under ultraviolet light [8]. 0.5 mg/mL 4E phage solution produced at 30 ℃ culture temperature (Lane 1), 0.5 mg/mL Y3E phage solution produced at 30 ℃ (Lane 2), and 0.5 mg/mL WT phage solution produced at 30 ℃ (Lane 3) were loaded into the wells of 1.8 % agarose gel. The individual mono-bands observed in the (**a**) original image and (**b**) inverted/contrast-regulated image of the gel confirmed that the purity of M13 phages produced at 30 ℃ culture temperature was identical regardless of genetic engineering, which was supported by (**c**) band intensity peaks analyzed using Gel-Analyzer.