Optimization of D-lactic acid production using unutilized biomass as substrates by Multiple Parallel Fermentation

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ABSTRACT This study investigated the optimization of D-lactic acid production from an unutilized biomass, banana peel and corncob by Multiple Parallel Fermentation (MPF) with *Leuconostoc mesenteroides* and *Aspergillus awamori*. The factors studied in this study consisted of Banana peel and Corncob, KH_2PO_4, Tween 80, $MgSO_4 \cdot 7H_2O$, NaCl, Yeast extract, and Diammonium hydrogen citrate to identify the concentration for optimal D-lactic acid production. Optimization of these component factors was performed by Taguchi method using an L8 Orthogonal Array. Optimal concentration for the effectiveness of MPF using biomass substrates were as follows: (1) Banana peel; D-lactic acid production was 31.8 g/L with a yield of 47.7% in medium containing 15% of a carbon source, 0.5% KH_2PO_4, 0.1% Tween 80, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NaCl, 1.5% Yeast extract, and 0.2% Diammonium hydrogen citrate: (2) Corncob; D-lactic acid production was 38.3 g/L with a yield of 59.4% in medium containing 15% of a Carbon source, 0.5% KH_2PO_4, 0.1% Tween 80, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl, 1.0% Yeast extract, and 0.4% Diammonium hydrogen citrate. Banana peel and Corncob have the potential as unutilized resources for D-lactic acid production. The results indicated that MPF with *Leu. mesenteroides* and *A. awamori* contributes to the potential industrial application of currently unutilized Banana peel and Corncob biomass for D-lactic acid production

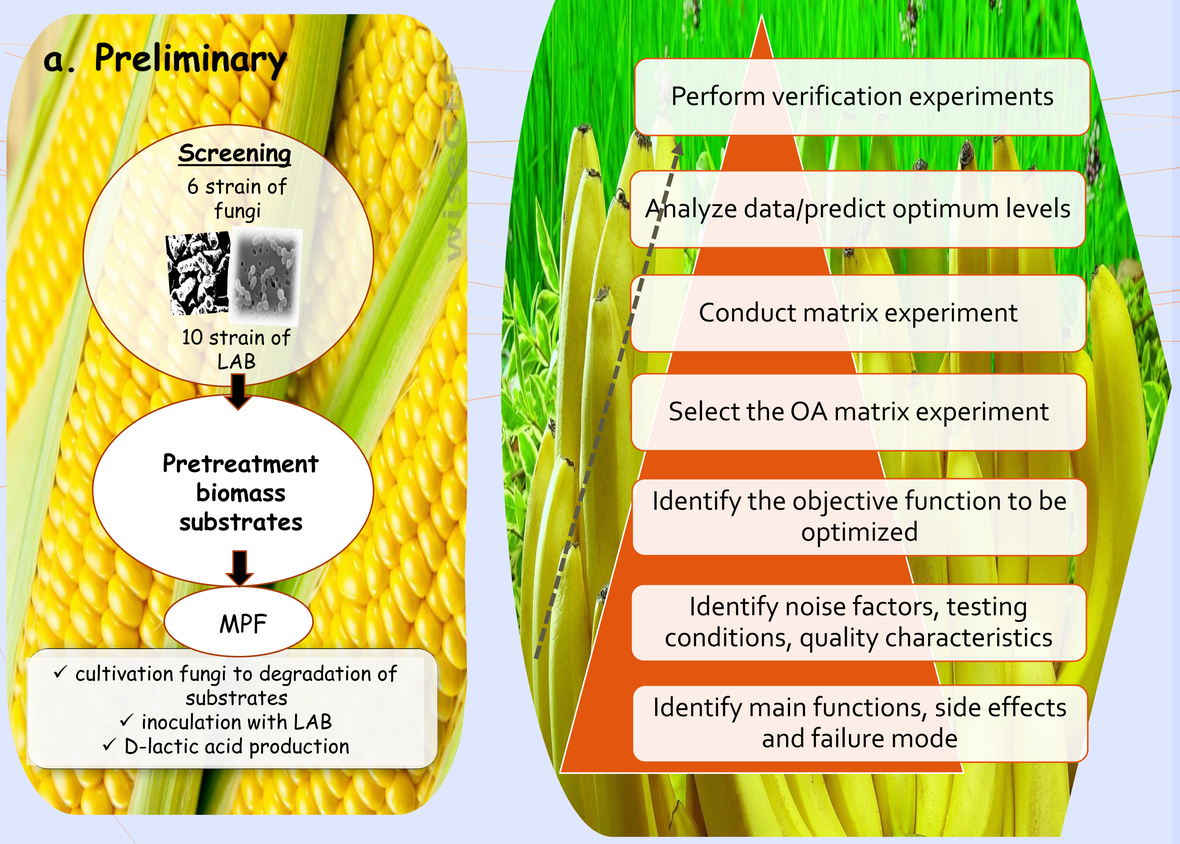
Introduction

D- lactic acid has many uses as a food chemical, preservative, a raw material of biodegradable polymers, an oxygenated chemical, a plant growth regulator, and an environmentally friendly solvent. Utilization of agricultural byproducts for lactic acid production is attractive because of their low price and conversion to a higher value material. Banana peel and corncob are agricultural residues, that provide abundant renewable energy resources. Cellulose, hemicellulose, starch, and pectin are present in banana peels and corncobs, and they constitute some of the most abundant renewable energy resources in the world. Lactic acid from unutilized biomass can be produced by saccharification and lactic acid fermentation. Although the technique of lactic acid production is uncomplicated, the type of biomass and hydrolytic enzymes used have an effect on its sustainable production. A pretreatment method for unutilized biomass also determines quantity. It was reported that high of D-lactic acid was produced by *Leu. mesenteroides* using an optimal combination of sugarcane juice and yeast auto-lysate. Although sugarcane juice is easily exploited as a base material for D-lactic acid fermentation by these lactic acid bacteria without a time- and labor-consuming pretreatment, sugarcane is also valuable, primarily as sweetener in the food industry. There have been no reports on D-lactic acid production from banana peel and corncob by *Leu. mesenteroides*.

Objective

To investigate optimal D-lactic acid production using banana peel and corncob as fermentation substrates by a combination of *Leuconostoc mesenteroides* and *Aspergillus awamori*, *koji* mold used in Japanese spirit brewing.

Methodology



c. Enzyme determination

substrates 1%	250 µl
KPB 100mM, PH 7	50 µl
Enzyme	100 µl
Distilled water	100 µl
	500 µl

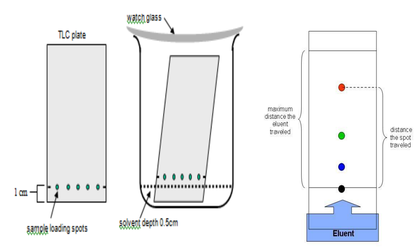
30 C, 15 minute

500 µl NaOH 0.1M

12000 RPM, 5 min, 4 C → Nelson somogy (540 nm)

Soluble Protein was assayed by the method of Lowry

d. Thin layer chromatography



Results

Table 1 Condition of factors and its level in Taguchi method design for multiple parallel fermentation using banana peel and corncob as carbon sources

Factors	Level 1	Level 2
X1. Banana peels (a), Corncob (b); (%)	10	15
X2. KH_2PO_4 (%)	0.5	1
X3. NaCl (%)	0.05	0.1
X4. Tween 80 (%)	0.05	0.1
X5. Yeast extract (%)	1	1.5
X6. $MgSO_4.7H_2O$ (%)	0.05	0.1
X7. Diammonium hydrogen citrate (%)	0.2	0.1

The factors and their values were decided based on triplicate experiments (n = 3)

Table 2 Taguchi's experimental design for multiple parallel fermentation using L_8 (2^7) orthogonal array and their response with Signal to Noise Ratio (S/N Ratio) obtained after different trials

Run	Factors							*SNR of LTB [a]	*SNR of LTB [b]
	X1	X2	X3	X4	X5	X6	X7		
1	1	1	1	1	1	1	1	22.812 ± 0.003	25.713 ± 0.021
2	1	1	1	2	2	2	2	22.088 ± 0.023	25.555 ± 0.004
3	1	2	2	1	1	2	2	19.647 ± 0.007	25.342 ± 0.004
4	1	2	2	2	2	1	1	22.524 ± 0.011	25.660 ± 0.022
5	2	1	2	1	2	1	2	29.530 ± 0.013	29.115 ± 0.032
6	2	1	2	2	1	2	1	29.011 ± 0.022	29.327 ± 0.041
7	2	2	1	1	2	2	1	28.708 ± 0.033	25.889 ± 0.007
8	2	2	1	2	1	1	2	29.230 ± 0.014	31.233 ± 0.004

X1-X7 are name of each factor, XI : carbon source; Banana peel concentration [a] ; corn cob concentration [b] , X2 : KH_2PO_4, X3: NaCl, X4: Tween 80, X5 : Yeast extract, X6 : $MgSO_4.7H_2O$, X7 : Diammonium hydrogen citrate
The number (1 and 2) below each factor (X1-X7) indicates the level of each factor described in Table 1

Table 3 Optimal condition and performance in validation of D-lactic acid production using banana peel [a] and corncob [b]

(a)

Factor (%)	Level Description	Level	Contribution
Banana peel	15	2	8.102
KH_2PO_4	0.5	1	0.695
NaCl	0.05	1	0.198
Tween 80	0.1	2	0.327
Yeast ext	1.5	2	0.340
$MgSO_4.7H_2O$	0.05	1	1.034
Diam.Hyd.cit	0.2	1	0.178
Total contribution from all factors			10.870
Current grand average of performance			20.490
Expected result at optimum condition			31.360
Validation result			31.840 (g/L)

(b)

Factor (%)	Level Description	Level	Contribution
Corncobs	15	2	4.753
KH_2PO_4	0.5	1	0.281
NaCl	0.1	2	0.137
Tween 80	0.1	2	2.223
Yeast ext	1	1	2.138
$MgSO_4.7H_2O$	0.05	1	2.134
Diam.Hyd.cit	0.4	2	1.875
Total contribution from all factors			13.541
Current grand average of performance			23.740
Expected result at optimum condition			37.280
Validation result			38.260 (g/L)

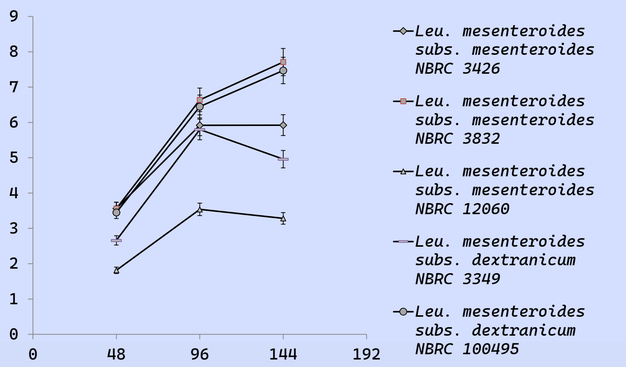
All calculations and analysis were performed using Qualitek-4 software for automatic design

Table 4 Activities of main polysaccharide–lytic enzymes produced in MPF

Carbon source	Day	Enzyme activity (U/mL)[a]				
		Xylanase	Pectinase	Amylase	ß-Glucosidase	Endo-glucanase
Banana peel	0 [b]	0.222 ± 0.001	0.311 ± 0.012	2.335 ± 0.023	6.281 ± 0.002	3.222 ± 0.038
	2	2.099 ± 0.008	0.314 ± 0.044	5.234 ± 0.024	2.112 ± 0.003	1.011 ± 0.022
	4	0.900 ± 0.011	0.330 ± 0.022	3.132 ± 0.003	0.035 ± 0.033	0.106 ± 0.014
Corncob	0 [b]	0.865 ± 0.015	0.031 ± 0.004	2.873 ± 0.005	3.561 ± 0.011	0.978 ± 0.002
	2	1.211 ± 0.031	0.031 ± 0.007	0.221 ± 0.006	2.112 ± 0.013	0.886 ± 0.011
	4	2.011 ± 0.011	0.031 ± 0.031	0.213 ± 0.008	0.651 ± 0.011	0.432 ± 0.017
	6	0.712 ± 0.023	0.031 ± 0.005	0.111 ± 0.010	0.312 ± 0.021	0.120 ± 0.013
	10	0.652 ± 0.022	0.031 ± 0.007	1.376 ± 0.005	ND [c]	ND [c]

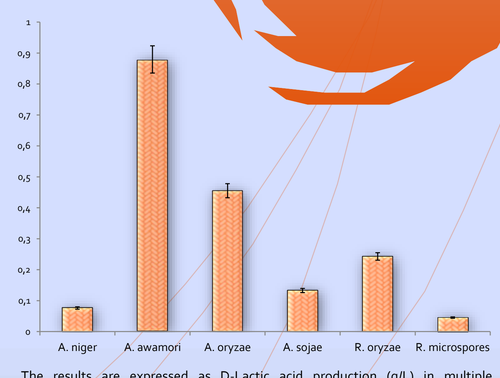
a) The values are expressed as enzyme activity (U/mL) and represent the mean ± SD (n = 3)
b) o day means the day at which *Leu. mesenteroides* NBRC 3832 was inoculated after 36-h cultivation of *A. awamori* NBRC 4388
c) ND : not detected

Figure 1 D-Lactic acid production in simple fermentation using various *Leu. mesenteroides* strains



The results are expressed as D-Lactic acid production (g/L) in simple fermentation using various strains of *Leu. mesenteroides*. MRS medium was used for standard cultivation of *Leu. mesenteroides*. The initial pH was adjusted to 6.3. A total of 20 mL of the inoculum and 3% of calcium carbonate were added to 300-mL Erlenmeyer flasks. Cultivation was carried out in flask cultures with a rotary shaker at 100 rpm and 30°C

Figure 2 D-Lactic acid production in multiple parallel fermentation using various fungi strains



The results are expressed as D-Lactic acid production (g/L) in multiple parallel fermentation using various fungi strains (*Aspergillus niger* NBRC 4414, *A. awamori* NBRC 4388, *Rhizopus oryzae* NBRC 4706, *R. microspores* NBRC 3200, *A. oryzae* NBRC 100959, and *A. sojae* NBRC33802). After 36-h cultivation of fungi strains, followed by inoculation of *Leu. mesenteroides* NBRC 3832 to D-lactic acid production by MPF. MPF was performed in the incubator with a gentle shaking at 100 rpm and 30°C

Conclusion The best condition using Taguchi method of an L8 OA enabled us to analyze the influence of factors and their interactions for D-lactic acid production using banana peel and corncob biomass substrates. This result indicated that utilization of such biomass substrates in MPF has a high potential for D-lactic acid production.

Future plan Characterization and purification of Xylanase from *Bacillus licheniformis* YN15 and YN11-1 applied in Multiple Parallel Fermentation to investigate of D-lactic acid production

Acknowledgment We are grateful to *Monbukagakusho*, The Ministry of Education, Culture, *Sports*, Science and Technology in *Japan* (*MEXT*), for financial support (*MEXT scholarships)* and my laboratory member in the enzyme technology laboratory, Graduated school of life sciences, Ritsumeikan University, Shiga, Japan.