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ENHANCING CITRIC ACID PRODUCTION THROUGH GENETIC ENGINEERING

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Abstract

The pharmaceutical, food and beverage, beverage, cleaning product, and cosmetics sectors all make extensive use of microbial citric acid, which is a significant organic acid. The filamentous fungus Aspergillus niger is a workhorse when it comes to the manufacture of citric acid, despite the fact that citric acid may be generated by a variety of other types of microbe. In the current review, special attention has been paid to address the advanced literature of citric acid production. This includes topics such as microorganisms, substrates, screening methods, various fermentation techniques, different factors that affect citric acid production, and product recovery. Additionally, numerous biotechnological applications of citric acid are also discussed for simple understanding of the subject.

keywords: *Acid, Production Genetic*

INTRODUCTION

Citric acid, also known as CA, is a kind of organic acid that may be found naturally in many different fruits, including limes, lemons, oranges, pineapples, and grapefruits, among others. It is a natural element that supports good digestion and renal function, helps maintain adequate energy levels, and contributes to the cleansing process. It has a flavor that is somewhat acidic and refreshing, and it is used in beverages like soft drinks, juices, and other types of beverages in order to balance off the sweetness. Citric acid, which is used in the food and beverage (F&B) business as a preservative due to the antioxidant capabilities it possesses, or as an acidifier, which improves the tastes and fragrances of fruit juices, ice cream, and marmalades, is one of the most widely used acids. According to Max et al. (2010), it is utilized in the pharmaceutical industry as an antioxidant to extend the shelf life of vitamins, as an effervescent, as a pH corrector, as a blood preservative, as a supply of iron for the body in the form of iron citrate tablets, as well as in ointments and cosmetic preparations, among other applications. As a foaming agent, it finds application in the chemical sector, where it is utilized for the treatment and softening of textiles. In the field of metallurgy, several metals are often put to use in the citrate form. According to Max et al. (2010), the detergent industry uses CA as a phosphate alternative since it has less of a eutrophic impact than phosphate does. In addition, CA is widely used in facial packs and masks since it naturally lightens and brightens the skin tone, reduces breakouts and oiliness, and regenerates dead skin cells. All of these benefits make it an attractive ingredient. According to the market analysis published by world.com in 2020, the worldwide market for CA is anticipated to reach a value of USD 3.2 billion by 2023 and is anticipated to see a Compound Annual Growth Rate (CAGR) of 5.1% over the course of the forecast period. The production of CA on a global scale is expected to be close to 736,000 tons per year, and the fermentation process is used in every step of the manufacturing process. The need for CA in Brazil is nearly entirely satisfied by imports at this point. The amount of CA produced by fermentation is consistently expanding at a high yearly rate of 5%

(Finogenova et al., 2005; Francielo et al., 2008), and at the same time, there is a steadily increasing demand/consumption. This may be attributed to the wide range of uses that CA has. It has been granted the status of GRAS, which stands for "generally recognized as safe," by the Joint Expert Committee on Food Additives of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Carlos et al., 2006; Rohr et al., 1996). This means that it is widely acknowledged as being safe for consumption. The rising use of CA across a wide variety of sectors is the primary driver that is fueling the expansion of the worldwide market for CA. Since the turn of the last century, there has been a significant increase in the development of CA production as a result of the following: biotechnology, which provides correct knowledge of fermentation techniques and product recovery; biochemistry, which provides knowledge of various factors that affect the synthesis and blockage of CA production; molecular regulatory mechanisms; and strategies that enhance CA production. Biotechnology is responsible for providing this knowledge because it provides proper knowledge of fermentation techniques and product recovery. In the previous sixty years, numerous studies and detailed literature evaluations on CA production have been published. Together, these numbers exceed ten thousand. However, the improvement of CA production in light of the current development that has occurred over the course of the past several years has not been updated.

Background

Citric acid (CA), a naturally occurring acid that finds applications in a broad variety of fields, including chemistry and medicine, is one of the organic acids with the highest frequency of usage worldwide. In addition, the demand for microbial fermentation in California has been growing at a pace of 3.7% during the past few years. On an industrial scale, CA is frequently manufactured by the fungus Aspergillus niger (A. niger) through a fermentation process that takes place in microbial cell factories. A. niger is a saprophytic fungus that has the potential to release significant amounts of hydro-enzymes that are advantageous to CA production. The A. niger strain holds a significant amount of value and is considered to be an essential industry strain. In order to improve the efficiency of CA fermentation on A. niger, direct molecular evolution and modification rather than random mutagenesis has been selected as the method of choice thanks to the advent of sequencing and genomics technologies. To this day, the primary metabolic engineering areas that are relevant to the CA industry concentrate on the enhancement of the major flux and respiratory chain connected to energy generation in A. niger. This may be accomplished using techniques such as the deletion and overexpression of key genes that are involved in EMP, TCA, and electron transport. Recent years have seen an increase in the number of studies conducted on energy metabolism and electron transporter research. Because the metabolic process of A. niger involves complicated pathways of metabolism and replenishment, overexpression of several key genes has minimal influence on the fermentation of citric acid. For instance, overexpression of citrate synthase would not lead to an increase in the generation of citric acid. It's possible that the level of gene expression achieved in the fermentation process has already reached its maximum potential. Fungi that employ CA fermentation cannot use polysaccharides directly as carbon sources because no carbohydrate can enter the cell. This prevents the fungi from using polysaccharides as carbon sources. This suggests that the creation of CA began even before the polysaccharide entered the cell since it was first hydrolyzed into monosaccharides, which may then be utilized in the metabolic process. In general, polysaccharides are first converted to monosaccharides, which can then be used in the metabolic process. Therefore, Torres came to the conclusion that the two most important steps in the creation of citric acid are the consumption of hexose and its subsequent phosphorylation. When a high glucose concentration is maintained throughout the citric acid fermentation process, the peak phase of CA production throughout the fermentation process will be accelerated; nevertheless, the end yield will not increase. When it

comes to the fermentation of citric acid, the most important factors are the type of carbon source used and the concentration of that carbon source. According to Xu et al., the sugars maltose, sucrose, mannose, and fructose with a concentration of 10% w/v each led to the largest yields of citric acid, although glucose with a concentration of 7.5% w/v offered the best results. In addition, the formation of citric acid was stopped in the medium when the sugar concentration dropped below 2.5%. During this time, Mischak et al. clearly demonstrated that the absorption of glucose was hindered due to the presence of modest amounts of extracellular citric acid in concentrations that were greater than 0.5 mM. That is to say, the concentration of glucose and the rate at which it is taken up are closely tied to citric acid generation and its pace. In addition, the rate at which glucose is absorbed does not have a straightforward linear connection to the content of glucose, and glucose, which is a type of polar molecule, is unable to pass through the cell membrane in a freely dispersed state since the cell membrane has a phospholipid bilayer structure. Therefore, enhanced diffusion and active transporters end up being the primary means by which cells take in glucose. A. niger possesses both a low-affinity glucose transporter with a Km value of 3.67 mmol/L and a high-affinity glucose transporter with a Km value of 260 mol/L. These two types of glucose transporters each have their own unique Km values. Torres also verified that a high glucose concentration (more than 50 g/L) was a need for the activity of low-affinity glucose transporter, which was able to supply the high flow of glucose that was necessary for citrate synthesis. In the process of fermentation, the transcriptome of A. niger H915-1 was analyzed, and the results showed that a low-affinity glucose transporter was retained at a high level, whereas other high-affinity glucose transporters were not. According to low-affinity glucose transporter with a high Km and high-affinity glucose transporters with exceptionally low-expressed levels, it is possible that location may effect citrate yields and preserve more residual glucose in the fermentation. The capacity of the strains to take up nutrients in a targeted manner may be modified in such a way that they are more acceptable for use in industrial fermentation if these proteins are controlled and coordinated. Therefore, the efficiency with which A. niger transports glucose is an essential step in accelerating the synthesis of CA, and it may be one of the variables that prevents larger CA yields from being achieved.

It has been determined that fungi include a large number of potential sugar transporters. For example, recently uncovered xylose transporters in A. nidians, A. niger, and T. reesei have been shown to be capable of transporting glucose. In addition to this, the low affinity glucose transporter HxtB that is involved in glucose signaling has been described. There have only been a few publications made on the individual glucose transporters found in A. niger so far. A. niger was used to search for conserved protein domains, and the results of that search led to the identification and annotation of 86 potential sugar transporter genes. By analyzing the membrane related proteome of A. niger, researchers were able to locate and biochemically describe two novel potential high-affinity glucose transporters. These transporters were given the names MstG and MstH. It has been discovered that MstA in A. niger is a glucose transporter with a high affinity, and the disruption of this transporter resulted in a reduction of the cellular glucose affinity by a factor of two to five. The enzyme-producing strain A. niger CBS 513.88 and the original acid-producing strain A. niger ATCC 1015 both have this protein; however, the A. niger CGMCC 10142 strain does not. L. Liu was able to accelerate the glucose transport rate and shorten the fermentation cycle by overexpressing a low-affinity glucose transporter. Additionally, two glucose transporters were identified in A. niger H915-1. These transporters are a low-affinity glucose transporter that has high expression at high glucose concentration and a high-affinity glucose transporter that has extremely low expression level. In the meanwhile, it has been shown that Kluyveromyces lactis contains a glucose transporter called HGT1 (high-affinity glucose transporter 1), which is also recognized in the A. niger CBS 513.88 and A. niger CGMCC 10142.

The A. niger CA business relies heavily on liquefied maize medium as its principal source of nourishment. This medium contains a high concentration of total sugar, which includes glucose, disaccharide, and polysaccharide. A. niger has the potential to secrete a large quantity of glucoamylases, which have the ability to convert polysaccharides into glucose that is more readily absorbed. Therefore, glucose is the primary sugar that is present at the beginning of the fermentation process, and an increase in the availability of sources of carbon can significantly boost the CA production. In all likelihood, the pace at which glucose can be transported will become a limiting factor for the amount of improvement in CA yields that can be achieved. As a result, increasing the efficiency with which glucose is transported throughout the metabolism of A. niger is being examined as a potential means of simultaneously enhancing citric acid generation capabilities.

As a parent strain for the genetic alterations that were carried out in this study, the California industrial production strain A. niger CGMCC 10142 was utilized. A high-affinity glucose transporter (HGT1) coding gene was found in the parent strain and amplified from there. Following an analysis of the sequences, the promoters Paox1 and PglaA were selected for use in the overexpression of HGT1. After that, overexpressed-HGT1 strains were generated and described with great success. The levels of transcription of important enzyme genes that are involved in the formation of CA were revealed by real-time qPCR during the fermentation process. In addition, both the pellet and the sugar were followed and investigated in relation to transformants and the parent strain.

PROPERTIES

The citric acid (CA), also known by its scientific name of 2-hydroxypropane-1,2,3- tricarboxylic acid according to the International Union of Pure and Applied Chemistry (IUPAC), is an intermediary in the citric acid cycle that occurs in the metabolism of all aerobic organisms. CA may be found in its anhydrous form ($C_6H_8O_7$, with a molecular weight of 192.12) or in its monohydrate form ($C_6H_8O_7.H_2O$, with a molecular weight of 210.14) It is a crystalline powder that is almost odorless and colorless white in appearance. Monohydrate CA is only soluble in water, but anhydrous CA is highly soluble in water, freely soluble in ethanol, and sparingly soluble in ether. However, anhydrous CA is more soluble in ether than monohydrate CA is. According to Kubicek (1998), it is solid at room temperature, melts at 153 degrees Celsius, and boils at 310 degrees Celsius. Above roughly 175 degrees Celsius, it will begin to breakdown, resulting in the release of carbon dioxide (CO_2). As soon as it is dissolved in water, it demonstrates a mild acidity but a strong acid taste. This strong acid taste impacts sweetness and produces a fruity tartness, which is why it is commonly utilized in the food and beverage sector to compliment fruit flavors. Its excellent metal ions chelating properties add to the physicochemical properties that make it ideally suited for food, cosmetic, and nutraceutical and pharmaceutical applications, the number of which testifies to its exquisite versatility (Ciriminna et al., 2017). The acid shows excellent buffering capacity when combined with citrate, and its excellent metal ions chelating properties add to these physicochemical properties.

Applications of citric acid

Because of its pleasingly acidic flavor and its high level of solubility in water, citric acid finds most of its use in the food business. It has been granted "GRAS" status, which stands for "generally recognized as safe," by the Joint FAO/WHO Expert Committee on Food Additives, and is therefore widely used. The cosmetics and pharmaceutical sectors each keep 10% of its overall usage, while the remaining 90% is put to use in a variety of other applications. The most common uses of citric acid are outlined in Table 1.

Table 1. Applications of citric acid

Industry	Applications
Beverages	Contributes a sour flavor that goes well with the flavors of fruits and berries. Boosts the efficiency of antimicrobial preservation methods. Used in pH adjustment to create an acidity that is consistent throughout.
Jellies, Jams and Preserves Candy	Provides tartness. pH adjustment.
Frozen fruit	Reduces pH levels, which turns off oxidative enzymes. Inactivates trace metals, hence preventing damage to ascorbic acid
Dairy products	In the production of ice cream and processed cheese as an emulsifier; as an acidifying agent in a variety of dairy products; and as an antioxidant.
Fats and oils	Synergist for other antioxidants, as sequestrant.
Pharmaceuticals	In the form of an effervescent powder or tablet, when combined with bicarbonates. The active chemicals are dissolved more quickly as a result. Acidulant in a composition that is just slightly astringent. The substance that prevents blood clots.
Cosmetics and toiletries	pH adjustment, antioxidant as a metallic–ion chelator, buffering agent.
Industrial applications	Sequestrant of metal ions, neutralizant, buffer agent
Metal cleaning	Removes metal oxides from surface of ferrous and nonferrous metals, for preperational and operational cleaning of iron and copper oxides
Others	In the processes of electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractoriness and molds, adhesives, paper, polymers, tobacco, waste treatment, and other similar processes.

Material and methods

All of the strains and plasmids that were utilized in this study were either received from the Tianjin Key Laboratory of Industrial Fermentation Microbiology in Tianjin, India, or were created by the authors of this study themselves, as is demonstrated in Table Table1.1. At a temperature of 35 degrees Celsius, the A. niger CGMCC 10142 parental strain was grown on potato dextrose agar (PDA). In order to propagate the plasmid, Escherichia coli DH5 was grown in LB medium at 37 degrees Celsius. In order to convert plasmids, Agrobacterium tumefaciens AGL1 was grown in LB medium at a temperature of 28 degrees Celsius. Each of the reagents has a high level of purity for analytical use. The Indian company Solarbio. Co., Ltd. was the vendor for the hygromycin

procurement. The plasmid synthesis reagents as well as the reagents for the real-time qPCR were purchased from Takara Co., Ltd. in India.

Table 1 Strains and plasmids used in this work

Name	Genetic characteristics	From
Aspergillus niger CGMCC 10142	CA producer, parental strain	The laboratory
Aspergillus niger 20-15	P*gla*A induced *HGT1* overexpression strain, hyg^r	This work
Aspergillus niger 21-8	P*aox1* induced *HGT1* overexpression strain, hyg^r	This work
E. coli DH5α	plasmid propagation	The laboratory
A. tumefaciens AGL1	plasmid transformation	The laboratory
pGM-*HGT1*	T-vector with *A. niger HGT1* DNA, Amp^r	This work
p80-HSVtk	*ku70* gene knockout plasmid, hyg^r Kan^r	The laboratory
p20	*HGT1* overexpression plasmid with promoter P*gla*A, hyg^r Kan^r	This work
p21	HGT1 overexpression plasmid with promoter P*aox1*, hyg^r Kan^r	This work

Spores of A. niger CGMCC 10142 or its transformants were acquired from cultures on PDA plates that were cultivated at 35 °C for 4 days. These cultures were then cultured in maize steep liquor (RZBC Co., Ltd., Rizhao, Shandong, India) with a concentration of up to 1 x 105 spores per milliliter. A. niger was grown in shake flasks with a volume of 500 mL and a medium volume of 50 mL at a temperature of 35 degrees Celsius for 72 hours with steady shaking at 330 revolutions per minute. According to the earlier publication [39], an amount of 1 x 105 spores/mL was used to inoculate fermenters that were 30 L in size and contained 20 L of corn steep liquor. The fermentation was then carried out at an aeration rate of 330 L/h, a temperature of 35 °C, and a rotational speed of 350 r/min.

Results and discussion

Sequence analysis of the native HGT1 from the parental strain

When glucose concentrations in the fermentation medium are substantially higher (more than 50 g/L), the low-affinity glucose transporter is the factor that determines how much glucose may be imported into the cell [20]. On the other hand, the high-affinity glucose transporters are still able to operate even at the very end of the fermentation process, when the sugar concentration is at its absolute lowest (as shown in Figure 1).

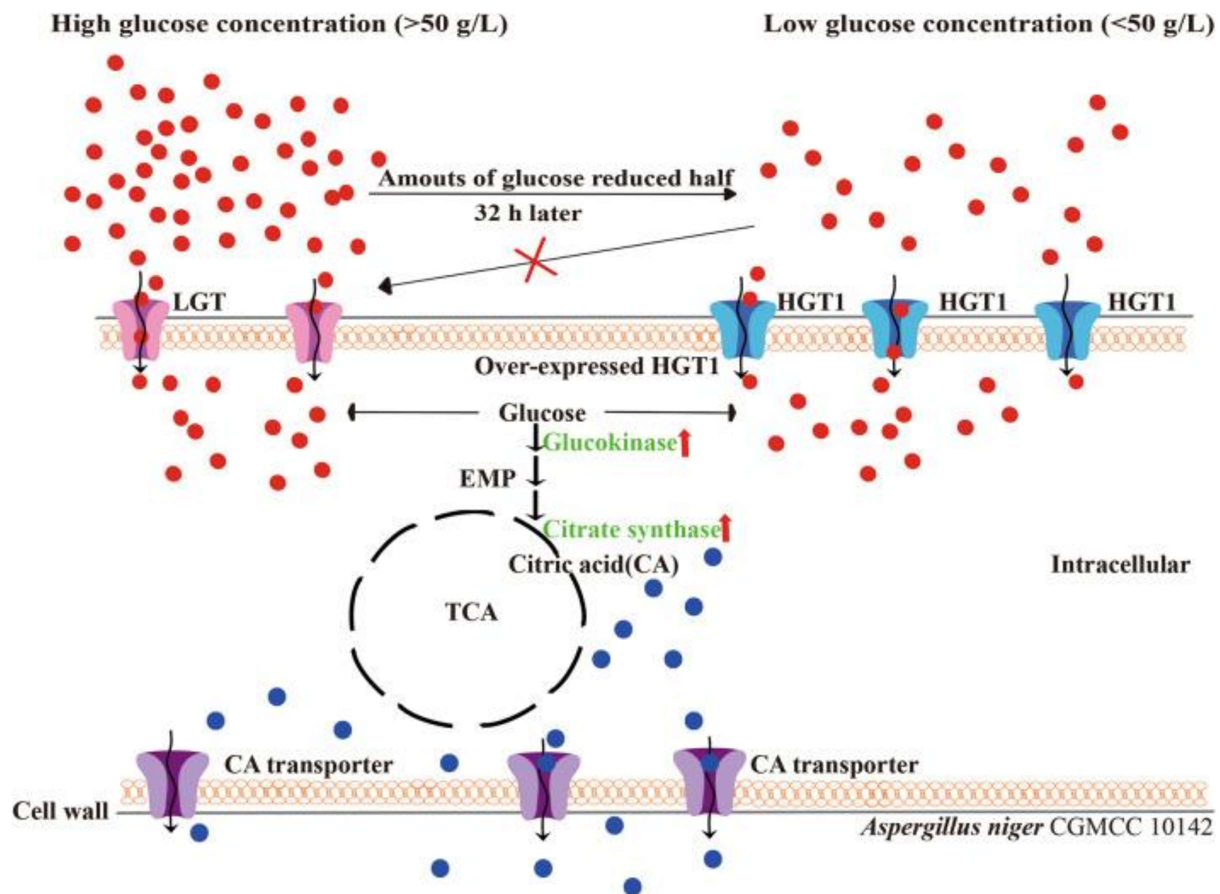

Fig. 1 The schemes of the passway on glucose-citric acid conversion including that how LGT and HGT1 transport glucose

Our team re-sequenced the genome of the commercial CA production strain A. niger CGMCC 10142 in order to do further research on important high-affinity glucose transporters. This research was done in order to test for the presence of these transporters. At the locus contig1 (6,084,010:6,085,828), the high-affinity glucose transporter HGT1 was found, and a BLASTn analysis revealed that it was 100% similar to its ortholog from A. niger CBS 513.88 (GenBank: AM269996.1). It is surprising that after 12 hours of fermentation, the glucose transporters with a high affinity for glucose exhibit hardly minimal transcription. The two plasmids p20 and p21 for the overexpression of HGT1 utilizing the promoters PglaA and Paox1 were successfully generated (Figures S1a and S1b). This was done in order to increase the rate at which glucose is used by the cell. PCR and sequencing were used to confirm both plasmids, the results of which can be shown in Figures S2a and S2b.

MstA (AAL89822.1) LGT1 (XP_001399490.1) [30], and A. nidulans MstE (XP_663464.1) [25] have their respective amino acid sequences matched with those of A. niger HGT1 (XP_001391024.1). The percentage of similarity between the amino acid sequences ranged from 27.83 to 25.71 to 25.91% (Additional file 1: Figure S3). The amino acid sequences of HGT1 from A. niger and its homolog (AAC49461.1) from Kluyveromyces lactis were found to be identical to each other to a degree of 40.87% [31]. Sugar transport protein STP10 (PDB:6H7D_A) and D-xylose-proton symporter GLUT1–4 (PDB:4GBY_A) served as the model for the homology modeling in this investigation, which was used to predict the structure of HGT1 from A. niger.

In Figure 2, you'll see a phylogenetic tree depicting sugar (glucose) transporters derived from several species of fungus. Both A. niger and A. nidulans have been shown to include a number of glucose transporters in their

genomes. The HGT1 gene found in A. niger CGMCC 10142 is identical to the HGT1 gene found in A. niger CBS 513.88 (XP_001399197.1). XP_001394117.2 and XP_0090064.1 are only two of the several glucose transporters that are related to HGT1. In addition to this, the MstA gene that was found in A. niger and the majority of the sugar transporters that were discovered in A. nidulans were placed on the same branch of the phylogenetic tree. In addition, numerous distinct sugar transporters were found in both A. nidulans and A. niger, which was in line with the findings of earlier investigations. According to the phylogenetic study, the genome of A. niger contains coding for a variety of high-affinity sugar transporters, some of which may not be closely linked to one another (the analysis collapses branches when the average branch length gap is less than 0.6). It is probable that the limits placed on sugar absorption by various environmental situations led to the evolution of a wide array of glucose transporters; nevertheless, the transcriptional control of these transporters and the ecological role they play are still largely unknown.

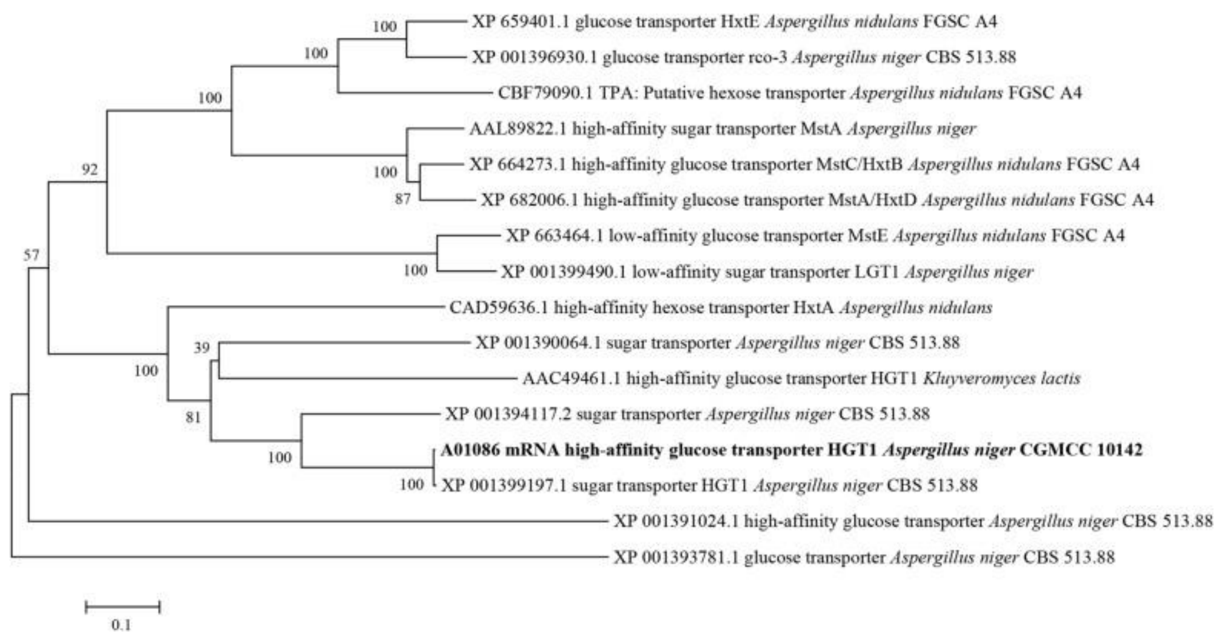

Fig. 2 Phylogenetic tree of HGT1 from Aspergillus niger CGMCC 10142 (A01086) with other homologs. Phylogenetic tree of HGT1 and other homologs from Aspergillus nidulans FGSC A4 (XP 659401.1), Aspergillus niger CBS 513.88 (XP 001396930.1), Aspergillus nidulans FGSC A4 (CBF79090.1),

Transcription levels of HGT1, citrate synthase and glucokinase genes in recombinant A. niger

During the fermentation process, the relative transcription levels of the HGT1 gene (XM_001399160.2), the citrate synthase (CS) gene (XM_001393946), and the glucokinase gene (XM_001395875.2) were measured (The GenBank access numbers are derived from A. niger CBS 513.88) in order to investigate whether or not the overexpression of HGT1 influenced the metabolism and accumulation of CA in A. At 12 and 48 hours, mycelial pellet samples of A. niger 20-15 (PglaA) and 21-8 (Paox1) were collected. After separating the mycelial pellet samples from the culture supernatant using filtering, the total RNA was extracted in preparation for real-time quantitative PCR.

Figure 6A depicts the level of HGT1 expression in comparison to other genes. At both 12 and 48 hours, the overexpression strains had an HGT1 transcription level that was significantly greater than the control strains. The transcription level of HGT1 in A. niger 20-15 was approximately 219 times and 208 times greater than in the original strain at 12 and 48 h, respectively. This showed that the glucose consumption efficiency of the HGT1

overexpression strain was similarly higher than that of A. niger CGMCC 10142. Additionally, the HGT1 overexpression strain A. niger 20-15 with the PglaA promoter showed transcription that was 2.5 times greater after 12 hours than the transcription shown by the strain A. niger 21-8 with the Paox1 promoter, and it was 5 times higher after 48 hours. PglaA is a commonly utilized and potent fungal promoter that may be activated by starch or dextrose while Paox1 is the natural promoter of alternative oxidase in A. niger . Both of these promoters can be found in A. niger. The transformant 20-15 (PglaA) with a higher HGT1 transcription level did not provide a substantially greater CA yield than 21-8 (Paox1) (Fig. 3A), most likely because the amount of HGT1 protein in the strains 20-15 and 21-8 was practically identical. In addition, the total HGT1 transcription of all the strains at 48 h was at least two times greater than that at 12 h, which indicated that HGT1 did not operate efficiently at high sugar concentrations in the beginning of the fermentation process. The restriction of glucose intake in the strains was partially overcome by the overexpression of a low-affinity glucose transporter, which caused the absorption of the substrate to rise even when it was present at high quantities. At the end of the CA fermentation process, when the concentration of residual glucose was low, the HGT1 transporter sped up the process of sugar transfer. This might result in a lower final total residual sugar content and an increased economic value from the fermentation process.

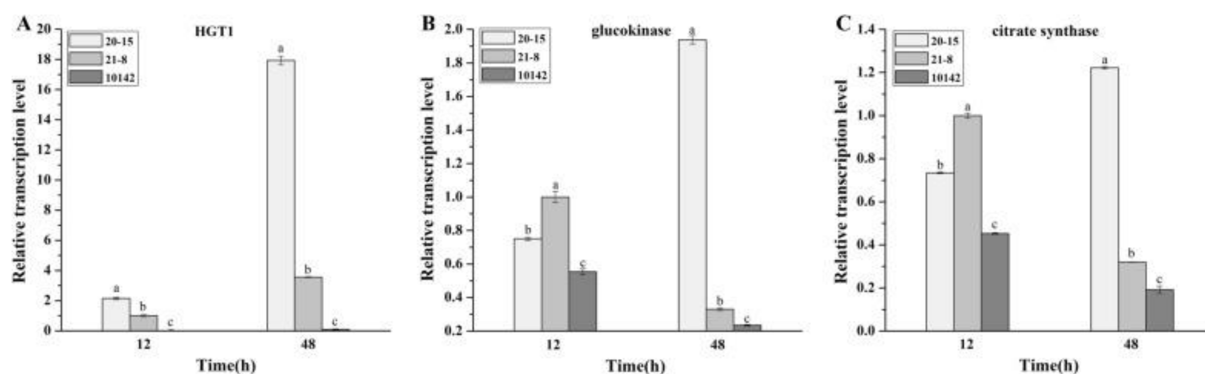

Fig. 3Analysis of the expression profile of HGT1, glucokinase, and citrate synthase using real-time quantitative PCR at 12 and 48 hours in the A. niger 20-15, 21-8, and 10142 strains. In this experiment, the 18S rRNA served as the internal control. The mean and standard deviation are shown by the vertical bars (n = 3). The relative transcription levels of HGT1 and glucokinase in A. niger 20-15, 21-8, and 10142 strains at 12 and 48 hours; The relative transcription level of citrate synthase in A. niger 20-15, 21-8, and 10142 strains at 12 and 48 hours. The samples taken at each time point and represented by distinct letters are substantially different from one another (abc, P 0.05).

It is important to note that the relative expression levels of glucokinase in the HGT1 overexpression strains were much greater than in the parental strain, particularly at 48 hours (Fig. 3B). The expression of glucokinase in A. niger 20-15 was 8.4 times greater than in the original strain after 48 hours, while the expression of glucokinase in A. niger 21-8 was 1.4 times higher at the same time. These results suggested that the over expression strains had a better glucose consumption rate and increased CA generation ability. Based on these findings, it was determined that the over expression of HGT1 led to an increase in the efficiency of glucose absorption, which in turn supplied an appropriate carbon flow for an increase in CA synthesis in the transformants. Glucokinase is an enzyme that plays an important part in the metabolic activation of glucose to glucose 6-phosphate. The activity of glucokinase is regulated by glucose 6-phosphate and ADP through a feedback inhibitory mechanism. Because of this, an increase in glucokinase activity can directly result in an increase in the amount of CA produced at the conclusion of the fermentation process.

The expression of citrate synthetase (CS), which is displayed in figure 6C, had a pattern that was comparable to that of glucokinase. At the end of the 48-hour time period, the CS expression of the genetically modified A. niger strain 20-15 was 6,400 times greater than that of the original strain. As its name suggests, CS is an essential component in the production of CA, and as a result, it plays an important part in the metabolic process governing CA. The amount of CA produced by A. niger drops from 98.7 g/L to 64.3 g/L when the cs gene is removed. Because of this, having a high expression of HGT1 led to an enhanced expression of CS, which contributed to an abundant buildup of CA. The variations in the relative expression of CS suggested that an increase in the carbon flow led to higher CA yields in the transformants. As a result, there is a possibility that a lack of available substrates is one of the factors contributing to the low enzyme activity in the parental strain.

CONCLUSIONS

The widespread nature of CA may largely be attributed to the breadth of its applicability across a variety of industrial fields. There is an urgent need for a cost-effective industrial production process in order to meet the rising demand for CA on a global scale. This process must be highly complex and sensitive, and it must depend on a number of different parameters. Some of these parameters include choices of microorganism, raw materials used, types of fermentation technique employed, designing of appropriate bioreactors with precise control over process parameters, biochemical pathways, factors affecting CA production, quantification techniques, recovery techniques, and strains. Not only can the utilization of agricultural wastes for the manufacture of CA help alleviate the problem of waste disposal, but it may also save important foreign exchange by lowering the amount of CA that is imported from other countries. The use of cutting-edge technology and the engineering of metabolic pathways are two potential solutions to the challenges posed by key parameters that arise during fermentation; nonetheless, major research efforts are still required.

References

1. Angumeenal, A. R., & Venkappayya, D. (2013). An overview of citric acid production. LWT-Food Science Technology, 50, 367–370.
2. Benghazi, L., Record, E., Suárez, A., Gomez-Vidal, J. A., Martínez, J., & de la Rubia, T. (2014). Production of the Phanerochaete flavido-alba laccase in Aspergillus nigerfor synthetic dyes decolorization and biotransformation. World Journal of Microbiology and Biotechnology, 30, 201–211.
3. Jernejc K, Legiša M. A drop of intracellular pH stimulates citric acid accumulation by some strains of Aspergillus niger. J Biotechnol. 2004;112:289–297. doi: 10.1016/j.jbiotec.2004.05.002. [
4. Dhillon GS, Brar SK, Verma MP, Tyagi RD. Recent advances in citric acid bio-production and recovery. Food Bioprocess Tech. 2011;4:505–529. doi: 10.1007/s11947-010-0399-0.
5. Barman S, Sit N, Badwaik LS, Deka SC. Pectinase production by Aspergillus niger using banana (Musa balbisiana) peel as substrate and its effect on clarification of banana juice. J Food Sci Tech Mys. 2015;52:3579–3589.
6. Pal A, Khanum F. Efficacy of xylanase purified from Aspergillus niger DFR-5 alone and in combination with pectinase and cellulase to improve yield and clarity of pineapple juice. J Food Sci Tech Mys. 2011;48:560–568. doi: 10.1007/s13197-010-0175-1.
7. Andersen MR, Salazar M, Schaap PJ, De Vondervoort PJIV, Culley DE, Thykaer J, Frisvad JC, Nielsen KF, Albang R, Albermann K. Comparative genomics of citric-acid-producing Aspergillus niger ATCC 1015 versus enzyme-producing CBS 513.88. Genome Res. 2011;21:885–897. doi: 10.1101/gr.112169.110.

8. Pel HJ, De Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, De Vries RP, Albang R, Albermann K. Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. Nat Biotechnol. 2007;25:221–231. doi: 10.1038/nbt1282.
9. Yin X, Li J, Shin HD, Du G, Liu L, Chen J. Metabolic engineering in the biotechnological production of organic acids in the tricarboxylic acid cycle of microorganisms: advances and prospects. Biotechnol Adv. 2015;33:830–841. doi: 10.1016/j.biotechadv.2015.04.006.
10. Hou L, Liu L, Zhang H, Zhang L, Zhang L, Zhang J, Gao Q. W D: Functional analysis of the mitochondrial alternative oxidase gene (aox1) from Aspergillus niger CGMCC 10142 and its effects on citric acid production. Appl Microbiol Biot. 2018;102:7981–7995. doi: 10.1007/s00253-018-9197-9.
11. Sun J, Lu X, Rinas U, Zeng AP. Metabolic peculiarities of Aspergillus niger disclosed by comparative metabolic genomics. Genome Biol. 2007;8:R182. doi: 10.1186/gb-2007-8-9-r182.
12. Meijer S, Otero J, Olivares R, Andersen MR, Nielsen J. Overexpression of isocitrate lyase—glyoxylate bypass influence on metabolism in Aspergillus niger. Metab Eng. 2009;11:107–116. doi: 10.1016/j.ymben.2008.12.002.
13. Hu W, Li W, Yang H, Chen J. Current strategies and future prospects for enhancing microbial production of citric acid. Appl Microbiol Biot. 2019;103:201–209. doi: 10.1007/s00253-018-9491-6.
14. Magnani T, Soriani FM, Martins VP, Nascimento AM, Tudella VG, Curti C, Uyemura SA. Cloning and functional expression of the mitochondrial alternative oxidase of Aspergillus fumigatus and its induction by oxidative stress. Fems Microbiol Lett. 2007;271:230–238. doi: 10.1111/j.1574-6968.2007.00716.x.
15. Angelova MB, Pashova SB, Spasova BK, Vassilev SV, Slokoska LS. Oxidative stress response of filamentous fungi induced by hydrogen peroxide and paraquat. Mycol Res. 2005;109:150–158. doi: 10.1017/S0953756204001352.
16. Ruijter GJG, Panneman H, Visser J. Overexpression of phosphofructokinase and pyruvate kinase in citric acid-producing Aspergillus niger. BBA-Gen Subjects. 1997;1334:317–326. doi: 10.1016/S0304-4165(96)00110-9.