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Estimating effects of yeast extract compositions on *Escherichia coli* growth by a metabolomics approach

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Bioprocess stability depends on the variety of yeast extract, which varies from lot-to-lots and between brands, thereby leading to variable bacterial growth and productivity in manufacturing processes. As a model experiment for good stability of bioprocesses, *Escherichia coli* growth in media containing different brands of yeast extract was evaluated and predicted using component-profiling and multivariate data analysis (metabolomics approach). The components of yeast extract were extracted from media containing varying concentrations of yeast extract and analyzed using gas chromatography-mass spectrometer. The yeast extract was categorized into three clades by principal component analysis (PCA). The *E. coli* growth using yeast extract showed approximately 30% difference at equivalent amount of supplementation. The bacterial growth in the media was estimated for the component profiles by partial least squares regression analysis (PLS-R). A predictive model was developed from the relationship between bacterial growth (as subjective attributes) and component profiles (as objective attributes), and correlation coefficients were calculated. Most of the amino acids in the media stimulated growth; however, methionine had negative effect on growth. In a culture validation, Asp, Val, Glu, and Try stimulated the bacterial growth, but Met inhibited. The other amino acids tested, Ser, Ile, Asp, Lys, Phe, Leu, Thr, and Gly did not show significant effects on the growth. The results indicate that the metabolomics approach can provide useful feedback information to improve the cultivation.

[**Key words:** Amino acid; *Escherichia coli*; Growth; Metabolome; Partial least squares regression analysis; Principal component analysis; Yeast extract]

Yeast extract (YE) is a complex raw material, usually produced from baker's or brewer's yeast through autolysis at ~50°C in the presence of solvents or salts (1,2). It is used in food industries as food flavor, additives, and vitamin supplements (3,4), and is also used as a supplemental component for microbial media (5,6). Recently, YE is also used as a supplemental component in serum-free media for mammalian cell cultivation and IgG production (7,8). It consists of a mixture of carbohydrates, amino acids, peptides, vitamins, and trace elements (9). The composition can vary between brands and influence production, for example, in the secondary metabolites of *Fusarium* (10). The lot–to-lots difference in yeast extract can lead to up to a 50% variation in the levels of biomass and growth rate of *Escherichia coli* (11). Saksinchai et al. (12) reported that iron, which is present in YE, is a limiting factor for growth, by-product formation, and sporulation, of *Bacillus thuringiensis* subsp. *kurosaki*. Therefore, YE quality affects the cell growth and productivity. In an industrial bioprocess, the instability of growth and production is, thus, a very serious problem. Therefore, to assure stable YE quality across batches, it become necessary to perform a laboratory screening prior to purchasing the optimal one, and to ensure a desirable performance in industrial processes.

Previously, some research studies were carried out for improving the optimized microbial processes. Potvin et al. (11) used an automated turbidimetric system to screen YE for *Lactobacillus plantarum* growth. Biomass level and μ_{max} indicated similar trends between automated turbidimetric system and shake-flasks, however, the automated turbidimetric system gave 30–50% lower values than those in controlled tank reactors. Correlation of recombinant fermentation yield with YE composition was characterized by near-infrared (NIR) spectroscopy, with models for predicting cell mass in 15-L fermentors and 2-L shake flasks (13). However, it is mechanistically difficult to suggest any insight for YE quality using NIR.

A metabolomics approach including food fingerprinting has been applied to predict geographical origin of hazelnuts (14), Japanese green tea (15,16), and sake (17) ranking, and to find discriminant markers for authentication of Asian palm civet coffee (Kopi Luwak) (18). Recently, Harada et al. (19) evaluated influences of yeast and lactic acid bacterium on soy source fermentation by gas chromatography–mass spectrometry (GC–MS) based non-targeted metabolic profiling. The approach can be applied for predicting microbial growth on the various brands and lots of YE. However, the feasibility of predicting microbial growth has never been presented, until now.

This paper describes a model experiment for predicting bacterial growth from hydrophilic low-molecular materials profiling,

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named substratome, by GC–MS analysis. A detailed study was carried out to observe the difference in growth of *E. coli* cultured in media containing YE derived from different manufacturers, using GC–MS, and the data were analyzed by statistical analysis to evaluate the influence of YE components on growth.

MATERIALS AND METHODS

Regents and yeast extract Methoxyamine hydrochloride was purchased from Wako Pure Chemical, Ltd. (Osaka, Japan). *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). YE was purchased from Becton, Dickinson and Company (BD Japan, Tokyo, Japan), Sigma–Aldrich Japan (Tokyo, Japan), Kyokuto Pharmaceutical Industrial, Co., Ltd. (Tokyo, Japan), and Nihon Pharmaceutical Co., Ltd. (Tokyo, Japan). Two different lots of YE were kindly provided from Oriental Yeast Co. Ltd. (Tokyo, Japan). YE samples were randomly assigned by alphabets (YE_a, YE_b, TE_c, YE_d, YE-e, and YE_f); fermentation performances will never be revealed.

Microorganism *E. coli* NBRC 3301 (synonym K-12) was purchased from the Bioresource Center of the National Institute of Technology and Evaluation (NITE), Japan. The strain was cultivated in LB broth, overnight at 37°C with continuous shaking, and the inoculum used in the experiments below. The culture broth was stored as frozen stocks with 30% glycerol at −80°C before use.

Culture conditions The frozen stocks (100 μl) inoculated in 50 ml LB in 500-mL shaking flasks at 30°C for 1 day with 200 rpm of orbital shaking, and the cultured cells were used for the experiments in this study. To evaluate the effect of yeast extract in stimulating bacterial growth, different concentrations of YE (0.5, 1.0, 2.0, and 5.0 g/l) were added to M9 broth (comprising 6.0 g/l Na_2HPO_6, 3.0 g/l KH_2PO_4, 0.5 g/*l*NaCl, 1.0 g/l NH_4Cl, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 4.0 g/l glucose, 30 mg/l $CaCl_2 \cdot 2H_2O$, and 20 mg/l thiamin hydrochloride), and the resulting media were used as the growth media. The above inoculum (each 1 ml) was transferred to 50 ml of the growth media in 500-ml shake flasks and cultivated at 30°C at 200 rpm. Growth in the various media were monitored at intervals of 3 h by measuring the absorbance at 600 nm using a spectrophotometer (V-630, JASCO Corporation, Tokyo, Japan). In the culture performed for model validation, 0.5 g/l of each amino acid (filter-sterilized) was added to growth media using YE-d. The other culture conditions were the same as those described above.

Analysis of hydrophilic compounds in YE by GC–MS analysis To avoid the high analytical background of medium components except YE, YE samples were prepared at final concentrations in water, and autoclaved at 121°C, 20 min. The autoclaved samples were used for GC–MS analysis. The samples (100 μl) and 20 mg/ml ribitol (60 μl) as an internal standard were well mixed. An extraction solvent consisting of a mixture of water/methanol/chloroform (1/2.5/1) was added to the mixture. After thorough mixing and centrifugation at 16,000 ×*g* at 4°C for 5 min, 600 μl of supernatant was transferred to a new tube, and the organic solvent was removed by using a centrifuge concentrator (MX-307, Tomy Seiko, Tokyo, Japan) for 2 h. The samples were then freeze-dried using a lyophilizer. Methoxyamine hydrochloride (20 mg/ml in pyridine) was added to the lyophilized samples, and incubated at 30°C for 90 min. After the incubation, *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide was added and incubated at 37°C for 30 min. The samples were directly injected to GC–MS for analysis.

Agilent GC–MS system, 7980B and 5977A MSD, was used for the hydrophilic component analysis. HP-5 ms UI (30 m × ϕ 0.25 mm × i.d. 0.25 μm) column was used. Helium gas was used as carrier gas at a rate of 5.0 ml/min. The oven temperature was set at 80°C for 2 min, elevated to 330°C at the rate of 15°C/min, and maintained at 330°C for 13 min. Split ratio was set at 25:1 (v/v). The transfer line and the ion source temperatures were set at 200°C and 230°C, respectively. Ions were generated by 70 eV electron beam, and 10 scans per second were recorded over the mass range of 85–1000 m/z. The acceleration voltage was turned on after solvent delay of 180 s.

Peaks were obtained from total ion chromatograms. Peak annotation were carried out using mass fragment peaks with assisted by the NIST database. To identified part of significant components, amino acids including L-Asp, L-Val, L-Glu, L-Trp, L-Tyr, L-Ser, L-Ile, L-Lys, L-Phe, L-Thr, Gly, L-Asn, L-Ala, and L-Met, used as references. The peak area of ribitol as internal standard was used for normalization of peaks. The calibrated area was standardized before performing partial least squares regression (PLS-R) analysis.

Multivariate analyses Principal component analysis (PCA) (20) and PLS-R (21) were performed by R 3.3.2 software. PCA were performed using correlation matrix (option; scale = T). PLS-R was performed with component profile of YE as explanatory variables and turbidity at 18 h after inoculation as the response variable in a model to predict growth. The number of latant variable used in the PLS-R analysis was determined using cross-validation procedure (validation = CV) up to comp 10, when root mean square error of prediction (RMSEP) was the smallest value. To evaluate the learning data qualities, different six patterns of learning and predicting data were used (Table 1). Latent variable was set at the number when the regression coefficient is the best value. Predicted data were visualized by plotting observed and predicted growth on the vertical and lateral axes. Correlation coefficients of each component were calculated from the model.

TABLE 1. Learning and test sample patterns for PLS-R analysis.

YE	Concn. (g/*l*)	Pattern 1	Pattern 2	Pattern 3	Pattern 4	Pattern 5	Pattern 6
YE_a	5.0	T	L	L	L	L	L
	2.0	T	L	L	L	L	L
	1.0	T	L	L	L	L	L
	0.5	T	L	L	L	L	L
YE_b	5.0	L	T	L	L	L	L
	2.0	L	T	L	L	L	L
	1.0	L	T	L	L	L	L
	0.5	L	T	L	L	L	L
YE_c	5.0	L	L	T	L	L	L
	2.0	L	L	T	L	L	L
	1.0	L	L	T	L	L	L
	0.5	L	L	T	L	L	L
YE_d	5.0	L	L	L	T	L	L
	2.0	L	L	L	T	L	L
	1.0	L	L	L	T	L	L
	0.5	L	L	L	T	L	L
YE_e	5.0	L	L	L	L	T	L
	2.0	L	L	L	L	T	L
	1.0	L	L	L	L	T	L
	0.5	L	L	L	L	T	L
YE_f	5.0	L	L	L	L	L	T
	2.0	L	L	L	L	L	T
	1.0	L	L	L	L	L	T
	0.5	L	L	L	L	L	T

L, learning data; T, test data.

RESULTS AND DISCUSSION

Flask cultivation To confirm the difference in the growth-stimulating performance of each YE variety/bland, YE was added to M9 minimal media at different concentrations and the culture was performed. Fig. 1 shows the growth curves of *E. coli* cultured in the YE-added media. In case of control experiment without YE, bacterial growth gradually increased to 2.45 in 24 h. The growth increased with the amount of YE added. All concentrations of YE, except for that in YE-d, resulted in increased cell growth to a turbidity of more than 9.0. However, the turbidity, in the case of 5.0 g/l YE-d addition, reached only 6.95 ± 0.08 at 24 h. At 18 h, turbidity in the media YE-a, YE-b, and YE-c increased to 10.80 ± 0.28, 10.50 ± 0.21, and 10.86 ± 0.32, respectively. Turbidity in YE-e and YE-f increased to 9.60 ± 0.50 and 9.13 ± 0.16, respectively, whereas that in YE-d was only 6.83 ± 0.09 at 18 h. In this study, we used turbidity at 18 h as a representative data of the bacterial growth in the various media for the following data analyses.

Component analysis of GC–MS To confirm the difference in low-molecular-weight hydrophilic components, GC–MS analysis was performed. Of the total 165 peaks detected from all samples, 109 peaks were assigned. Of those, 6 sugars, 26 amino acids, 20 glycosides, and 3 fatty acids were estimated, including different degrees of TMS derivatives. The remaining peaks were not classified. In the present study, TMS derivatives were treated as individual peaks in the following analysis. The composition of the component profiles are summarized in Table 2.

Similarity analysis of YE using PCA PCA was used to analyze the GC–MS data. PCA is a dimensional compression method with minimum loss of multivariate information. Principal component (PC) 1 is selected to have the greatest variance and PC2 is selected to have the greatest variance in subspace perpendicular to PC1. PCA score plots on the basis of component analysis by GC–MS are indicated in Fig. 2A. The PC1 represented the YE concentration added. When YE added more than 2.0 g/L used, the

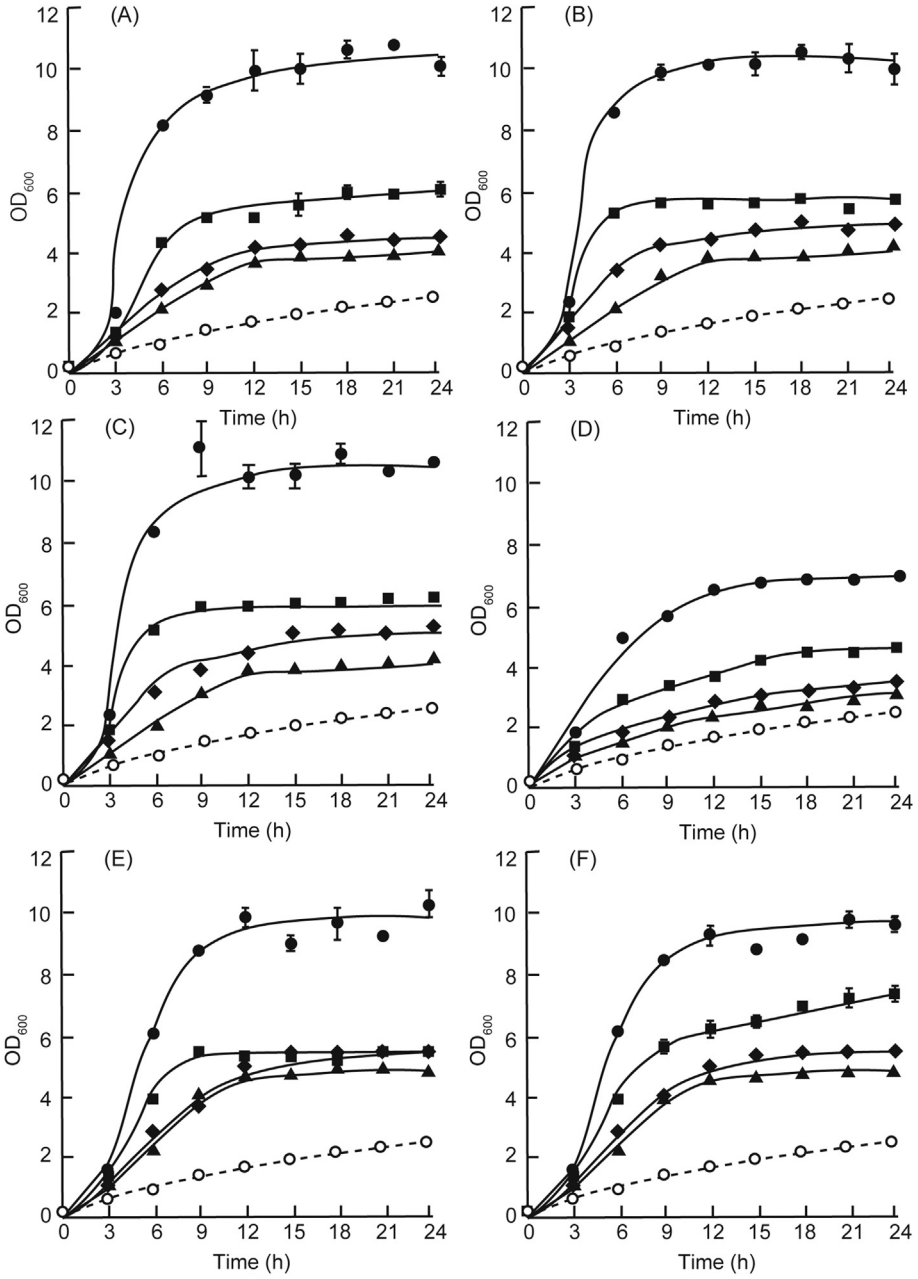

FIG. 1. Growth curves of *Escherichia coli* NBRC 3301 (K-12) in M9 supplemented with yeast extract (YE) of different brands and lots. (A) YE_a; (B) YE_b; (C) YE_c; (D) YE_d, (E) YE_e; (F) YE_f. Symbols: open circles, without YE (control); closed circles, 5.0 g/l YE added; closed squares, 2.0 g/l YE added; closed diamonds, 1.0 g/l YE added, closed triangles, 0.5 g/l YE added.

score plots demonstrate clustering of three groups: group 1 including YE-a, YE-b, and YE-c; group 2 including YE-d; group 3 including YE-e and YE-f. Lower concentration samples indicated the degree of similarity and higher concentration samples (5.0 g/l) demonstrated large gaps in PC2 between the clusters. However, the bacterial growths of group 1 were the largest in the three groups (Fig. 1). Therefore, the bacterial growth using the high concentration YE was not dependent on PC2. The loading plot of PC2 for amino acids and fatty acids detected (Fig. 2B), was indicative of the difference between the YE. Leu, Met, Phe, and Tyr, and fatty acids in group 3 were larger in comparison to group 1, and Ala, Val, Ile, Gly, Ser, Thr, Asp, Glu, Asn, Lys, and Trp in group 2 were larger when compared to group 1. The results indicate that amino acids and fatty acids may have an effect on the growth.

Construction of predictive models of GC–MS data and identification of significant compounds by PLS-R To identify the significant compounds responsible for causing the difference of *E. coli* growth, PLS-R was performed. PLS is a type of multiple regression analysis in which latent variables are calculated and used instead of the original explanatory variables in order to circumvent the problem of multicollinearity between variables. To evaluate the effect of moldering data on the correlation between prediction and observed values, six patterns of data sets were used. Series concentrations of each YE was used for test data, and those of the others were used for modeling data. Fig. 3 shows the results of PLS-R, relation of the observed growth and predicted growth for each of the cases. Good correlations between observed growth and predicted growth were observed in modeling data in all cases. The degree of agreement between

TABLE 2. Peaks assigned by NIST library search and the correlation coefficient values calculated by PLS-R.

RT (min)	Assigned chemicals	Probable (%)	Correlation coefficient
3.188	Lactic acid, 2TMS	42.68	0.030
3.344	Glycolic acid, 2TMS	38.18	0.021
3.433	Valine, TMS	94.07	0.055
3.645	Alanine, 2TMS	78.52	0.051
3.824	Glycine, 2TMS	85.30	0.063
3.947	Ethyl(2-hydroxyethyl)carbamic acid, 2TMS	33.17	−0.035
4.069	3-Hydroxypropionic acid, 2TMS	42.95	−0.00011
4.181	Leucine, TMS	89.73	0.055
4.282	Carbitol, TMS	30.99	0.032
4.404	Isoleucine, TMS	57.37	0.059
4.873	Valine, 2TMS	82.24	0.039
5.041	Ethanolamine, 2TMS	37.11	−0.0068
5.085	Urea, 2TMS	45.53	−0.019
5.275	Serine, 2TMS	97.41	0.051
5.375	Ethanolamine, 3TMS	59.78	0.020
5.442	Leucine, 2TMS	66.67	0.052
5.487	Phosphoric acid, 3TMS	81.73	0.0097
5.666	Isoleucine, 2TMS	74.16	0.036
5.777	Glycine, 3TMS	83.71	0.046
5.822	Succinic acid, 2TMS	87.21	−0.026
5.945	2,3-Dihydroxy-2-methylpropanoic acid, 3TMS	83.51	0.067
6.012	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-1,3-dimethyl, TMS	34.15	−0.029
6.079	Uracil, 2TMS	77.49	0.035
6.134	2-Butenedioic acid, (E)-, 2TMS	31.24	0.021
6.268	Amphetamine, 2TMS	71.44	0.040
6.324	Serine, 3TMS	93.18	0.033
6.581	Threonine, 3TMS	96.43	0.032
6.67	Methionine, TMS	67.41	−0.012
6.826	Aspartic acid, 2TMS	97.15	0.045
6.905	3-Aminoisobutyric acid, 3TMS	39.56	−0.081
7.329	Aspartic acid, 3TMS	45.89	0.053
7.452	Malic acid, 3TMS	55.47	0.032
7.552	Monoamidoethylmalonic acid, 3TMS	32.62	0.030
7.675	5-Oxoproline, 2TMS	77.05	0.042
7.708	Aspartic acid, 3TMS	84.56	0.046
7.82	Phenylalanine, TMS	89.79	0.067
7.931	5-Hydroxymethyl-2-furoic acid, 2TMS	46.55	0.022
8.255	Asparagine, 2TMS	54.45	0.036
8.49	Glutamic acid, 3TMS	82.67	0.050
8.88	Asparagine, 3TMS	88.12	0.039
9.137	Lysine, 3TMS	46.58	0.037
9.505	Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, TMS	32.7	−0.033
9.628	Glycerophosphoric acid, 4TMS	76.47	0.031
9.952	Ornithine, 4TMS	64.67	0.047
10.008	Citric acid, 4TMS	50.27	0.0049
10.309	Asparagine, 4TMS	39.78	0.029
10.644	Lysine, 4TMS	54.31	0.034
10.755	Tyrosine, 3TMS	59.50	0.025
11.336	Palmitic acid, TMS	78.44	0.040
11.827	*Myo*-Inositol, 6TMS	71.28	0.024
11.927	Guanine, TMS	49.51	0.026
12.285	Tryptophan, 1TMS	31.70	0.033
12.486	Tryptophan, 3TMS	72.77	0.0061
12.509	Stearic acid, TMS	67.30	0.036
12.709	Glycerophosphoric acid, 4TMS	38.23	0.025
13.792	Uridine, 4TMS	67.48	0.026
14.751	Adenosine, 4TMS	98.17	0.034

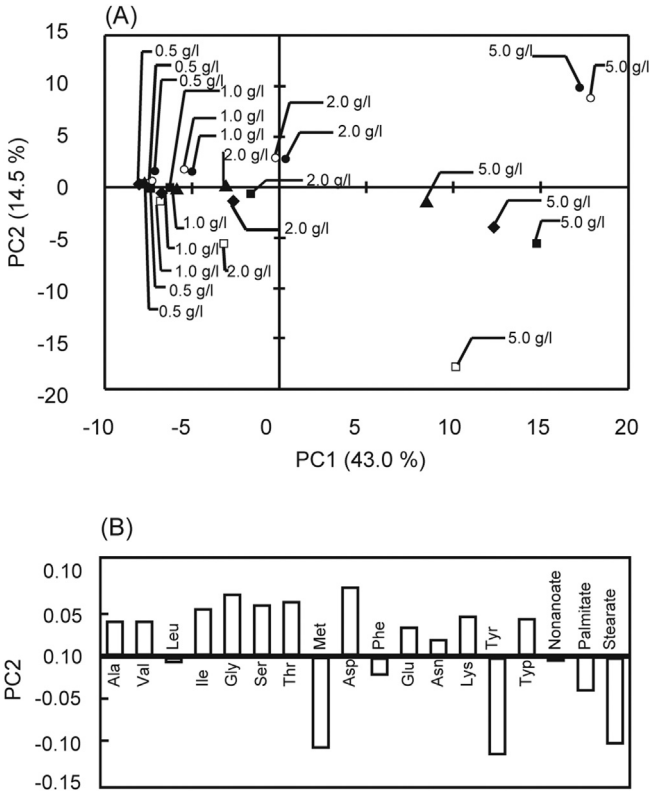

FIG. 2. (A) Score plots of PCA on the basis of detected GC–MS peaks. Symbols: closed diamonds, YE_a; closed triangles, YE_b; closed squares, YE_c; open squares, YE_d; closed circles, YE_e; open circles, YE_f. (B) Part of loading factors against PC2 of PCA.

the derived model and the data, R^2, were indicated in the range between 0.986 and 0.997. However, the predictive ability of the derived model, Q^2, were varied in the datasets. In case of YE-d used as test data (pattern 4), Q^2 indicated low value of 0.324, although Q^2 in the other cases were larger than 0.850. Generally, an R^2 of 0.65 or more and Q^2 of 0.5 or more indicate satisfactory quantitative predictive ability in PLS (22). In case of YE-d used as test data (pattern 4), the modeling data was not covered by the test data, as shown in Fig. 1. The difference between modeling and test data would lead the low Q^2 value. The others showed sufficient Q^2 values. Root mean square error (RMSE) was also calculated and the tendency was corresponding to that of Q^2 values. According to the results, accuracy of the predicted growth test data increased when similar modeling data was used for the modeling.

PLS-R can also be used to identify, through use of correlation coefficient of each compound, important variables that contribute substantially to model construction. Assigned peaks, the probable values of which showed >35% match with those in the NIST library, and identified peaks that corresponded to that of a standard compound were sorted as presented in Table 2. Compounds such as 3-aminoisobutyric acid (3-ABA), butanedioic acid (succinate), urea, Met, ethanol amine, and 3-hydroxypropionic acid (3HP) were estimated as growth-inhibitory compounds. Normal aminobutyric acids including 3-ABA have been reported as growth inhibitors of *E. coli*, and the inhibitory effects were not observed in the presence of amino acids such as Ala, Val, Leu, and Ile (23). 3HP has been previously reported to be toxic to *E. coli* over concentrations of 100 mM (24). Coexistence of succinate and glucose enhance the growth rate without stimulating glucose-uptake via inhibition of cAMP synthesis (25). Urea did not inhibit *E. coli* growth when the dose used was less than 5.0 g/l (26). 2,3-Dihydroxy-2-methylpropanoic acid was estimated as the best growth activator; however, the effect of the compound on *E. coli* growth has not been reported yet. Most amino acids, including Phe, Gly, Ile, Leu, Val, Asn, Ser, Glu, Asp, Lys, Tyr, 5-oxoproline, and ornithine, were also predicted as growth activators. Xiang et al. (27) reported that Arg and Ser increase *E. coli* cell growth in

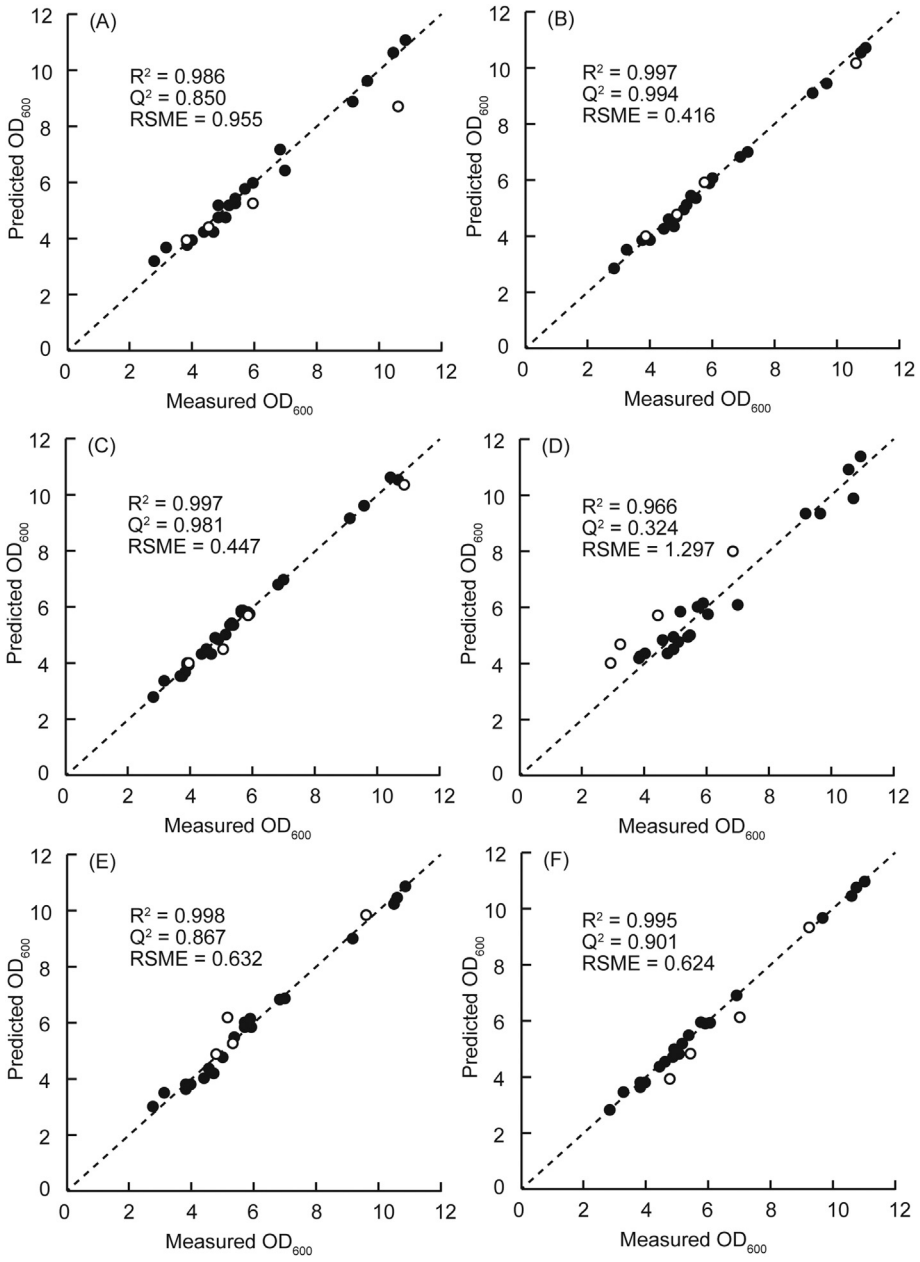

FIG. 3. Measured and predicted OD_{600} plots. (A) Pattern 1, (B) pattern 2, (C) pattern 3, (D) pattern 4, (E) pattern 5, (F) pattern 6. R^2 and Q^2 indicate validation metrics for learning data and for predicting data, respectively. RMSE: root mean square error. Symbols: closed circles, estimated by learning data; open circles, estimated by test data.

minimal medium using glycerol as the sole carbon source by 63% and 53%, respectively. The amino acids promote glycerol utilization and shorten the lag-phase. Nam et al. (28) reported that in a β-carotene producing recombinant *E. coli*, DH5α, growth was reduced in the presence of Met and Val, but increased in the presence of Gln, Ile, Leu, Lys, Phe, and Thr in the range of 0.2–4 g/l in a synthetic medium. Val is well known to inhibit the growth of *E. coli* K-12 and prevents the synthesis of Ile and Leu, when its concentration is above 4 mg/ml (29). However, when 20 mg/l Leu coexists with 40 mg/l Val, it did not have an effect on the growth of *E. coli* K-12. In the present case, as Val and Leu and/or Ile coexist in YE, the growth inhibition of Val could be observed in the analysis. Fatty acids, nucleosides, sugars, and sugar alcohols were also extracted as activators. Monosaccharides assigned as hexoses, including fructose, sorbose, mannose, and galactose, were detected at retention times of 10.454 and 10.543 min, the coefficients were indicated by 1.79×10^{-3} and 4.23×10^{-3}, respectively. Although most of the saccharides were not assigned, these components correlated roughly in positive with growth. Palmitic acid inhibits growth of *E. coli* O57:H7 in early growth phase (10 h) in a narrow concentration range (30); however, the small amounts used in this study did not fall within this inhibitory range (data not shown). In some cases, there is a possibility that the correlation between component concentrations and growth hardly represent the growth stimulation or inhibition effects of each component, because PLS-R can mention the correlation independent of the relationship between explanatory and response values, only when the relationship exists. However, the results seem to roughly evaluate how each component effects the cell growth.

Culture for model validation In order to confirm the accuracy of the PLS-R estimation, we performed a cultivating validation

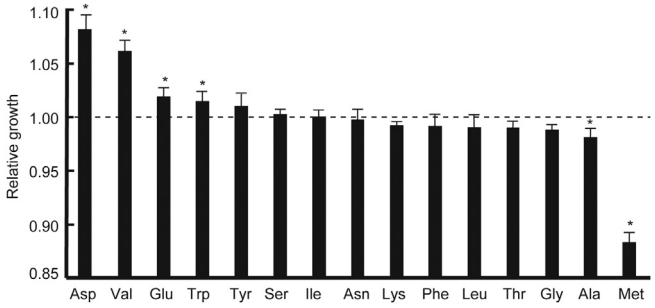

FIG. 4. Results of cultivating validation. Relative growth is the ratio of growth in media with amino acids to that in media without amino acids. The amount of amino acids was 5.0 g/l in each case. Asterisks indicate significant differences observed against a control experiment, determined by a *t*-test ($p \leq 0.05$, $n > 3$).

test using supplemental amino acids. Fifteen amino acids, including L-Asp, L-Val, L-Glu, L-Trp, L-Tyr, L-Ser, L-Ile, L-Lys, L-Phe, L-Thr, Gly, L-Asn, L-Ala, and L-Met (0.5 g/l), were added to YE-d, which indicated the least growth stimulation. Here, the amount of amino acids added were set at equivalent level to confirm simply their enhancing or inhibiting effects. Fig. 4 shows the effect of the different amino acids on the relative growth of the bacteria at 18 h; relative growth is the ratio of growth in media with amino acids to that in media without amino acids. L-Val stimulated 6% of the growth. Although there were no statistically significant differences, L-Asp, L-Val, L-Glu and L-Try showed tendency of growth stimulation in the range of 1.3–8.0%. L-Met obviously inhibited the growth; a 12% decrease in growth was observed. The results support the above observation using PLS-R. Although it is unknown why L-Met inhibit the *E. coli* growth, Nam et al. (28) have been reported that Met decreases cell growth of a recombinant strain producing β-caroten production. Unfortunately, L-Ala inhibited the growth slightly, although the compounds had been estimated as growth stimulator by PLS-R. The other amino acids tested had little effect on growth. The difference observed in the results of culture and PLS-R estimation could be due to the initial condition used for modeling. In PLS-R modeling, it is presumed that all independent variables (i.e., the GC–MS data), correlate to the dependent variables (the growth data). According to culture validation, most of the medium components might have little effect on growth. The gaps between modeling and realistic situation can be reduced by increasing learning data and improving data sampling.

In conclusion, we applied a metabolomics approach using GC–MS analysis to estimate the effect of YE components on *E. coli* growth, as a new approach for optimizing cultivation and its quality control. The statistical model between YE composition and culture data using PLS-R gave representative components with a high correlation between the variations of the concentrations and growth. These components would be the key components for culture optimization and YE quality control. The statistical selection of components have great advantage in increasing the total throughput against a trial-and-error exploring, although different experiments are necessary to confirm their behavior. Therefore, the new procedure demonstrated in this study could change the methodology in culture optimization and YE quality control, thus reducing laborious non-targeted exploring. Furthermore, statistical analyses of substratome seem to be compatible with the automated optimizing systems of culture and quality control that are currently used in the industry, and therefore, have the potential to enhance bioprocess productivities in manufacturing processes.

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