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RECENT DEVELOPMENT IN L(+)-LACTIC ACID BIOTECHNOLOGY

Summary: Lactic Acid is known from 1780 as a sour component of milk. It is widely used in food, cosmetic, pharmaceutical industry. Now one of its enantiomers (L(+)-) is being used as a source material of poly-lactic acid (PLA). PLA is used for the production of biodegradable and compostable plastics. This review focuses on recent works leading to decrease the costs of production of L(+)-lactic acid and to optimize the conditions and medium composition of fermentation.

Keywords: L(+)-Lactic Acid, fermentation, biosynthesis, poly-lactic acid (PLA).

1. Introduction

Lactic acid and its derivatives are widely used as a preservatives, pH regulators and taste-enhancers in food industry, for implants and suture in the medical practice, as a reagent for polylactic and polyacrylic acids synthesis for biodegradable polymers. Lactic acid can be manufactured either by chemical synthesis or by microbial fermentations. Chemical synthesis results in racemic DL lactic acid whereas stereospecific (L(+), D(-) and DL mixture) form can be produced by fermentation using specific microbial strain [1-6].

Recently, demand for lactic acid has been increasing considerably. Apart from wide applications in mentioned industries L(+)-lactic acid is a source material of poly-lactic acid (PLA) that can be used for the production of biodegradable and compostable plastics. Biodegradable plastics from biomass are expected as 'green plastics', because they are decomposed into CO_2 and H_2O by microorganisms and return to the earth. Since PLA is one of the most promising polymers that will play an important role in solving worldwide environmental problems, i.e., disposing of waste plastics derived from petroleum feedstocks, worldwide demand for L(+)-lactic acid is increasing year by year [2; 7-11].

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Mainly PLA is commercially produced from cornstarch via lactic acid fermentation and its potential as a 'green plastic' has been discussed. It generates resource conflicts, because cornstarch is also a resource for food production for humans and livestock [12].

One of the main obstructions in the large-scale L(+)-lactic acid production is the cost of raw material. It generates 3 to 5 times higher cost than those used by production of conventional plastics [3; 11].

Yeast extract (YE) satisfies the nutritional requirements of (+)-lactic acid bacteria that are commonly used in lactic acid fermentations, but the cost of this supplement contributes significantly to L(+)-lactic acid production costs. It is estimated to be approximately 38% of total production costs of L(+)-lactic acid. Consequently, many studies have attempted to find cheaper supplements, which could be used as alternatives or in combination with YE. Significant advantage over chemical synthesis is that biological production can also use cheap materials such as kitchen wastes, waste water sludge, food processing wastes, waste office paper, crop residues, whey, molasses, starch waste, beet, cane sugar and other carbohydrate rich materials. Whey for example is a by-product of the cheese industry which was often disposed as a waste in the past, causing high environmental problem [4; 13; 14].

The aim of this study is presentation of recent works on L(+)-lactic acid biosynthesis. Research works recapped in this article are both leading to decrease costs of production of L(+)-lactic acid and optimize conditions and medium composition of fermentations.

2. Recent investigations

2.1. Materials and methods

Microorganisms

Many researchers used microorganism strains isolated from diary processing industry, starch processing industry or contaminated soil in many cases. List of organisms described in viewed articles is given in Table 1. Majority of researchers worked on bacterial strains but some of them also worked on fungi strains e.g. *Rhizopus* sp. MK-96-1196 [15; 16].

In most cases L(+)-lactic acid producing bacteria operates in anaerobic conditions with the exception of *Bacillus coagulans* SIM-7 DSM 14043*, Bacillus coagulans* NBRC 12583*, Bacillus licheniformis* TY7*, Lactococcus lactis ssp. lactis* B84 which are being reported as oxygen-tolerant strains [5; 10; 17; 18].

Table 1. Microorganisms used in L(+)-lactic acid production

Microorganism	References
Bacillus coagulans SIM-7 DSM 14043	[17]
Bacillus coagulans NBRC 12583	[10]
Bacillus licheniformis TY7	[18]
Enterococcus faecalis RKY1	[7]
Lactobacillus amylophilus GV6	[4; 6]
Lactobacillus casei KH-1	[13]
Lactobacillus casei LA-04-1	[2]
Lactobacillus delbrueckii NCIM 2025	[3]
Lactobacillus delbrueckii ssp. lactis DSM 20073	[17]
Lactobacillus rhamnosus NBRC 3863	[9]
Lactococcus lactis ssp. lactis B84	[5]
Rhizopus sp. MK-96-1196	[8; 15]
Streptococcus bovis 148	[19]
Streptococcus bovis JCM 5802	[11]

2.2. Growth media

Most lactic acid bacteria require a wide range of growth factors including specific minerals, amino acids, vitamins, fatty acids, purines, and pyrimidines for their growth and biological activity [2]. Application of fungi however such as *Rhizopus* for the commercial production of L(+)-lactic acid do not require organic nitrogen sources in growth medium [8].

Most novel works on L(+)-lactic acid production is focused on decreasing costs of source materials. Therefore most often researchers are experimenting with agricultural and municipal wastes. Table 2 shows the combination of carbon and nitrogen sources indicated in discussed articles.

Table 2. Carbon and nitrogen sources used in L(+)-lactic acid production*

Microorganism	Carbon source	Nitrogen source	References
B. coagulans SIM-7 DSM 14043	G	YE, YA	[17]
B. coagulans NBRC 12583	MKR	MKR	[10]
B. licheniformis TY7	MKR	MKR	[18]
Ec. faecalis RKY1	G, CSL, CS	YE	[7]
Lb. amylophilus GV6	WB	CH_3COONa, $Na_2HPO_4 \cdot 2H_2O$	[4]
Lb. amylophilus GV6	WB	P, YE, TC, $NaH_2PO_4 \cdot 2H_2O$	[6]
Lb. casei KH-1	G, CSL	YE	[13]
Lb. casei LA-04-1	G	YE, SP, $(NH_4)_2SO_4$	[2]
Lb. delbrueckii NCIM 2025	Inert SCB, CB	NH_4Cl, YE	[3]
Lb. delbrueckii ssp. lactis DSM 20073	G	YE	[17]
Lb. rhamnosus NBRC 3863	G, RB	RB, YE, $(NH_4)_2SO_4$	[9]
Lc. lactis ssp. lactis B84	PS	MRS without YE, ME	[5]
Rhizopus sp. MK-96-1196	G, CS	$(NH_4)_2SO_4$	[8]
Rhizopus sp. MK-96-1196	CS	$(NH_4)_2SO_4$	[15]
Sc. bovis 148	G, RS	n/a	[19]
Sc. bovis JCM 5802	G	P	[11]

Abbreviations listed and explained at the end of article.

Some of carbon sources have to be saccharified first (e.g. starch, bagasse, bran, agricultural wastes, etc.) which is carried out by hydrolysis using enzymes such as α-amylase and glucoamylase [19; 20]

3. Results of fermentations

Industrial production of L(+)-lactic acid is complicated and currently not economically feasible because of high costs of source material. In present work Table 3. shows effects of recent works mainly focused on lowering production costs.

Table 3. Comparison of the best fermentation results for L(+) Lactic Acid production.

Microorganism	T (ºC)	t (h)	Q_P (g dm^{-3} h^{-1})	$Y_{P/S}$ (g g^{-1})	References
B. coagulans SIM-7 DSM 14043	45	10	9.90	0.92	[17]
B. coagulans NBRC 12583	55	120	1.36	0.98	[10]
B. licheniformis TY7	50	24	2.50	n/a	[18]
Ec. faecalis RKY1	38	57	1.65	0.93	[7]
Lb. amylophilus GV6	37	120	n/a	0.96	[4]
Lb. amylophilus GV6	37	144	n/a	0.36	[6]
Lb. casei KH-1	37	24	0.70	0.81	[13]
Lb. casei LA-04-1	42	84	2.14	0.90	[2]
Lb. delbrueckii NCIM 2025	37	144	n/a	0.25	[3]
Lb. delbrueckii ssp. lactis DSM 20073	56	24	5.60	0.86	[17]
Lb. rhamnosus NBRC 3863	42	22	2.66	0.98	[9]
Lc. lactis ssp. lactis B84	33	144	n/a	0.31	[5]
Rhizopus sp. MK-96-1196	30	48	2.60	0.87	[8]
Rhizopus sp. MK-96-1196	30	96	n/a	0.82	[15]
Sc. bovis 148	37	96	n/a	0.88	[19]
Sc. bovis JCM 5802	37	14	1.00	0.88	[11]
Min.	30	10	0.70	0.25	
Max.	56	144	9.90	0.98	

Encouraging results were obtained in L(+)-LA production with alternative nitrogen sources as substituents for high in cost peptone and yeast extract used usually as a source materials. Michelson et al. reports that substitution of yeast extract (1.75 g) by yeast autolysate (9 ml) resulted in the increase of mean value of productions rate by 18%. Duration of fermentation was also shortened by 16%. During their experiments Michelson et al. also compared *B. coagulans* SIM-7 DSM 14043 to *Lb. delbrueckii ssp. lactis* DSM 20073 with regard on L(+)-LA production. In optimal conditions (Table 3.) the use of *B. coagulans* SIM-7 DSM 14043 strain is resolutely

much more efficient. *Lb. delbrueckii ssp. lactis* DSM 20073 fermented the same initial glucose under optimal condition 2,4 times longer than *B. coagulans* SIM-7 DSM 14043, with the yield lower by 6% [17].

Sakai et al. reports that L(+)-LA producer *B. coagulans* NBRC 12583 was able to converse municipal kitchen refuse (MKR) into highly fine L(+)-LA in temperature 55ºC (optical activity of L(+)-LA: 98,5%). Lower fermentation temperatures resulted in lowering optical activity; conversion of MKR was also conducted under 45ºC and 37ºC and resulted 44.2% and 1.4% respectively. Sakai et al. operated under unsterile conditions and only at high temperature (55ºC) *B. coagulans* NBRC 12583 was able to dominate the environment [10].

Sakai et al. investigated also *B. licheniformis* TY7 strain for the production of L(+)-LA from MKR. This strain operated in high temperature too (50ºC), but in contradistinction to *B. coagulans* NBRC 12583 is rather thermotolerant then thermophilic. Under sterile conditions *B. licheniformis* TY7 was able to generate 2.8-fold higher maximum productivity then *B. coagulans* NBRC 12583 investigated earlier (2.5 g dm^{-3} h^{-1}) with similar optical activity (97%) [18].

Wee et al. investigated production of L(+)-lactic acid using electrodialysed wastewater as a base for fermentation broth. Addition of glucose (100 g/l) and YE (15 g/l) resulted in the productivity of 1.65 g dm^{-3} h^{-1} when *Ec. faecalis* RKY1 was used [7].

Altaf et al. and Naveena et al. carried fermentation with *Lb. amylophilus* GV6 and stated that those strain is able to convert raw materials like starch, wheat bran (without saccharification) directly to L(+)-LA. Altaf et al. used mixture of wheat bran with starch and reported yield of L(+)-LA 2.66-higher then Naveena et al. working only with wheat bran. Both researchers conducted their fermentations in the same conditions [4; 6].

Both Ha et al. and Ding et al. used *Lb. casei* in their experiments. Two different viewpoint were presented. Ha et al. analyzed L(+)-LA production by optimizing culture broth composition focused on addition of CSL supplement to YE/G broth. They worked under lower temperature than Ding et al. – 37ºC and reported lower maximal productivity and yield, 0.70 g dm^{-3} h^{-1} and 81% respectively. However the duration of fermentation was 4 times shorter then at work of Ding et al. research team. Ding et al. investigated different fed-batch feeding strategies and worked only on G/YE broth. The autors noted higher maximal productivity and yield – 2.14 g dm^{-3} h^{-1} and 90% respectively, but fermentation lasted 84 h instead of 24 h [2; 13].

John et al. investigated solid-state fermentation of agro wastes with use of *Lb. delbrueckii* NCIM 2025. They proved that under anaerobic conditions *Lb. delbrueckii* NCIM 2025 is able to produce optically active L(+)-LA with 25% yield. Low yield is rewarded by low energy costs and the ease of extraction of L(+)-LA after fermentation [3].

Gao et al. – another research group that investigated low-cost source materials to produce optically active L(+)-LA using *Lb. rhamnosus* NBRC 3863. The best effect

was obtained when rice bran (RB) and YE with initial pH of culture broth of 1.0 was used. Fermentation took 22 h and during it L(+)-LA with 98% yield was produced. Maximum productivity reached 2.66 g dm^{-3} h^{-1} [9].

Another L(+)-LA producer that is able directly convert starch to optically active L(+)-LA was reported by Petrov et al. *Lc. lactis ssp. lactis* B84 was capable to accomplish full starch saccharification. The best results were obtained by carrying out fermentations on MRS broth with addition of soluble potato starch. After 144 h *Lc. lactis ssp. lactis* B84 was able to hydrolyze completely starch and produce L(+)-LA with the yield of 31% [5].

L(+)-LA has been reported to be obtain from corncob by fungi mutant strain *Rhizopus sp.* MK-96-1196 fermentation in airlift bioreactor. Miura et al. report that *Rhizopus sp.* MK-96-1196 aided by *Acremonium thermophilus* ATCC 24622 was able to produce optically active L(+)-LA on satisfactory levels. *Acremonium thermophilus* ATCC 24622 was responsible for enzymatic hydrolysis of corncob. Next the hydrolyzate was fermented by *Rhizopus sp.* MK-96-1196. They achieved 82% yield. Tha idea was very interesting because of low costs of operation. Airlift bioreactors (ARB) are much less energy-consuming than stirred tank reactors (STR). In addition fungi like *Rhizopus sp.* MK-96-1196 do not require organic nitrogen sources and the biomass is easily separated from the culture broth in the process of recovery and purification of L(+)-LA produced [8; 15].

Ghofar et al. and Narita et al. used *Sc. bovis* strain to produce L(+)-LA. Narita et al. report that *Sc. bovis* 148 is able convert raw starch to optically pure L(+)-LA. *Sc. bovis* 148 secretes α-amylase, which efficiently hydrolyzate raw starch. Authors did not show maximal productivity, but stated that in 96 h fermentation's 88% yield was achieved. However the fermentation took 96 h. Ghofar et al. converted fresh cassava roots slurried with tofu liquid waste supplemented by peptone. However best results were noted when mentioned agro residues were substituted by glucose. It took *Sc. bovis* JCM 5802 only 14 h to convert 1.25 % w/w glucose into L(+)-LA. The similar 1.39 % w/w initial sugar content was converted in 15 h. In both cases yield was similar and amounted to 88% and 85% using glucose, tapioca and fresh cassava roots respectively [11; 19].

4. Conclusions

Researchers concerned with optically active L(+)-LA production are looking for economically feasible source materials. Fast, cheap method is needed to increase world production of L(+)-LA and to exchange plastics with renewable biodegradable plastics from PLA.

Except the need of finding new cheaper raw materials for L(+)-LA synthesis researchers are reporting many factors that can affect biosynthesis of this product. In general, homofermentative organisms have the conversion efficiency of 1 mol glucose to 2 mol lactic acid.

Currently various mesophilic lactic acid bacteria (LAB) strains are tested for production of LA while mainly *Lactobacillus* (*Lb.*) strains are used in industrial LA production. Mesophilic strains are not feasible for the industrial production of LA because of high contamination risks. By using thermophilic strains, LA can be produced under unsterile conditions that decreases expenses for LA fermentation [17].

Application of fungi such as *Rhizopus* for the commercial production of L(+)-LA is also well advised because they do not require organic nitrogen sources and are easily separated from the culture broth in the process of recovery and purification of the lactic acid produced [8; 21].

Abbreviations used in text in alphabetical order:

CB – cassava bagasse
CS – corn starch
CSL – corn steep liqour
G – glucose
ME – meat extract
MKR – municipal kitchen refuse
P – peptone
PS – potato starch
RB – rice bran
RS – raw starch
SCB – sugarcane bagasse
SP – soya peptone
TC – tri-ammonium citrate
WB – wheat bran
YA – yeast autolysate
YE – yeast extract

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NAJNOWSZY ROZWÓJ W BIOTECHNOLOGII KWASU L(+)-MLEKOWGO

Streszczenie: Kwas mlekowy znany jest od 1780 roku jako kwaśny składnik mleka. Znajduje szerokie zastosowanie w przemyśle spożywczym, kosmetycznym, farmaceutycznym. Jeden z jego enencjomerów (L(+)-) jest używany jako substrat do wytwarzania kwasu polimlekowego (PLA). PLA może być wykorzystywany do produkcji biodegradowalnych polimerów. Niniejszy artykuł stanowi przegląd ostatnich prac naukowych skupiających się na ograniczeniu kosztów produkcji kwasu L(+)-mlekowego na drodze optymizacji składu podłoża i warunków hodowli.