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Review

Current Progress on Microbial L-malic Acid Production

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ABSTRACT: As an important intermediate in the tricarboxylic acid (TCA) cycle, L-malic acid (L-MA) is also one of the 12 important platform bulk chemicals with high added value. Owing to its various applications in food, pharmaceuticals, cosmetics and industry, the global L-MA market size is growing year by year. Over the last few decades, increasing concerns regarding fossil fuels depletion and excessive CO_2 emissions have led the global commitment to fostering a green economy and sustainable development. Alternatively, the sustainable microbial fermentation of L-MA has gradually attracted more and more attention. Here, this review summarizes the common L-MA biosynthesis pathways and compares the differences between different chassis microorganisms as well. Moreover, regulation strategies of genetic metabolic engineering and fermentation process to boost the L-MA production are summarized, and the research status of L-MA production from the cheaper substrates is also discussed. Finally, the direction of further exploration of industrialized L-MA biosynthesis is proposed, which provides a theoretical guidance on promoting technological innovation in industrial L-MA production.

Keywords: L-malic acid; Microbial biosynthesis; Strain engineering; Process optimization

1. Introduction

Malic acid, also known as 2-hydroxysuccinic acid, is a well-known C4 dicarboxylic acid, which has been widely applied in food, pharmaceuticals, oil, wastewater treatment, metal cleaning, textile finishing etc. Due to the asymmetric carbon atoms in the molecular structure of malic acid, there exist two stereoisomers, namely L-malic acid (L-MA) and D-malic acid (D-MA) [1]. However, only the L-MA can be metabolized by living cells [1]. Given the significant commercial value of L-MA, the United States Department of Energy has recognized it as one of the 12 important bulk chemicals with the highest added value. According to a report by IMARC Group, the global malic acid market size reached $211.7 million in 2022, and is expected to reach $283.7 million by 2028, with a compound annual growth rate of 5.1% between 2023 and 2028.

On one hand, natural extraction efficiency of L-MA from immature fruits such as hawthorns, apples, and grapes were quite low (only 0.4~0.7%). On the other hand, chemical synthesis process has the disadvantage of using petroleum-based products and requiring high temperatures and pressures, resulting in severe environmental pollution and excessive carbon emissions [1]. Besides, the final products of chemical synthesis are usually a racemic mixture D,L-malic acid, needing extra separation and extraction [2]. Alternatively, the microbial synthesis provides an environmentally friendly and cost-effective way to synthesize high-purity L-MA. Bacteria, yeasts and filamentous fungi are common chassis for L-MA production due to their characteristics. For example, yeasts are competent in low pH fermentation and environmentally friendly production while filamentous fungi can utilize affordable and abundant lignocellulose to synthesize L-MA (Figure 1).

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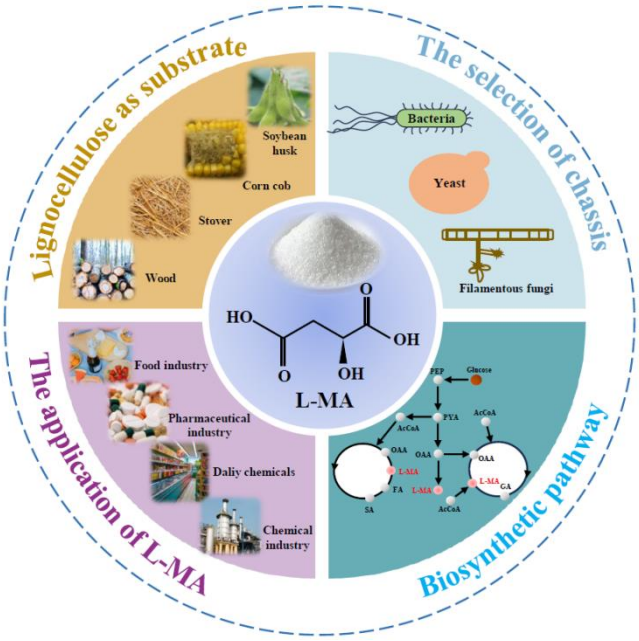

Figure 1. Panoramic view of microbial production of L-MA.

To better understand the state-of-art of microbial L-MA production, this review will introduce common biosynthesis pathway and chassis microbes for L-MA production. What is more, the differences between different chassis microorganisms will also be compared. Besides, metabolic engineering strategies on boosting L-MA production, such as reinforcement on reductive tricarboxylic acid (rTCA) cycle synthesis pathway, secretion enhancement, by-product reduction and cofactor regulation will be summarized. Furthermore, fermentation process optimization including pH regulation, fed-batch and two-stage strategy will also be concluded. Moreover, the concern on cheap feedstock utilization will also be discussed to save operating costs. Finally, the direction of further exploration in industrial L-MA production will be pointed out.

2. The Common Metabolic Pathway and Chassis Microbes for L-MA Production

2.1. Biosynthesis Pathways of L-MA

Generally, there are three main metabolic pathways for L-MA biosynthesis, namely the tricarboxylic acid (TCA) cycle, the glyoxylic acid route, and the rTCA cycle as Figure 2 shows. TCA cycle begins with oxaloacetate and acetyl-CoA, then citrate synthase converts the substances into citrate. After multiple oxidation and decarboxylation reactions, L-MA was finally formed, accompanied by the release of two carbon dioxide [3]. Thus, a theoretical L-MA yield of TCA cycle was 1 mol/mol glucose. Another L-MA biosynthesis pathway was glyoxylate shunt discovered by Hans Adolf Krebs in 1954, which is a branch pathway of the TCA cycle that enables microorganisms to utilize acetic acid, ethanol, and other C_2 substances for growth [4]. Since cyclic glyoxylate shunt completely synthesizes L-MA from acetyl-CoA, its theoretical yield was also 1 mol/mol glucose. Except the two mentioned-above L-MA biosynthesis pathway, another pathway that achieves a theoretical conversion of 2 mol L-MA/mol glucose by fixing CO_2 during reaction, namely rTCA cycle. According to a ^{13}C NMR analysis on the typical L-MA producer *Aspergillus flavus*, it has been confirmed that rTCA pathway was just a simple two-step reaction comprised of pyruvate-oxaloacetate-malate. Specifically, pyruvate carboxylase (*pyc*) and malate dehydrogenase (*mdh*) are the two critical enzymes, *pyc* realized the carboxylation of pyruvate to oxaloacetate, then reduction of oxaloacetate to malate was accomplished under *mdh* catalysis [2,5]. Especially, this efficient synthesis pathway is located in the cytoplasm, L-MA can be exported into the extracellular more easily than the TCA pathway, which was located in mitochondria. Hence, the rTCA pathway becomes a popular pathway introduced into cell factories for the efficient L-MA production.

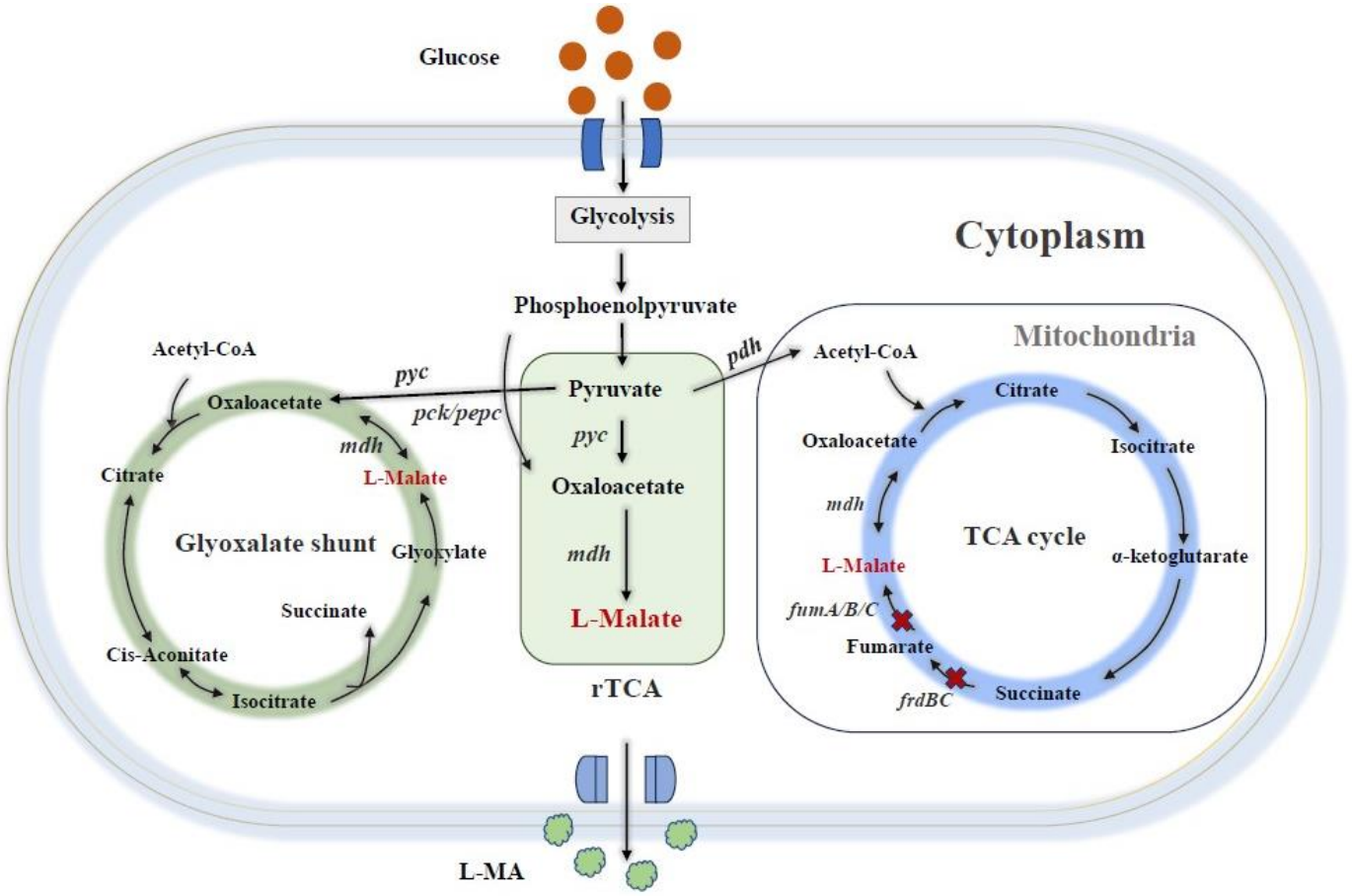

Figure 2. Microbial biosynthetic pathways of L-MA.

2.2. Typical L-MA Producing Microbial Chassis

As reported, although there exist several fungi and yeasts as natural L-MA producers, the performances of these wild types are far from the requirements of industrial production scale. In the meantime, selecting an appropriate starting chassis is the prerequisite to obtain a high-producing strain. Hence, this section describes unique properties of different microorganisms and discusses advantages and disadvantages of the commonly used hosts, providing the references on the efficient L-MA production.

2.2.1. Bacteria

Bacterial L-MA production primarily depended on *Escherichia* and *Bacillus* owing to their advantage of clear genetic background, facile genetic manipulation and rapid metabolic rates [6]. However, due to the limited L-MA producing capability, genetic engineering is required. As reported, overexpressing phosphoenolpyruvate carboxykinase (*pckA*) which mediates the conversion of phosphoenolpyruvate into oxaloacetate can increase oxaloacetate accumulation, contributing to the efficient organic acid production. The strategy also works for the microbial synthesis of L-MA. Soo et al. found that overexpressing gene *pckA* derived from *Mannheimia succiniciproducens* in *E. coli* produced 9.25 g/L of L-MA after 12 h in a 5-L bioreactor. Of course, the appropriate source of *pckA* is also important, and another engineered *E. coli* which overexpressed native *pckA* only produced 1.42 g/L L-MA [7]. To further improve the L-MA production, *mdh* and *pck* were co-overexpressed in *E. coli* BA040 and 13.14 g/L of L-MA with a yield of 0.73 g/g was produced, which obtained an increase of 199.3% and 143.3% than that of the parent one, respectively. What is more, its final L-MA concentration achieved 28.50 g/L with a yield of 0.69 g/g through a scale-up fermentation in 5-L fermentor [8].

2.2.2. Yeasts

Compared to bacteria, several kinds of yeasts are the natural L-MA producers, such as *Zygosaccharomyces rouxii*, however the amount of L-MA accumulation was still far from the industrial requirements [2]. Picking up a suitable synthesis pathway is important to further improve the L-MA production, and rTCA pathway was the most selected one. For instance, through simultaneously overexpressing *pyc2* and *mdh3* in *Saccharomyces cerevisiae*, the final L-MA

production was improved to 59 g/L with a productivity of 0.18 g/L/h in a 500-mL flask [9]. Besides, high acid tolerance of yeasts was a prominent advantage for simplifying downstream recycle process, reducing the overall cost and environmental pollution [10,11]. For example, Sun et al. continuously sub-cultured *S. cerevisiae* under extremely low pH (decreased to pH 2.3) for 1710 generations. After a series of metabolic pathways modifications, the mutant could finally realize 232.9 g/L of L-MA titer with a productivity of 1.62 g/L/h a 3-L fermentor at 144 h without any neutralizer addition [12].

2.2.3. Filamentous Fungi

Compared to the bacteria and yeasts, fungi are a kind of natural L-MA producers, which have a quite high-producing ability. For instance, a typical L-MA producing fungus is *A. flavus*, whose titer of L-MA reached 113 g/L with a productivity of 0.59 g/L/h after fermentation condition optimization in a 16-L fermentor [13]. However, it was found that *A. flavus* produced carcinogen aflatoxin during the fermentation process, which is harmful to human health and unsuitable for food-grade L-MA production. Hence, other lower L-MA producing capacity but safer fungi were selected as the starting chassis, such as *A. oryzae*. To improve its L-MA productivity, native *pyc* and *mdh* in *A. oryzae* NRRL 3488 were overexpressed, improving the L-MA titer from 26.1 g/L to 42.3 g/L with a 28.3% increase [14]. Then, after going through further genetic modifications, *A. oryzae* NRRL 3488 produced 165 g/L L-MA with a productivity of 1.38 g/L/h in 3-L fed-batch culture, achieving a competitive L-MA productivity [14].

Apart from the advantage of containing native L-MA biosynthetic pathways in some fungal species, fungi have another striking trait of degrading and utilizing cheap substrates. For instance, as an excellent natural lignocellulose degrader, *Myceliophthora thermophila* produced 181 g/L L-MA from Avicel in a 5-L fed-batch fermentor through co-overexpression of malate transporter (*mae*) and *pyc* from *A. oryzae* [15].

3. Metabolic Engineering to Boost the L-MA Production

3.1. Reinforcement on L-MA rTCA Synthesis Pathway

As described above, since rTCA pathway is the most selected one for microbial L-MA biosynthesis owing to its high theoretical yield. Hence, picking up appropriate sources for *pyc* and *mdh* is a prerequisite to obtain L-MA hyper-producing strain. Xi et al. combinatorically expressed two source-derived *pyc* (*Pichia kudriavzevii* and *A. oryzae*) and five source-derived cytoplasmic *mdh* (*P. kudriavzevii*, *Z. rouxii*, *Corynebacterium glutamicum*, *S. cerevisiae* and *A. oryzae*) in *P. kudriavzevii*. And among these ten generating strains, MA006-10 whose *pyc* and *mdh* from *A. oryzae* produced the highest L-MA titer of 43.8 g/L in 250-mL shake [3]. Apart from screening optimal gene sources, selecting proper isozyme also count a lot. For example, cytoplasmic MDH2 was prone to be inactivated by glucose catabolism, while isozyme MDH3 which was derived from the peroxisome has overcome this (MDH3ΔSKL). Hence, overexpressed MDH3ΔSKL in *S. cerevisiae* has a higher MDH activity, achieving 4.2 g/L L-MA titer with a 3.6-fold increase [12]. Apart from exploring malate dehydrogenase with higher activity, inactivation enzymes which is harmful to it also counts. For example, glucose-induced degradation defective (GID) complex facilitated the degradation of MDH2, which is detrimental to L-MA synthesis. And the missense mutation in GID4 subunit in *S. cerevisiae* resulted in the inactivation of GID complex, contributing to a 14.2-fold increase in L-MA production [16].

3.2. Secretion Enhancement

In spite of introducing pivotal genes to promote L-MA generation, strengthening the cytoplasmic export of L-MA into the external by overexpressing transporter genes also have a significant effect on L-MA productivity. For example, by introducing a malate transporter gene *SpMAE1* derived from *Schizosaccharomyces pombe* to an acid-tolerant yeast *P. kudriavzevii*, the L-MA titer was improved to 43.8 g/L from below 3 g/L in 250-mL flask [3]. Similarly, Sun et al. verified that a *SpMae* variant enhanced the export capacity of L-MA successfully, contributing to 59.9 g/L L-MA with a 25% increase and a reduction of fermentation time by 72 h in *S. cerevisiae* [12]. Apart from affecting L-MA production in yeasts, introduction of carboxylic acid transporters also works in fungi. For instance, overexpressing the native C4-dicarboxylic acid transporter in *A. oryzae* NRRL 3488 improved L-MA concentrations to 60 g/L, roughly two-fold of that of wild-type (27 g/L) after 72 h [5]. Moreover, simultaneous overexpression of *A. oryzae*-derived C4-dicarboxylate transporter gene and *S. pombe*-derived L-MA permease gene improved the L-MA titer of *A. oryzae* from 58.5 g/L to 89.5 g/L [14].

3.3. By-products Reduction

For efficient L-MA production, enhancing carbon flux to target products is not enough, it is also requisite to suppress the shunt of the by-product formation to reduce competition on carbon metabolic flow. Besides, by-products mixture brought out more difficulty in downstream separation and extraction. As reported, the most accumulated by-product during L-MA biosynthesis are fumaric acid, succinic acid, lactic acid, formic acid, citric acid as well as acetic acid [2].

Specifically, gene *fumB* (coding fumarase), *frdABCD* (coding fumarate reductase), *ldhA* (coding lactate dehydrogenase), *pflB* (coding pyruvate formate-lyase) and *poxB* (coding pyruvate oxidase) was respectively related to fumaric acid, succinic acid, lactic acid, formic acid and acetic acid biosynthesis. Thus, destruction of these by-product generating pathway may boost the L-MA production. For example, Dong et al. sequentially knocked out *frdBC*, *fumB* and *fumAC* in *E. coli* F0511 in order to inhibit the conversion of L-MA to succinate, resulting in succinate accumulation was decreased by 64.9%, 76.9%, and 91.5%, respectively compared to the parent one. Accordingly, the highest L-MA titer was increased up to 7.78 g/L [17].

3.4. Cofactor Regulation

In addition to the strategies mentioned above, cofactor regulation could be considered as another working strategy on L-MA production improvement. For example, introducing soluble pyridine nucleotide transhydrogenase *SthA* derived from *E. coli* (EcSthA) into engineered *P. kudriavzevii* successfully improved L-MA production to 38.3 g/L in 250-mL flask. The underlying mechanism was that EcSthA provided adequate cytosolic NADH, which is important to activate malate dehydrogenase and the by-product pyruvic acid accumulation was decreased to 2.9 g/L from 7.9 g/L at the same time [3]. Similarly, overexpressing of NADH oxidase LlNOX from *Lactobacillus lactis* leads to a 36.7% decrease on succinic acid unexpectedly [18]. Aside from NADH content regulation, cofactor NADPH also has an effect on L-MA improvement. For instance, Dong et al. introduced gene *pos5* coding NADH kinase into *E. coli* W3110 to provide more NADPH, and this engineered *E. coli* W3110 produced 21.65 g/L L-MA with a yield of 0.36 g/g in a 5-L bioreactor with a 183.4% increase [17].

4. Culture Process Optimization to Increase the Microbial L-MA Production

4.1. pH Regulation

It is well-known that fermentation conditions play key roles in L-MA production, especially pH. Generally, near-neutral pH (6.0–6.5) was considered as optimal pH for effective L-MA production, no matter for bacteria, yeasts and fungi [3,9]. However, the medium pH decreases markedly due to the overproduction of acidic products, which severely inhibits the metabolic activity and the production of cells [12]. Zambanini et al. found that dissolved L-MA in the medium does cause a product inhibition as soon as the concentration reached 100 g/L and addition of neutralizing agent to the broth precipitated out the L-MA into carbonate form, greatly alleviating product inhibition and toxicity on strain [19]. And among various kinds of neutralizers, $CaCO_3$ is the most used one. A study has proved that L-MA titer reached 129 g/L under the $CaCO_3$-buffered system while the titer just reached 4.0 g/L in MES-buffered system [20]. The reason is that $CaCO_3$ not only has the ability of maintaining fermentation broth at appropriate pH, but also supplies the CO_2 required for the L-MA production. For instance, enhanced L-MA titer of 195 g/L and productivity of 0.74 g/L/h was achieved in *Ustilago trichophora* TZ1 in a 2.5-L bioreactor with $CaCO_3$ buffer system, while the titer and productivity of the initial one was 142 g/L and 0.54 g/L/h, respectively [20]. What is more, the addition amount of neutralizer also accounts. For example, a modified yeast *P. kudriavzevii* produced only 53.2 g/L L-MA with 20 g/L $CaCO_3$ addition, whereas 199.4 g/L of L-MA with a yield of 0.94 g/g was achieved in a 3-L fermentor when $CaCO_3$ neutralizer increased to 70 g/L [3].

4.2. Fed-batch Strategy

Another major limitation of organic acid production during fermentation is the substrate inhibition, especially when using glucose as the substrate. Hence, fed-batch strategy is a commonly adopted method. For example, the maximum L-MA titer reached 165 g/L at 120 h in a 3-L fed-batch fermentor by an engineered *A. oryzae* strain, which was increased by 17.9% and 13.1% compared with that in 250-mL shake flasks and in batch culture, respectively [14]. Similarly, an

engineered *Trichoderma reesei* produced 220.5 g/L L-MA with the productivity of 1.15 g/L/h in a 5-L fed-batch culture while only produced 105 g/L L-MA in 250-mL flask culture [21].

4.3. Two-stage Regulation Strategy

The two-stage regulation strategy was another commonly used strategy to maintain a balance between microbial growth and metabolite formation, bringing in higher target titer. The underlying mechanism of two-stage strategy is that aerobic phase supported growth of the strain, while anaerobic phase was designed to minimize the carbon flux to biomass while maximize the carbon flow to the target products. For example, Zhang et al. adapted a two-stage strategy during L-MA fermentation by *E. coli*, namely cells grown aerobically (1.0 vvm air) for 16 h and then shifted to anaerobic conditions for L-MA production (72 h). With this approach, 33.9 g/L L-MA was produced with a yield of 1.42 mol/mol glucose in 3-L bioreactor, higher than 21.9 g/L of L-MA without two-stage regulation [22]. Similarly, Jiang et al. conducted a dual-phase fermentation process of the recombinant *E. coli* BA063 for higher L-MA titer. Specifically, aerobic culture was finished when DCW reached around 11 g/L, then shifted into anaerobic phase by sparging CO_2. Finally, L-MA concentration of this engineered *E .coli* reached 28.50 g/L with a yield of 0.69 g/g within 67 h in a 5-L fermentor [8]. Besides, anaerobic condition also had an effect on ATP level and intracellular NAD(H) pool enhancement, resulting in L-MA yield increase.

5. Microbial L-MA Production from Cheap Substrate Utilization

5.1. Utilization of Glycerol or Methanol as the Feedback

From the above sections, it can be deduced that glucose is the most widely used fermentable substrates in microbial L-MA production cases. However, to further meet the industrial interests, cheaper carbon sources need exploring. One of potential carbon sources is crude glycerol, a major by-product accounting for approximately 10% of the total volume of produced biodiesel [23]. To make use of this kind of feedstock for L-MA production, Zambanini et al. obtained the *U. trichophora* RK089 which could synthesize L-MA through glycerol assimilation after screening 74 *Ustilago* strains [19]. And further adaptive laboratory evolution (ALE) improved glycerol intake and L-MA synthesis efficiency, the growth and production rate of *U. trichophora* TZ1 reached 0.26 g/L/h and 3.5 g/L/h, which were increased by 2.5- and 6.6-fold, respectively after domestication. Along with further medium optimization, the final titer of L-MA reached 196 g/L in 500-mL flask [20]. Apart from crude glycerol, methanol is another promising feedstock owing to its low price, abundance, energy richness and renewability [24]. Similar to the strategy above-mentioned, *A. niger* MTCC 281 was sub-cultured adaptively in the medium containing methanol (1–5%) and L-MA (40–80 g/L) for 22 weeks. Finally, with enhanced stress tolerance, the mutant *A. niger* MTCC produced 62.54 g/L of L-MA after 192 h, 4.45-fold higher than the control strain MTCC 281 [25].

5.2. Utilization of Lignocellulose as the Feedback

Lignocellulose is a kind of abundant and renewable resource, but the complex structure hinders its utilization [26]. Thus, the pretreatment on lignocellulosic substrates is commonly required [27,28]. However, as one of the major components, the poor utilization capability of xylose usually hinders the efficient total component sugars utilization of lignocellulose hydrolysate [29]. For example, *S. cerevisiae* CTMAE produced 70 mg L-MA/g xylose under 10 g/L xylose condition after the XR-XDH assimilation pathway import in 125-mL flask [30]. Furthermore, by eliminating the by-product pathway, enhancing the L-MA synthesis pathway, transport function and a fed-batch fermentation, L-MA titer of *S. cerevisiae* CTMAE reached 61.2 g/L with a yield of 0.23 g/g xylose in a 3-L bioreactor [31]. However, a problem worth noting is that lignocellulose hydrolysate usually contains quite amounts of inhibitors, such as furans, weak acids and phenolic aldehydes, having a negative effect on cell growth and product generation. Alternatively, some fungi with the strong cellulose degradation ability, becoming the promising chassis microorganisms. For example, after expressing an exogenous *pyc* derived from *C. glutamicum* ATCC 13032, 62.76 g/L of L-MA was directly produced from 100 g/L cellulose by a modified strain *Thermobifida fusca* muC in 3-L fermentor. What is more, it even could directly degrade milled corn stover and obtain 21.47 g/L of L-MA [32].

6. Conclusion and Prospects

With the expanding demand for L-MA, more and more attentions were paid to realize biological production for its environmental protection. And selecting an appropriate starting chassis is the fundamental step. Based on this review,

it can be deduced that bacteria have a relatively low L-MA titer than that of the yeasts and fungi as Table 1 shows. What is more, yeasts have an obvious advantage of significant acid-tolerance capacity, contributing to reduced neutralizer addition and lower investment cost. When it comes to fungi, which has a native ability of utilizing cheap lignocellulose feedbacks, saving overall cost as well. Collectively, yeasts and fungi chassis exhibit superior performance in L-MA production compared to bacterial ones.

Besides, it is also worth noting that filamentous fungal has another unique feature, namely its specific morphological is closely correlated with target production [14,33]. Hence, making full use of this trait is also a direction for L-MA production enhancement. For example, Chen et al. realized 142.5 g/L L-MA of *A. oryzae* in a 7.5-L fermentor, much higher than 105.5 g/L of the origin one through overexpression of gene *cdc14* (cell division cycle 14) with three copies [33]. Similarly, deletion of *gul1* which encodes a putative mRNA-binding protein related to hyphal morphology in *T. reesei* boosting the L-MA production reached 235.8 g/L from 170 g/L in 5-L fermentor [34].

Apart from breeding high-producing chassis through metabolic engineering, there still exist another three problems as for L-MA microbial fermentation process. One is a plenty of neutralizers should be added to maintain the neutral pH, supporting microorganism growth and organic acid generation in its carboxylate form. Consequently, inorganic acids sulfuric or hydrochloric acid was added to release carboxylic acids form, which bring the challenges for the downstream extraction process, such as the increased environmental pollution and overall production costs. Hence, realizing reduction on neutralizers usage is a main requirement especially when it comes to industrial production. And ALE is a promising strategy to breed acid-tolerant strains, making for neutralizers decrease. Besides, further genetic modifications could focus on membrane engineering to enhance microbial resistance to stress.

The other problem need solving is the difficulty in downstream separation and extraction process. As reported, the emerging innovation extraction techniques, namely in-situ product recovery (ISPR) system by trioctylamine in 1-octanol successfully improved L-MA titer of *A. niger* PJR1 to 131.48 g/L from 115.67 g/L through fed-batch extractive fermentation [35]. This approach decreases the adverse effects of stress caused by low pH and high L-MA concentrations on the physiological traits of the producing strain and simplified downstream process.

Last but not least, the costs of substrates should be reduced. Although the lignocellulose hydrolysate is considered as a promising substrate, the inhibitors hinder the cell growth and the final L-MA production. Hence, approaches towards elimination of inhibitors should be developed. As reported, detoxification treatment is a promising strategy [32]. Besides, ALE to improve the strain tolerance, genetic modifications on resistance ability enhancement both can be considered as promising strategies to overcome the inhibitor problem. Collectively, to further promote L-MA industrial production scale, ideal L-MA cell factories with high productivity and strong resistance ability need breeding through integrating disciplines. Furthermore, in conjunction with innovative separation and extraction techniques downstream process, the cost will be further optimized.

Table 1. A summary on typical chassis for L-malic acid production.

	Strain	Substrate	Titer (g/L)	Yield (g/g)	Productivity (g/L/h)	Fermentation Scale	Strategy	Reference
Bacteria	*E. coli*	Glucose	9.25	0.56	0.74	5-L fermentor	Δ*pta*, +*MspckA*	[7]
	E. coli	Glucose	28.50	0.69	ND	5-L fermentor	Δ*ldhA*, Δ*pflB*, Δ*frdABCD*, Δ*fumB*, +*mdh*, +*pck*	[8]
	E. coli	Glucose	21.65	0.36	0.30	5-L fermentor fed-batch	Δ*ldhA*, Δ*poxB*, Δ*pflB*, Δ*pta-ackA*, Δ*frdBC*, Δ*fumB*, Δ*fumAC*, +*AtME2*(C490S), +*Scpos5*	[17]
	E. coli	Glucose	33.9	1.06	0.47	3-L fermentor	Δ*ldhA*, Δ*adhE*, Δ*ackA*, Δ*focA*, Δ*pflB*, Δ*mgsA*, Δ*poxB*, Δ*frdBC*, Δ*sfcA*, Δ*maeB*, Δ*fumB*, Δ*fumAC*	[22]
	B. subtilis	Glucose	2.16	0.12	0.03	500-mL flask	+*Ecppc*, +*Scmdh2*, Δ*ldh*	[36]
Yeasts	*S. cerevisiae*	Glucose	59	0.31	0.29	500-mL flask	Δ*pdc1*, Δ*pdc5*, Δ*als*, Δ*pdc6*, Δ*ura3*, Δ*trp1*, +*ScMDH3ΔSKL*, +*ScPYC2*, +*SpMAE1*	[9]
	S. cerevisiae	Glucose	232.9	0.66	1.62	3-L femtentor	Adaptive laboratory evolution, +*ScPYC2*, +*ScMDH3ΔSKL*, +*SpMae1F253A*, +*ScPYK*, +*ScCYB2*	[12]
	S. cerevisiae	Xylose	61.2	0.23	0.32	3-L femtentor	Δ*alsS*, Δ*RtME1*, +*ScPYC1*, +*ScPYC2*, +*SpMAE1*, +*ScMDH3ΔSKL*, +*ScLEU2*, +*ScHIS3*, +*ScTRP1*, +*ScURA3*	[30]
	P. kudriavzevii	Glucose	199.4	0.94	1.86	3-L femtentor, fed-batch	Δ*URA3*, Δ *PDC1*, Δ*GPD1*, Δ*PCK1*, Δ*MDH2*, +*AoPYC*, +*AoMDH*, +*SpMAE1*, +*SpVHT1*, +*EcPPC*, +*EcSthA*, +*PkURA3*	[3]
Fungi	*A. flavus*	Glucose	113	0.95	0.59	16-L fermentor	Wild strain	[13]
	A. oryzae -	Glucose	165	0.68	1.38	3-L fermentor	+*Aopyc*, +*Aomdh*, +*Ecppc*, +*Ecpck*, +*AoC4T318*, +*Spmae1*, +*Aopfk*	[14]
	U. trichophora	Glycerol	195	--	0.74	2.5-L fermentor, fed-batch	Adaptive laboratory evolution	[20]
	U. trichophora	Glycerol	196	0.82	0.39	500-mL flask, fed-batch	Adaptive laboratory evolution	[19]

	T. fusca	Avicel	62.8	--	--	3-L fermentor	Adaptive laboratory evolution, *+Cgpyc*,	[26]
	A. niger	Glycerol	62.5	--	--	No specific flask size	Adaptive laboratory evolution	[25]
	A. oryzae	Glucose	142.5	0.75	1.08	30-L femtentor, fed-batch	*+AoCDC14(3)*	[33]
	M. thermophila	Avicel	181	0.99	--	5-L fermentor, fed-batch	*+Aopyc*, *+Aomae*	[15]
	T. reesei	Glucose	220.5	--	1.15	5-L fermentor, fed-batch	Δ*tre103451*, *+Aomae1*, *+Aopyc*	[21]
	T. reesei	Glucose	235.8	--	1.23	5-L fermentor	*+trmae1*, *+trpyc*, *+trmdh*, Δ*gul*, *+ trme*, *+trhxt*	[34]

Note: “ND” and “——” represent “not detected” and “not described”, respectively.

Author Contributions

Conceptualization, M.J. and Y.J.; Validation, W.J., W.Z., and F.X.; Writing—Original Draft Preparation, L.M.; Writing—Review & Editing, L.M. and M.Q.; Supervision, Y.J.; Project Administration, M.J.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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