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Research Article

Organic acids: An overview on microbial production

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ABSTRACT:
Increasing environmental concern and depletion of limited resources have led to the development of microbial approaches for the production of commodity chemicals for sustainable development. The advances in microbiology and fermentation technology have lead to the development of eco-friendly processes replacing the some of the conventional chemical methods. Microbial production of organic acids has been incessantly escalating over conventional chemical methods due to several advantages including enantioselectivity, high purity, less environmental pollution and cost effectiveness. The increasing knowledge on metabolism and pathway regulation of industrially relevant organisms has already proven to be invaluable for generating rational strain development with primarily industrial performances. In recent times, microbial production of building block chemicals is progressively expanding its market for the production of succinic, lactic, citric, itaconic, gluconic, lactobionic acids, etc. Organic acids are utilised directly or indirectly in a wide range of applications including food, healthcare, cosmetics, textile, solvents, and construction industries as well as for the manufacturing of biodegradable packaging materials. In this communication, we are presenting an overview of organic acids production by microbial approaches as well as their applications in different industries.

Keywords: organic acid, microorganisms, industry, production

INTRODUCTION

In recent times, escalating global energy and environmental problems has led to the rapid development in biotechnology to invent environmental friendly green methodologies for the production of wide variety of commodity chemicals. As a result, in recent years, microorganisms mediated production of commercially valuable products has continuously grown.

Since ancient time, organic acids have been used as food additives and preservatives for prolonging the shelf life of food ingredients. Organic acids constitute a key group among top platform chemicals that can be produced by microbial fermentation. Microorganisms mediated production of valuable products is preferred over conventional methods due to high purity, selectivity, cost effectiveness and eco-friendly nature [1-4]. Organic acids have a wide range of applications and are utilized as key ingredients or platform chemicals in a variety of applications including food, beverages, pharmaceuticals, textile, detergents, solvents, petrochemicals, dyes and adhesives, rubber, perfumes, and plastics. Additionally, they also used in the production of chemicals and materials used in automotive and construction industries [3, 5]. According to a specified market testimony, the organic acid market was expected to grow at a compound annual growth rate of 6.0% for the period 2016-21. Growing demand of vinyl acetate monomer in food packaging industry and high growth of pharmaceutical industry are among major driving factors for high growth in market [6]. Organic acids and their derived products are used on a large scale in the chemical and food industry. Different salts of gluconic acid are used for supplementation as well as for the treatment of the diseases caused by deficiency of minerals such as calcium, iron and zinc [7]. Biodegradability and biocompatibility of polylactic acid and lactobionic acid have been utilized enormously in pharmaceutical industry for the synthesis of prosthetic devices, sutures and internal drug dosing, and as well as in development of new innovative treatment of many diseases [2, 8]. Many organic acids are conveniently produced from renewable feedstock materials by fermentation or biotransformation using pure microbial cultures. Biocatalytic activities of numerous microorganisms have been used widely for the commercial production of different organic acids, such as citric acid, acetic acid, lactic acid, itaconic acid, tartaric acid, succinic acid etc [1, 9-12].

3-Hydroxypropionic acid is one of the most valuable chemicals from biomass and it has a huge market potential being used as precursor for the production of acrylic acid. The massive demand of acrylic acid is continuously surging up owing to widespread use in adhesive, paint, super absorbent and surface coating and it may reach 8 million tons in size with a net value of $18 billion in 2020. Other uses of 3-hydroxypropionic acid involve production of ethyl 3-hydroxypropionic acid, 1,3 propanediol, acrylonitrile and acrylamide, malonic acid and also in the production of polyesters, metal lubricants, textiles etc. Microbial production of 3-hydroxypropionic acid is considered more effective with mild operation conditions and reduced environmental loads than chemical catalysis based methods. Klebsiella pneumoniae and E. coli have been studied extensively for the microbial production of 3-hydroxypropionic acid using glycerol as a carbon source [3, 13-19].

Acetic acid is widely used in the production of commodity chemicals, such as vinyl acetate, acetic anhydride, cellulose acetate etc. These components are used in a wide range of industries, such as plastics, coatings, photographic, automobile, pesticides and food ingredients among others [20, 21]. In 2014, the acetic acid production was 12 million tons and is expected to reach volume 17 million tons by 2022 at a compound annual growth rate of 4.7%. In 2014, highest consumption of the global acetic acid was accounted for the production of vinyl acetate monomer, which is primarily used in adhesive and sealant industry [22]. A variety of microorganisms such as Acetobacter, Gluconacetobacter, and Gluconobacter are used for the biological production of acetic acid [23-26].

Citric acid is a biodegradable and ecofriendly chemical widely used in food, pharmaceutical, and cosmetic industries. It is the main additive used in food industry and approximately 70% citric acid of total global production is used as acidulant, antioxidant, preservatives and flavor enhancer in food and beverage industry [27-30]. Other major applications of citric acid involve its use in pharmaceutical industry, as antioxidant to preservatives, effervescent, and in textile industry as foaming agent for softening and treatment of textiles [31]. Approximately, 99% of global production occurs via microbial processes using surface or submerged cultures and a large number of microorganisms have been utilized for the citric acid production, but only a few of them are appreciable to produce citric acid at industrial scale [30]. The current industrial production relies on Aspergillus niger through submerged fermentation of starch-based or sucrose-based media [3, 32]. Many other species of Aspergillus and varieties of yeasts such as Candida catenula, Candida guilliermondii, Yarrowia lipolytica, and Candida tropicalis are also used in the production of citric acid [33]. Two metabolically engineered and closely related species of the Aspergillus, A. niger and A. wentii were reported for the commercial production of citric acid [31].

Gluconic acid, a multifunctional carbonic acid, is mainly utilized in construction, food and pharmaceutical industries. It is used as an additive in cement to control the setting time and for improved strength and water resistance. It is a nonvolatile, noncorrosive, nontoxic mild organic acid used for refreshing sour taste in food products [5]. Several microorganisms, such as filamentous fungi are involved in glycolic acid production. The bio-production of gluconic acid from glucose primarily utilizes fungal species, such as A. niger, and Penicillum and bacterial species, such as Pseudominas, Acetobacter and Gluconobacter. Different salts of gluconic acids are used in pharmaceutical industry for treatment of deficiency as well as supplement of calcium and iron. Zinc gluconate is used to cure various diseases such as mental lethargy caused by zinc deficiency. Its property of sequestering iron over a wide range of pH is exploited in the textile industry, where it prevents the deposition of iron and for desizing polyester and polyamide fabrics [7]. According to a study, the value of the global gluconic acid market is expected to grow at a CAGR of 9.1% during the period 2016-2021 [34].

Itaconic acid is an important component in the chemical industry. Itaconic acid and its derivatives have broad application spectrum in textile, chemicals, and pharmaceutical industries. It is used as building block for acrylic plastics, acrylic latexes, anti-scaling agents and super absorbents [35-37]. It also has other applications in food packaging, detergents, paints & coatings, pharmaceuticals, agriculture, emulsifiers, herbicides, and printing chemicals. Many microorganisms, such as Ustilago zeae, U. maydis, Candida sp., and Rhodotorula sp. have been reported to produce itaconic acid [38-40]. However, Aspergillus terreus is a preferred source in commercial production of itaconic acid up to 80 g/L [35]. The annual market of about 80,000 tons is currently met by fungal fermentation relying on natural Aspergillus spp. producers [41-43].

Lactic acid is an important commodity chemical utilized in a wide range of applications in food, pharmaceutical, cosmetic, and leather industries [3]. It has a great potential for the production of biodegradable and biocompatible polylactic acid polymer, which has vital applications in pharmaceutical industry for the construction of prosthetic devices, sutures and internal drug dosing [8, 44]. The poly lactic acid has other important applications in packaging, agriculture, automobile, textile, and other industries [45, 46]. Lactic acid production can be achieved by chemical method or through fermentation. Fermentative production is more advantageous as chemical method produce racemic mixture (±) of lactic acid. In addition, microbial productions utilize low cost renewable substrates and minimize energy consumption [20, 47]. Commercially pure lactic acid can be synthesized by microbial fermentation of the following carbohydrates, such as glucose, sucrose, lactose, and starch/maltose derived from feed-stocks such as beet sugar, molasses, whey, and barley malt [48, 49]. Lactobacillus strains have been particularly useful in the production of D-or L-lactic acid due to their high acid tolerance and ability to be engineered [47, 50-52]. Lactic acid bacteria are preferred over fungi due to the high yield of lactic acid by bacterial mediated fermentations. Stereochemically pure L-(+)-lactic acid is solely produced by Rhizopus oryzae [46]. Other microorganisms, such as Escherichia, Bacillus, Kluyveromyces and Saccharomyces are also involved in efficient production of lactic acid [48, 53].

Succinic acid, an intermediate metabolite of TCA cycle and also known as amber acid or butanoic acid or butanedioic acid, is widely utilized in the food industry, pharmaceuticals, and agriculture. It is used as a platform chemical for the synthesis of several chemical compounds including polybutylene succinate, adipic acid, 1,4-butanediol, tetrahydrofuran, and gamma butyrolactone that have industrial values [54]. The commodity chemicals, derived from succinic acid, are further utilized for the manufacturing of biodegradable polyesters, neutralizing agents, pesticides, herbicides, solvents in chemical, and batteries industries [19]. The demand of SA as a platform chemical is expected to rapidly increase to an anticipated-market size of >700 000 tons per year by 2020 [42, 55]. Conventional method for production of succinic acid is a high cost conversion process, which utilizes maleic anhydride derived from petroleum materials [54]. The biotechnological production of succinic acid is more advantageous over chemical approaches as microbial production offers high yield, purity and cost effectiveness [56, 57]. Though succinic acid is synthesized in almost all animals, plants and microbes, bacteria and but fungi are recognized for considerable commercial production of succinic acid due to technical and economical advantages. Several fungal, yeast and bacterial microbes have been investigated for succinic acid production and Actinobacillus succinogenes, Basfia succinicproducens, Corynebacterium glutamicum, Pichia kudriavzevii, Escherichia coli, Saccharomyces cerevisiae, and Mannheimia succiniciproducens are primarily used in large commercial production [55, 58]. Escherichia coli is one of the most studied microorganisms for succinate production [19].

In recent time, **lactobionic acid** has been recognized as a potential commodity chemical and bioactive molecule for a wide range of applications in food, cosmetics and pharmaceutical industries [2, 59, 60]. Lactobionic acid is amphiphilic, excellent nontoxic, biodegradable and biocompatible, and has ion-chelating abilities and therefore it can be used for innovative treatment of many potential diseases including cancer. Nanoparticles, coated with bio-functional LBA, have significant potential for biomedical applications such as detection, cancer therapies, bio-labeling and magnetic resonance imaging [2, 61]. It is widely used in medical products such as antibiotics and preservative solutions for organ transplantation. It is used as an acidifier agent, antioxidant, gelling agent, mineral absorption enhancer in dairy desserts and aging inhibitor for bread in food industry. In cosmetic products, it is applied as key component of anti-aging and regenerative skin care products [62]. Pseudomonas species are efficiently involved in the production of this acid since the first discovery of lactobionic producing bacteria in the late 1940s [63]. Strains such as P. mucidolens, P. myxogenes or P. fluorescens were found to be able to oxidize lactose directly without the need for prior hydrolysis or phosphorolysis. P. taetrolens has been observed with an excellent potential for cost effective production as it utilizes inexpensive feedstock cheese whey for the fermentative production of this bionic acid [2].

Fumaric acid is a naturally occurring organic acid, and was first isolated from Fumaria officinalis plant. Many microorganisms produce fumaric acid, which is an important intermediate of TCA cycle. It is 1.5 times more acidic than citric acid, and hence used as food acidulant. Many microbial strains, such as Rhizopus, Mucor, Cunninghamella, and Circinella are used for the production of fumaric acid, and among them Rhizopus species, namely Nigricans, Arrhizus, Aryzae and Formosa are preferred due to the high yield. It is also used to produce synthetic papers, plasticizers and eco-friendly polymers [19, 64-66].

CONCLUSION: Increasing depletion of fossil fuels and environmental concern have considerably expanded biotechnological horizon to search for new biobased routes to produce commodity chemicals. The present review aimed to provide an overview on production of organic acids using various microorganisms. Here, we have concisely described the role of building block chemicals and the microorganism for the production of industrially valuable commodity chemicals. Although, microbial production of organic acids has significant consideration, great advances have to be made to develop high performance strains for high yield and productivity for explored and unexplored commodity chemicals.

REFERENCES

1- Sauer, M., Porro, D., Mattanovich, D., & Branduardi, P. (2008) Microbial production of organic acids: expanding the markets. Trends in biotechnology 26(2):100-108.
2- Alonso, S., Rendueles, M., & Díaz, M. (2013) Bio-production of lactobionic acid: current status, applications and future prospects. Biotechnology advances 31(8):1275-1291.
3- Chen, Y., & Nielsen, J. (2016) Biobased organic acids production by metabolically engineered microorganisms. Current opinion in biotechnology 37:165-172.
4- Singh, R., Kumar, M., Mittal, A., & Mehta, P.K., (2016) Microbial enzymes: industrial progress in 21st century. 3 Biotech 6(2):174.
5- Ahmed, A.S., Farag, S.S., Hassan, I.A., & Botros, H.W. (2015) Production of gluconic acid by using some irradiated microorganisms. Journal of Radiation Research and Applied Sciences 8(3):374-380.
6- Anonymous (http://www.marketsandmarkets.com/Market-Reports/organic-acid-market-30190158.html.
7- Ramachandran, S., Fontanille, P., Pandey, A., & Larroche, C. (2006) Gluconic acid: properties, applications and microbial production. Food Technology and Biotechnology 44(2):185-195.
8- San□Martín, M., Pazos, C., & Coca, J. (1992) Reactive extraction of lactic acid with alamine 336 in the presence of salts and lactose. Journal of Chemical Technology and Biotechnology 54(1):1-6.
9- Goldberg, I., Rokem, J.S., & Pines, O. (2006) Organic acids: old metabolites, new themes. Journal of Chemical Technology and Biotechnology 81(10):1601-1611.
10- Yang, S., et al. (2006) Extractive fermentation for the production of carboxylic acids. Bioprocessing for Value-Added Products from Renewable Resources 16:421-446.
11- Straathof, A.J. (2013) Transformation of biomass into commodity chemicals using enzymes or cells. Chemical reviews 114(3):1871-1908.
12- López-Garzón CS & Straathof AJ (2014) Recovery of carboxylic acids produced by fermentation. Biotechnology advances 32(5):873-904.
13- Gokarn, R.R., et al. (2007) 3-hydroxypropionic acid and other organic compounds. (Google Patents).
14- Nikolau, B.J., Perera, M., Brachova, L., & Shanks, B. (2008) Platform biochemicals for a biorenewable chemical industry. The Plant Journal 54(4):536-545.
15- Kumar, V., Ashok, S., & Park, S. (2013) Recent advances in biological production of 3-hydroxypropionic acid. Biotechnology advances 31(6):945-961.
16- Della, Pina, C., Falletta, E., & Rossi, M. (2011) A green approach to chemical

building blocks. The case of 3-hydroxypropanoic acid. Green chemistry 13(7):1624-1632.

17- Chen, Y., & Nielsen, J. (2013) Advances in metabolic pathway and strain engineering paving the way for sustainable production of chemical building blocks. Current opinion in biotechnology 24(6):965-972.

18- Luo, H., et al. (2016) Production of 3-Hydroxypropionic Acid via the Propionyl-CoA Pathway Using Recombinant Escherichia coli Strains. PloS one 11(5):e0156286.

19- Sun, X., et al. (2015) Synthesis of chemicals by metabolic engineering of microbes. Chemical Society Reviews 44(11):3760-3785.

20- Dionisi, D., & Silva, I.M. (2016) Production of ethanol, organic acids and hydrogen: an opportunity for mixed culture biotechnology? Reviews in Environmental Science and Bio/Technology 15(2):213-242.

21- Yoneda, N., Kusano, S., Yasui, M., Pujado, P., & Wilcher, S. (2001) Recent advances in processes and catalysts for the production of acetic acid. Applied Catalysis A: General 221(1):253-265.

22- Anonymous (http://www.credenceresearch.com/report/acetic-acid-market.

23- Deppenmeier, U., Hoffmeister, M., & Prust, C. (2002) Biochemistry and biotechnological applications of Gluconobacter strains. Applied Microbiology and Biotechnology 60(3):233-242.

24- Demain, A.L. (2007) REVIEWS: The business of biotechnology. Industrial biotechnology 3(3):269-283.

25- Yamada, Y., & Yukphan, P. (2008) Genera and species in acetic acid bacteria. International journal of food microbiology 125(1):15-24.

26- Jang, Y.S., et al. (2012) Bio□based production of C2–C6 platform chemicals. Biotechnology and Bioengineering 109(10):2437-2459.

27- Kamzolova, S.V., et al. (2005) Lipase secretion and citric acid production in Yarrowia lipolytica yeast grown on animal and vegetable fat. Food Technology and Biotechnology 43(2):113-122.

28- Soccol, C.R., Vandenberghe, L.P., Rodrigues, C., & Pandey, A. (2006) New perspectives for citric acid production and application. Food Technology and Biotechnology 44(2):141.

29- Lancini, G., Donadio, S., & Marino, G. (2008) Parte I–L'uso industriale dei microrganismi. Storia e campi di applicazione. Biotecnologie Microbiche, Casa Editrice Ambrosiana, Milan:5-35.

30- Yalcin, S.K., Bozdemir, M.T., & Ozbas, Z.Y. (2010) Citric acid production by yeasts: fermentation conditions, process optimization and strain improvement. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 9:1374-1382.

31- Max, B., et al. (2010) Biotechnological production of citric acid. Brazilian Journal of Microbiology 41(4):862-875.

32- Angumeenal, A., & Venkappayya, D. (2013) An overview of citric acid production. LWT-Food Science and Technology 50(2):367-370.

33- Kubicek, C.P., & Karaffa, L. (2001) Organic acids. Basic Biotech. Cambridge University Press, Cambridge: 305-324.

34- Anonymous (https://www.mordorintelligence.com/industry-reports/gluconic-acid-market.

35- Steiger, M.G., Blumhoff, M.L., Mattanovich, D., & Sauer, M. (2013) Biochemistry of microbial itaconic acid production. Frontiers in microbiology 4:23.

36- Willke, T., & Vorlop, K-D. (2001) Biotechnological production of itaconic acid.

Applied microbiology and biotechnology 56(3-4):289-295.

37- Okabe, M., Lies, D., Kanamasa, S., & Park, E.Y. (2009) Biotechnological production of itaconic acid and its biosynthesis in Aspergillus terreus. Applied microbiology and biotechnology 84(4):597-606.

38- Haskins, R., Thorn. J., & Boothroyd, B. (1955) Biochemistry of the Ustilaginales: XI. Metabolic products of Ustilago zeae in submerged culture. Canadian journal of microbiology 1(9):749-756.

39- Tabuchi, T., Sugisawa, T., Ishidori, T., Nakahara, T., & Sugiyama, J. (1981) Itaconic acid fermentation by a yeast belonging to the genus Candida. Agricultural and Biological Chemistry 45(2):475-479.

40- Kawamura, D., Furuhashi, M., Saito, O., & Matsui, H. (1981) Production of itaconic acid by fermentation. JP Patent 56.

41- Becker, J., Lange, A., Fabarius, J., & Wittmann, C. (2015) Top value platform chemicals: bio-based production of organic acids. Current Opinion in Biotechnology 36:168-175.

42- Choi, S., Song, C.W., Shin, J.H., & Lee, S.Y. (2015) Biorefineries for the production of top building block chemicals and their derivatives. Metabolic engineering 28:223-239.

43- Werpy, T., et al. (2004) Results of screening for potential candidates from sugars and synthesis gas. Top value added chemicals from biomass.

44- Chahal, S. (1990) Lactic acid, Ullman's Encyclopedia of Industrial Chemistry vol A15. (VCH Verlagsgesellschaft, Weincheim).

45- Singhvi, M., Joshi, D., Adsul, M., Varma, A., & Gokhale, D. (2010) d-(−)-lactic acid production from cellobiose and cellulose by Lactobacillus lactis mutant RM2-2 4. Green Chemistry 12(6):1106-1109.

46- Martinez, FAC, et al. (2013) Lactic acid properties, applications and production: a review. Trends in food science & technology 30(1):70-83.

47- Abdel-Rahman, M.A., Tashiro, Y., & Sonomoto, K. (2013) Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances 31(6):877-902.

48- Ghaffar, T., et al. (2014) Recent trends in lactic acid biotechnology: A brief review on production to purification. Journal of Radiation Research and Applied Sciences 7(2):222-229.

49- Pang, X., Zhuang, X., Tang, Z., & Chen, X. (2010) Polylactic acid (PLA): research, development and industrialization. Biotechnology journal 5(11):1125-1136.

50- Benthin, S., & Villadsen, J. (1995) Production of optically pure D-lactate by Lactobacillus bulgaricus and purification by crystallisation and liquid/liquid extraction. Applied microbiology and Biotechnology 42(6):826-829.

51- Kylä-Nikkilä, K., Hujanen, M., Leisola, M., & Palva, A. (2000) Metabolic Engineering of Lactobacillus helveticus CNRZ32 for Production of Purel-(+)-Lactic Acid. Applied and environmental microbiology 66(9):3835-3841.

52- Lapierre, L., Germond, J-E., Ott, A., Delley, M., & Mollet, B. (1999) D-Lactate dehydrogenase gene (ldhD) inactivation and resulting metabolic effects in the Lactobacillus johnsonii strains La1 and N312. Applied and environmental microbiology 65(9):4002-4007.

53- Maas, R.H., et al. (2008) Lactic acid production from lime-treated wheat straw by Bacillus coagulans: neutralization of acid by fed-batch addition of alkaline substrate. Applied microbiology and biotechnology 78(5):751-758.

54- de Barrosa, M., Freitasb, S., Padilhaa, G.S., & Alegrea, R.M. (2013) Biotechnological Production of Succinic Acid by Actinobacillus Succinogenes Using Different Substrate. Chemical Engineering 32.

55- Ahn, J.H., Jang, Y-S., & Lee, S.Y. (2016) Production of succinic acid by metabolically engineered microorganisms. Current opinion in biotechnology 42:54-66.

56- Muzumdar, A.V., Sawant, S.B., & Pangarkar, V.G. (2004) Reduction of maleic acid to succinic acid on titanium cathode. Organic process research & development 8(4):685-688.

57- Vasudevan, D. (1995) Reduction of maleic acid at a Ti/ceramic TiO 2 cathode. Journal of applied electrochemistry 25(2):176-178.

58- Nishi, K., et al. (2011) Method of manufacturing succinic acid and ammonium succinate solutions. (Google Patents).

59- Green, B.A., Ruey, J.Y., & Van Scott, E.J. (2009) Clinical and cosmeceutical uses of hydroxyacids. Clinics in dermatology 27(5):495-501.

60- Gutiérrez, L-F., Hamoudi, S., & Belkacemi, K., (2012) Lactobionic acid: a high value-added lactose derivative for food and pharmaceutical applications. International Dairy Journal 26(2):103-111.

61- Knopp, D., Tang, D., & Niessner, R. (2009) Review: bioanalytical applications of biomolecule-functionalized nanometer-sized doped silica particles. Analytica Chimica Acta 647(1):14-30.

62- Graber, E.M., & Thiboutot, D. (2016) Over□the□counter Acne Treatments. Cosmetic Dermatology: Products and Procedures, Second: 501-508.

63- Stodola, F.H., & Lockwood, L.B. (1947) The oxidation of lactose and maltose to bionic acids by Pseudomonas. Journal of Biological Chemistry 171(1):213-221.

64- Engel, CAR, Straathof, A.J., Zijlmans, T.W., van Gulik, W.M., & van der Wielen, L.A. (2008) Fumaric acid production by fermentation. Applied microbiology and biotechnology 78(3):379-389.

65- Wen. F., Sun, J., & Zhao, H. (2010) Yeast surface display of trifunctional minicellulosomes for simultaneous saccharification and fermentation of cellulose to ethanol. Applied and environmental microbiology 76(4):1251-1260.

66- Engel, CAR., Van Gulik, W.M., Marang, L., Van der Wielen, L.A., & Straathof, A.J. (2011) Development of a low pH fermentation strategy for fumaric acid production by Rhizopus oryzae. Enzyme and microbial technology 48(1):39-47.