



Comparison and optimization of conjugated linoleic acid production by *Lactobacillus plantarum* and *Lactobacillus plantarum subsp. plantarum*

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KEYWORDS

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 Castor oil;
Lactobacillus plantarum;
 Optimization;
 Washed cell.

Abstract. Conjugated Linoleic Acid (CLA) was produced from castor oil using washed cells of *Lactobacillus plantarum* PTCC 1058 and *Lactobacillus plantarum subsp. plantarum* PTCC1745 as the catalyst. Under the optimal reaction conditions, the washed cells of *Lactobacillus plantarum* PTCC1058 produced 1661.26 mg of CLA/L reaction mixture (36% yield of production) from 4.6 mg/ml of castor oil after using 15% (w/v) cell for 121 h. The resulting CLA was a mixture of two CLA isomers, cis-9, trans-11 (or trans-9, cis-11)-octadecadienoic acid (CLA1, 44% of total CLA) and trans-10, cis-12-octadecadienoic acid (CLA2, 46% of total CLA). The total production of CLA is extracellular in all of the reactions performed with *Lactobacillus plantarum* PTCC1058. Also, *Lactobacillus plantarum* PTCC1745 produced 1590.31 mg of CLA/L reaction mixture (16.5% production yield) from 9.6 mg/ml of castor oil after using 15% (w/v) cell for 121 h. Therefore, *Lactobacillus plantarum* PTCC1058 was suggested for CLA production due to its more production ability and CLA extracellular production.

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1. Introduction

Pharmaceutical and chemical industries used Lactic acid bacteria widely in foods and drugs. *Lactobacillus plantarum* is one of the strains of Lactic acid bacteria capable to produce nutritious and valuable products such as Conjugated Linoleic Acid (CLA). CLA is a natural derivative of the essential fatty acid (linoleic acid) and has received increasing attention in recent years due to its health effects potential [1], such as having anticarcinogenic activity [2-4], enhancing the immune system, or decreasing body fat content [5]. CLA is not a single substance but actually a mixed group of positional and geometrical isomers of linoleic

acid(C18:2) – conjugated double bonds position may vary from 7 to 14 carbon atoms – including all possible geometric configurations of trans-cis, cis-trans, cis-cis, and trans-trans isomers [6,7]. The major isomer present in food (> 80%), such as beef, milk, and dairy products, is cis-9, trans-11 CLA; therefore, the majority of the research has focused on the cis-9, trans-11 CLA. Moreover, the c9, t11 CLA has been associated with a multitude of health promoting effects, such as anti-atherogenic activity [8].

Other isomers, namely, trans-10, cis-12 (t10, c12 CLA), are found in much smaller quantities. c9, t11 CLA and t10, c12 CLA are considered to be the most bioactive isomers [1,9]. However, the low concentration of CLA found in these food products does not seem to be sufficient to have any significant therapeutic effect and, thus, efforts are running in order to obtain CLA-enriched foods [1]. Using CLA for medicinal and food purposes requires a safe and selective process.

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The use of biological reactions to produce CLA is recommended. Nowadays, CLA is applicable in food products and is usually produced by chemical synthesis method. One of the disadvantages of chemical synthesis method is production of various isomers. From this point of view, the biotechnological techniques and the use of microorganisms are highly considered because of the minimum production of byproducts as well as outputting of natural products. Compared to the formation of CLA in the rumen, bacterial synthesis of CLA by the biotechnological process has a number of advantages, such as independence from atmospheric conditions and season of the year, the possibility of accurate monitoring of the process, and the possibility of a continuous production. The bacteria that have common and well-known culture conditions and are Generally Recognized As Safe (GRAS) are especially attractive [10]. An excellent review of the microbial synthesis of CLA was presented by Ogawa et al. [11]. The findings of Ogawa et al. led to the establishment CLA production by lactic acid bacteria from ricinoleic acid and, furthermore, from castor oil [11]. Only two CLA isomers, c9, t11(CLA1) and t10, c12-CLA(CLA2), were produced from ricinoleic acid or castor oil by lactic acid bacteria, suggesting that the biological CLA production processes are more isomer-selective than the chemical ones [11]. Ricinoleic acid is abundant in plant oil and castor oil. Castor oil is an economical source of ricinoleic acid. About 88% of the total fatty acids in castor oil is ricinoleic acid. However, CLA was produced from castor oil in an unexpected low yield [12]. Lactic acid bacteria were found to only use the free form of ricinoleic acid for CLA production, not its triacylglycerol form, which is mainly found in castor oil. Thus, Ando et al. (2004) investigated the conditions for practical CLA production from castor oil in the presence of lipase with *Lactobacillus plantarum* JCM 1551 to increase CLA productivity [13].

In this research, the washed cells of *Lactobacillus plantarum* PTCC 1058 and *Lactobacillus plantarum* subsp. *plantarum* PTCC1745, which have the potential for CLA synthesis, were employed as the catalyst for CLA production. Three main factors including cell concentration, substrate concentration, and cultivation time, which could impact the yield and amount of CLA production, were also studied.

2. Materials and methods

2.1. Materials

Lactobacillus plantarum PTCC 1058 and *Lactobacillus plantarum* subsp. *plantarum* PTCC1745 were obtained from Iranian Research Organization for Science and Technology Co. (Tehran, Iran); and Lipase enzymes were obtained from Merck (Lipase 24548, Native lipase from *Chromobacterium viscosum*, Germany)

and Renco (Lipase 20522.6, New Zealand). Castor oil (triacylglycerol: ricinoleic acid 89.1%) and standard fatty acids methyl ester CLA (FAME) (c9, t11-octadecadienoic acid 42%, t10, c12 octadecadienoic acid 44%, c10 c12-octadecadienoic acid 10%, and others 5%) and linoleic acid (c9, c12-octadecadienoic acid) were purchased from Sigma (St. Louis, MO). Methanolic boron trifluoride was supplied by Fluka (Switzerland). Tween 80 and all other chemicals were of analytical grade and from Merck (Darmstadt, Germany).

2.2. Microorganisms, cultivation, and preparation of washed cells

Lactobacillus plantarum PTCC 1058 and *Lactobacillus plantarum* subsp. *plantarum* PTCC1745 were selected as potent CLA producers from ricinoleic acid and utilized in this study. The strains were cultivated in MRS broth medium at 37°C for 24 hr, the medium was supplemented with 0.04% (w/v) linoleic acid. Cells were harvested by centrifugation (4000 rpm, 10 min), washed twice with physiological serum 0.85%, centrifuged again, and then used as the washed cells for the cultivations.

2.3. Reaction conditions

The reaction mixture, 1 ml in a 25 ml Flask, comprised 10.0, 15.0, and 20.0 mg/ml castor oil mixed with Tween 80 (0.1% w/v), 0.5 M phosphate buffer (pH 6.5), 100 U/ml lipase, and 10, 15, 20% (wet cell w/v) washed cells. The cultivations were carried out with Gentle shake and 120 rpm at 37°C for 72, 120, and 170 hr.

2.4. Lipid extraction and analyses

Lipid extraction was performed by the methods of Bligh and Dyer [14], followed by incubation; the medium was mixed with 7.5 ml of chloroform: methanol (2:1, v/v) and shaken for 1 min. Then, 2.5 ml of chloroform was added to the solution and shaken for 1 min. After that, 2.5 ml of deionized water was added and shaken for 1 min, and the mixture was centrifuged at 4000 rpm for 15 min. To study the intracellular production of CLA, cells were collected and stored in phosphate buffer solution [15].

The lower layer was transferred to a 50 ml balloon and evaporated with a rotary evaporator at 30°C. The residue was saponified with 1 ml of 1.0 N sodium hydroxide in methanol in a 100°C water bath for 15 min. As described in the methylation procedures, Chin et al. used acid catalyzed methanolysis; 6 ml of 4% HCl in methanol was added after cooling and methylated at 60°C for 20 min [16]. The mixture was concentrated under a stream of nitrogen at room temperature and redissolved in 3 ml of hexane. Finally, CLA in hexane extract was quantified by capillary GC MSD. GC MSD analysis instrumentation used for the analyses was as follows: Agilent 5975c (Agilent Technologies, Santa

Clara, CA, United States of America) equipped with Hp-5 MS fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness). The injection volume was 1.0 μl . The temperature of the GC oven was programmed at 110°C for 2 min and from 110°C to 230°C was programmed at the rate of 5°C/min and held for 5 min, then from 230°C to 270°C was also programmed at the rate of 10°C/min and held for 5 min. The injector and detector temperatures were 270°C. Helium was used as the carrier and make-up gas. The flow rate of make-up gas was 30 ml/min. The pressure was maintained at 10 psig on the column in order to obtain the flow rate of the carrier gas at 1.2 ml/min. The split ratio was set at 1:100. The c-9, t-11 CLA methyl ester eluted for 23.14 min was identified by comparing the retention time with that of methylated CLA standard (Sigma Chemical Co., St. Louis, MO, USA). To analyze the quantity of FAME, the calibration of standard methyl esters of CLA in linear correlation was illustrated with good accuracy ($R^2 = 0.996-9$). To verify the accurate location of each fatty acid, the amount of one micro liter of standard liquids was injected to the system and retention time was set to recognize each peak.

2.5. Optimization of processing

Optimization of production amount and yield of CLA is the most important issue in the microbial synthesis of CLA. Thus, optimization of the process for maximum production was studied. For optimization of the process in order to maximize production yield, the surface response method was utilized. Also, planning of the tests was performed based on central composite design with three factors, each factor in 3 levels with 3 iterations in the central point containing 17 tests. Second order factorial experimental design was chosen due to its capability to save time and work (minimizing the number of performed experiments) [17]. The quantities of independent variables consisting of oil concentration (mg/ml), cultivation time (hr), and the quantity of cells (%) were converted to the encrypted codes +1, 0, -1 that represented high, medium, and low levels, respectively, which are presented in Table 1 [18].

For the statistical analysis, consisting of regression, variance analysis, the graphical shapes, and optimization, design expert 8 software was used [19]. The yield of CLA production was evaluated with the following equation:

yield of CLA production =

$$\frac{\text{CLA concentration}}{\text{Castrol oil concentration}} * 100. \quad (1)$$

Polynomial models were used for adjusting of experimental data [17]:

$$Y = a_0 + \sum_{i=1}^3 a_i X_i + \sum_{i=1}^3 a_{ii} X_i^2 + \sum_{i < j} a_{ij} X_i X_j, \quad (2)$$

where Y represents each of the system responses (dependent variables) studied; X_i and X_j are the independent variables defined in Table 1; and a_0 , a_i , and a_{ij} are the fitting parameters (obtained by multiple regression).

3. Results and discussion

According to the research [1,11], *Lactobacillus plantarum* has the highest CLA production; therefore, in this study, washed cells of *Lactobacillus plantarum* PTCC1058 and *Lactobacillus plantarum subsp. plantarum* PTCC1745 were selected to study CLA production. Using resting cells (washed cells) of lactic acid bacteria helped to avoid cell growth inhibitory effect of unsaturated fatty acids such as linoleic acid.

In Figure 1, the growth curves of *Lactobacillus plantarum* in MRS medium and MRS medium with 0.04% LA are presented. The maximum CLA production occurred in the beginning of stationary phase of Cell growth. After 24 hr of growth, the cells were collected and transferred into MRS medium with 0.04% LA for 24 hr, leading to adaptation of cells with LA. As it is presented in Figure 1, when bacteria are grown in MRS medium with 0.04% linoleic acid, it reaches the stationary phase in a shorter time.

Table 1. Independent variables and their levels for the combinational central plan in CLA production process.

Independent variables	Symbols	Variable levels encrypted for L.P.1058*			Variable levels encrypted for L.P.1745*		
		-1	0	+1	-1	0	+1
Cultivation time (hr)	X_1	72	121	170	72	121	170
Quantity of cell (%)	X_2	10	15	20	10	15	20
Oil concentration (mg/ml)	X_3	5	10	15	10	15	20

*The following formulae were used for converting of real independent variables to encrypted variable levels $A = (\text{time} - 121)/49$, $B = (\text{the quantity of cell} - 15)/5$ and $C_{1058} = (\text{oil concentration} - 10)/5$ or $C_{1755} = (\text{oil concentration} - 15)/5$.

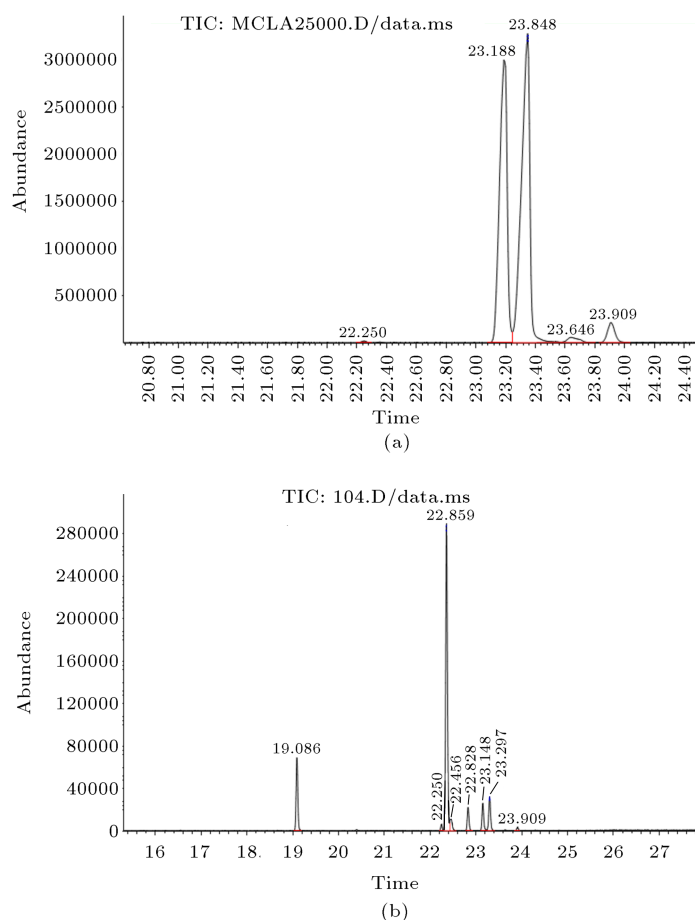


Figure 1. The growth curves of *Lactobacillus plantarum* (▲ *subsp. plantarum* PTCC1745 × PTCC1058) in (a) MRS medium and (b) MRS medium with 0.04% LA.

Table 2. Specific growth rate of lactobacillus strains in MRS medium and MRS with linoleic acid.

Strain	MRS		MRS+LA	
	$\mu(h^{-1})$	R^2	$\mu(h^{-1})$	R^2
<i>Lactobacillus plantarum</i> PTCC1745	0.349	0.96	0.4457	0.790
<i>Lactobacillus plantarum</i> PTCC1058	0.320	0.992	0.124	0.999

Specific growth rates of *Lactobacillus* strains in MRS medium and MRS with linoleic acid are compared in Table 2. *Lactobacillus plantarum* PTCC1058 had less specific growth rate in MRS medium and MRS with linoleic acid, respectively.

Amount and yield of CLA production was evaluated by GC-MSD after CLA separation and free fatty acids methylation. Figure 2 shows the chromatogram of Fatty Acids Methyl Ester CLA (FAME). The maximum CLA was produced in the medium by *Lactobacillus plantarum* PTCC1058. After injection of methylated sample into GC-MSD, retention time for Palmitic acid, Linoleic acid, Oleic acid, Stearic acid, Cis9, trans11-CLA(CLA1), trans10, cis12-CLA(CLA2), and Ricinoleic acid methyl ester were 19.09, 22.25, 22.35, 22.82, 23.14, 23.3, and 25.67, respectively.

Design-expert software was used to optimize CLA production. Using RSM (Response Surface Method), the effects of three variables, namely, oil concentration (mg/ml), cultivation time (hr), and the quantity of cells (%), on the amount of CLA production and yield were studied. In RSM, uncoded variables were changed to coded variables (Tables 3 and 4) with a zero mean and standard deviation that had been defined to be dimensionless. Tables 3 and 4 show experimental settings of the cultivation factors for the Central Composite Design (CCD) arrangements and responses for the CLA production yield. Also, the relation between coded independent variables and each of the system responses (dependent variables) by the method of least square multiple regressions is presented in the Table 5.

Table 5 shows different parameters of the models and their significance levels. a_2 , a_3 , a_{12} , and a_{33} were significant parameters in the CLA production and CLA

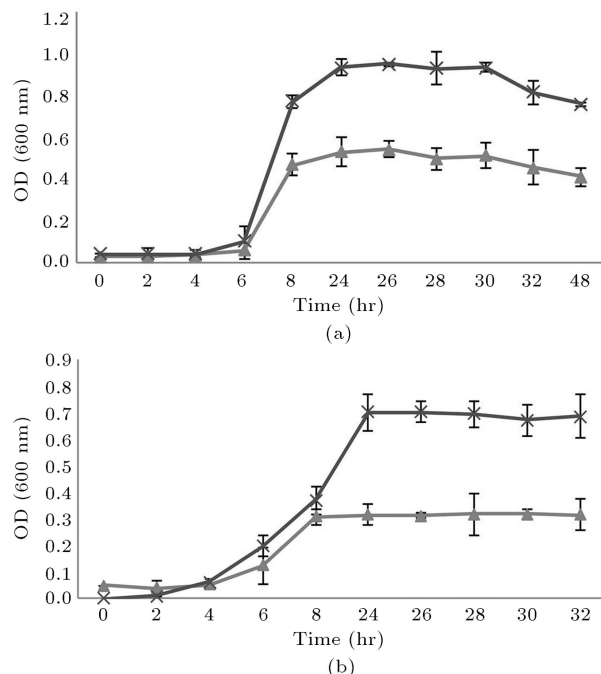


Figure 2. Chromatogram of (a) maximum CLA production by PTCC 1058 and (b) standard fatty acids methyl ester CLA (FAME) (c9, t11-octadecadienoic acid 42%, t10, c12-octadecadienoic acid 44%, c10 c12-octadecadienoic acid 10%, and others 5%).

yield by PTCC1745 bacteria. a_1 , a_3 , and a_{33} were significant parameters in the CLA production and CLA yield by PTCC1058 bacteria.

The results from the designed tests by Design-expert software show that for *Lactobacillus plantarum* PTCC1058, the production yield increased to 36.11% with decreasing oil concentration to 4.6mg/ml and increasing cells concentration to 15% (w/v) after 121 hr. Also, for *Lactobacillus plantarum subsp. plantarum* PTCC1745, the production yield increased to 16.5676 with decreasing oil concentration to 9.6 mg/ml and increasing cells concentration to 15% (w/v) after 121 hr.

The production yield of *Lactobacillus plantarum* PTCC1058 was higher than that of *Lactobacillus plantarum* PTCC1745; thus, this strain had more production ability. CLA production yield increased by decreasing oil concentration as high concentration of castor oil exhibited toxic effect on cells. According to the authors' investigation, the higher efficiency in producing CLA isomers from LA confirms the hypothesis that microorganisms perform isomerization as a detoxification process because of high toxic nature of LA [20,21].

Increasing cell concentration up to 15% (w/v) was appropriate. At lower cells concentration, the quantity of cells was insufficient and castor oil showed more toxic effect on cells. At higher cells concentration, decrement in available nutrients led to lower CLA production. Reaction time effect on production and yield is presented in Figure 3. CLA production increased by increasing reaction time. However, prolonged reaction time did not increase CLA production because of detoxification

Table 3. Experimental settings of the cultivation factors for the Central Composite Design (CCD) arrangements and responses for the amounts of CLA production and yield for *Lactobacillus plantarum subsp. plantarum* PTCC1745.

Run	Factor 1 A: time hr	Factor 2 B: cell concentration %w/v	Factor 3 C: oil concentration mg/ml	Response 1 CLA concentration mg/L	Response 2 CLA yield %	CLA1 concentration mg/L	CLA2 concentration mg/L
1	72	10	20	1767.15	8.83574	725.01	1042.11
2	72	20	10	1637.71	16.3771	667.69	969.99
3	121	15	15	1753.09	11.6873	728.85	1025.05
4	173.92	15	15	1831.71	12.2114	751.01	1080.7
5	121	15	20.4	1712.78	8.56389	719.3676	993.4124
6	121	15	15	1765.31	11.7687	759.0833	1006.227
7	170	10	10	1677.83	16.7783	687.9103	989.9197
8	72	20	20	1784.25	8.92125	740.4638	1043.786
9	72	10	10	1603.63	16.0363	705.5972	898.0328
10	170	20	20	1654.18	8.27089	699.7181	954.4619
11	121	9.6	15	1776.53	11.8435	751.4722	1025.058
12	68.08	15	15	1734.24	11.5616	728.3808	1005.859
13	170	20	10	1616.67	16.1667	679.0014	937.6686
14	170	10	20	1952.29	9.76147	878.5305	1073.76
15	121	20.4	15	1746.11	11.6407	785.7495	960.3605
16	121	15	9.6	1590.31	16.5657	683.8333	906.4767

Table 4. Experimental settings of the cultivation factors for the Central Composite Design (CCD) arrangements and responses for the amounts of CLA production and yield for *Lactobacillus plantarum* PTCC1058.

Run	Factor 1 A: time hr	Factor 2 B: cell concentration %w/v	Factor 3 C: oil concentration mg/ml	Response 1 CLA concentration mg/L	Response 2 CLA yield %	CLA1 concentration mg/L	CLA2 concentration mg/L
1	72	20	5	1620.58	32.4115	891.319	939.9364
2	121	15	15.4	1950.26	12.664	1107.358	1131.151
3	121	15	4.6	1661.26	36.1144	950.7391	963.5308
4	121	9.6	10	1612.57	16.1257	677.2794	935.2906
5	72	10	5	1606.29	32.1257	674.6418	931.6482
6	68.08	15	10	1615.19	16.1519	678.3798	936.8102
7	72	10	15	1824.2	12.1613	766.164	1058.036
8	121	15	10	1727.44	17.2744	725.5248	1001.915
9	121	15	10	1697.44	16.9744	712.9248	984.5152
10	170	10	15	1938.11	12.9207	814.0062	1124.104
11	72	20	15	1814.22	12.0948	761.9724	1052.248
12	173.92	15	10	1877.6	18.776	788.592	1089.008
13	170	20	5	1699.6	33.992	713.832	985.768
14	170	20	15	1872.64	12.4843	786.5088	1086.131
15	170	10	5	1685.41	33.7081	707.8722	977.5378
16	121	20.4	10	1740.2	17.402	730.884	1009.316

Table 5. Regression parameters for variables, signification of regression equations and goodness of fitting.

Fitting parameters	Name	CLA production 1058	CLA yeild 1058	CLA production 1745	CLA yeild 1745
a_0		1722.98*	17.32*	1751.98*	3.42*
a_1	Cultivation time	59.41*	0.69*	20.66*	0.02**
a_2	Quantity of cell	8.8**	0.14**	-33*	-0.029*
a_3	Oil concentration	111.24*	-10.44*	73*	-0.53*
a_{12}		-6.95	-0.046	-51.31*	-0.048*
a_{13}		1.77	-0.25	0.24	-3.08E-03
a_{23}		-12.99**	-0.13	-31.74**	-0.025
a_{11}		15.15	0.038	29.94	0.022
a_{22}		-44.87	-0.56	11.38	4.70E-03
a_{33}		66.05	5.98*	-82.74*	0.066*
R^2		0.9144	0.9983	0.9250	0.9983
$R^2(\text{adj})$		0.8059	0.9958	0.8126	0.9957

* Significance level < 0.05; ** Significance level < 0.10.

mechanism of free linoleic acid. When reaction time was extended, CLA was converted to stearic acid (18:0) via trans vaccenic acid (18:1).

Optimization for maximum CLA production showed that both strains at optimum state presented similar productions and conditions (the optimum conditions and the amounts of CLA production were similar for both strains). *Lactobacillus plantarum* PTCC1058 produced 1938.11 mg of CLA/L (produc-

tion yield of 12.92%) at 10% (w/v) cell concentration, 15 mg/ml of oil concentration, and 170 hr of reaction time. Also, *Lactobacillus plantarum subsp. plantarum* PTCC1745 produced 1952.29 mg of CLA/L (production yield 9.7) at 10% (w/v) cell concentration, 20 mg/ml of oil concentration, and 170 hr of reaction time. Therefore, *Lactobacillus plantarum* PTCC1058 was a better selection due to higher production yield and production concentration.

Table 6. The comparison of CLA production with that in the other research.

Strain	Culture type	Medium	Substrate	Total CLA (mg/L)	Reference
<i>L. plantarum</i> A6-1F	Washed cells	KPB*	Linoleic acid (1.5 g/L)	276	[24]
<i>L. plantarum</i> Ip5	Growing cells	MRS**	Linoleic acid (600 mg/L)	142	[25]
<i>L. plantarum</i> L12	Growing cells	MRS	Linoleic acid (1 g/L)	57	[26]
<i>L. plantarum</i> JCM 1551	Washed cells	KPB	Lipase treated castor oil (30 g /L)	7500	[13]
<i>L. plantarum</i> AKU 1009a	Washed cells	KPB	Ricinoleic acid (4 g /L)	1650	[27]
<i>Lactobacillus plantarum</i> PTCC1058	Washed cells	MRS	Lipase treated castor oil (4.6 mg/ml)	1661	This work
<i>Lactobacillus plantarum</i> PTCC1745	Washed cells	MRS	Lipase treated castor oil (9.6 mg/ml)	1590	This work

* KPB: Potassium phosphate buffer; ** MRS, Man, Rogosa and Sharp medium.

Figure 3 shows the perturbation diagram for CLA yield (Figure 3(a)) and CLA concentration (Figure 3(b)) for *Lactobacillus plantarum* PTCC1058, which represents the effect of three coded variables on CLA yield and CLA concentration. Castor oil con-

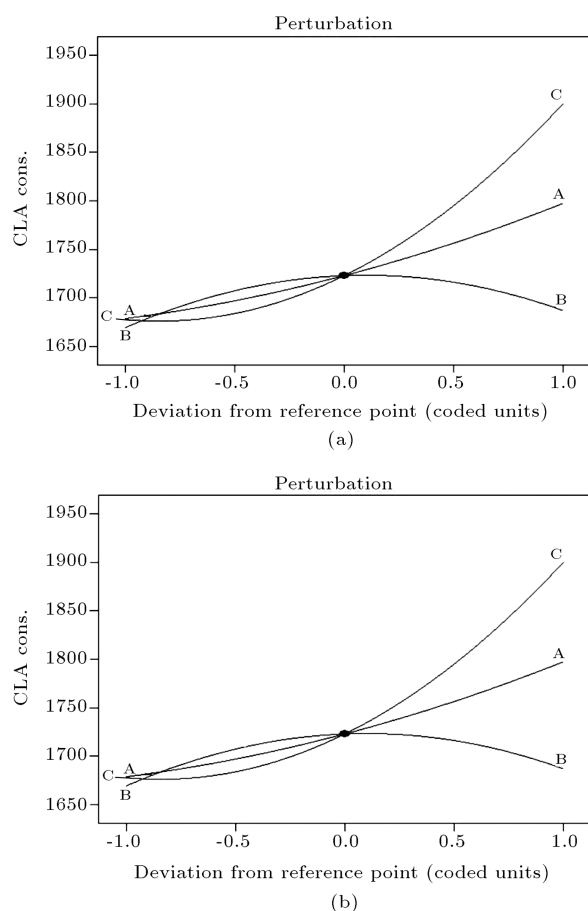


Figure 3. Perturbation diagram for CLA yield (a) and CLA concentration (b) for *Lactobacillus plantarum* PTCC1058 (A, B, and C are the coded variables, listed in Table 1).

centration is the parameter that challenges CLA yield and CLA concentration. The higher level of castor oil increased the amount of CLA production and decreased CLA production yield. Table 6 shows the comparison of this production with that in other research. As this table presents, washed cells of *Lactobacillus plantarum* PTCC1058 produced a considerable amount of CLA under optimal conditions. Therefore, in order to have more attractive and economical bioconversion, the use of alternative sources of linoleic acid, such as ricinoleic acid is desirable.

As some studies report that most of the produced CLA is associated with cells, mainly as free fatty acid [22], the intracellular CLA production has also been investigated. As shown in Table 7, under some reaction conditions with *Lactobacillus plantarum subsp. plantarum* PTCC1745, a significant amounts of CLA was associated with the cells. However, in all of the reactions performed with *Lactobacillus plantarum* PTCC1058, the CLA production was extracellular. Thus, as *Lactobacillus plantarum subsp. plantarum* PTCC1745 was used as the reaction catalyst, cells were broken to release the CLA associated with cells. Therefore, its use is more facile and recommended to produce extracellular CLA with *Lactobacillus plantarum* PTCC1058.

Two ways could be considered to produce CLA enriched foods by the use of food grade microorganisms, either as starter cultures for CLA-enriched fermented food products or as biocatalysts to produce an additive for the natural food products. The CLA produced in this study can be used as a substrate for transesterification to produce enriched triacylglycerols and nutraceutical foods [1,23].

4. Conclusion

Using RSM (Response Surface Method), the effects of three variables, namely, oil concentration (mg/ml),

Table 7. Intracellular and extracellular production of CLA by *Lactobacillus plantarum* PTCC 1058 and *Lactobacillus plantarum subsp. plantarum* PTCC1745.

Bacteria	Reaction condition			CLA Production	
	Quantity of cells %w/v	Oil concentration mg/ml	Time (hr)	Intracellular CLA (mg/l)	Extracellular CLA (mg/l)
<i>L. plantarum</i> PTCC1058	15	10	68.08	0	1076.712446
<i>L. plantarum</i> PTCC1058	20	15	72	0	1209.305418
<i>L. plantarum</i> PTCC1058	10	15	72	0	1215.954572
<i>L. plantarum</i> PTCC1058	10	5	72	0	1070.857277
<i>L. plantarum</i> PTCC1058	9.6	10	121	0	1086.285531
<i>L. plantarum</i> PTCC1058	10	15	121	0	1101.954326
<i>L. plantarum subsp. plantarum</i> PTCC1745	10	10	72	426.25488	1069.011561
<i>L. plantarum subsp. plantarum</i> PTCC1745	15	9.6	121	0	1060.134836
<i>L. plantarum subsp. plantarum</i> PTCC1745	20	20	72	0	1189.340516
<i>L. plantarum subsp. plantarum</i> PTCC1745	10	20	72	0	1177.947408
<i>L. plantarum subsp. plantarum</i> PTCC1745	20.4	15	121	0	1563.652834
<i>L. plantarum subsp. plantarum</i> PTCC1745	15	15	121	623.896167	1076.784855
<i>L. plantarum subsp. plantarum</i> PTCC1745	10	10	170	0	1118.445711

cultivation time (hr), and the quantity of cells (%), on the amount of production and yield of CLA were studied. The higher level of castor oil increased the amount of CLA production and decreased CLA production yield. Under the optimal reaction conditions, washed cells of *Lactobacillus plantarum* PTCC1058 produced 1661.26 mg CLA/L reaction mixture (36% yield of production) from 4.6 mg/ml castor oil in 121 h by using 15% (w/v) cell. The resulting CLA was a mixture of two CLA isomers, c9, t11-octadecadienoic acid (CLA1, 44% of total CLA) and t10, c12-octadecadienoic acid (CLA2, 46% of total CLA). The total CLA production was extracellular in all the reactions performed with *Lactobacillus plantarum* PTCC1058. Also, *Lactobacillus plantarum* PTCC1745 produced 1590.31 mg CLA/L (16.5% yield of production) from 9.6 mg/ml castor oil in 121 h by using 15% (w/v) cell. Considering higher production yield by *Lactobacillus plantarum* PTCC1058, this strain is suggested for CLA production as it is capable to provide higher CLA production and higher yield; therefore, this strain could be recommended for scale-up system for industrial and economical levels as the product is considered to be a functional food and a health aid.

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