Mini Review

Application of Synthetic Biology for the Production of Amino Acids

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Abstract | Amino acids perform several vital functions in food, pharmaceuticals, and agriculture. Previously, amino acids were obtained by chemical synthesis or fermentation, both of which were expensive and had a severe impact on the environment, and not all of these processes could be employed commercially. Synthetic biology, rather than traditional methodologies, is now helping to make microbial systems more accurate to maximise productivity, efficiency, and sustainability. It examines current advances in utilising synthetic biology to create amino acids, covering the key genetic and computational technologies produced, notable examples of success, and ongoing problems. It discusses breakthroughs in cell-free systems, AI-supported metabolic pathway design, and the production of non-canonical amino acids. The advancements in synthetic biology are opening up new avenues for producing amino acids, which will drive the growth of a greener bioeconomy.

Keywords | Amino acid production, Synthetic biology, Host organisms, Computational tools, Challenges, Gene editing

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INTRODUCTION

Amino acids, which make up all life forms, are encoded by DNA. Other important macromolecules in our body such as structural proteins and enzymes are made from amino acids. In addition, amino acids have many uses in the food and pharmaceutical industries. Certain amino acids are important for the body because they can only be supplied by outside sources. They are found in animal feed additives (lysine, methionine, threonine), used as ingredients to improve the taste of foods (aspartic acid, monosodium glutamate, serine) and added to both cosmetic products and medicines. The main customers for amino acids are food and animal feed businesses, which means these mixes account for a very small percentage of the annual supply, yet their high purity is needed (Tang *et al.*, 2024). In addition, aspartame, an artificial sweetener, is made using amino acids. In the past, essential amino acids were important in transfusion, but now extra amino acids are added from blood products. Amino acids are used by the liver to remove ammonia from the blood during liver damage, heart problems, peptic ulcers and male sterility. For the production of antibiotics, several amino acids function as intermediate substances (Ikeda and Takeno, 2020). Many dietary supplements depend on using amino acids in their ingredients. Individual amino acid products can be bought, but they are usually given as mixes. Arginine, tryptophan, tyrosine, glutamine and lysine are usually the top amino acid supplements available. Taking amino acids in food supplements is beneficial for developing the body, assisting in sleep, helping with depression, controlling PMDD, quitting smoking, treating bruxism and dealing with ADHD (Guo *et al.*, 2024; Jacob *et al.*, 2024).

In order to provide enough amino acids for many industries, we must switch to more environmentally friendly and productive ways of producing them. Making amino acids conventionally with chemicals and extraction is limited

by many things that make them both inefficient and unsustainable. Because these technologies are costly, can harm the environment and result in small yields, they add several challenges when used by industries (Rittmann *et al*., 2008). Because chemical synthesis generally fails to generate the proper stereoisomers, amino acid bioactivity suffers and makes chemically synthesised amino acids less useful in treatments that require these isomers. Finding chemical residues in the product can reduce its safety and limit its use, so using chemically synthesised amino acids is mainly done in animal feed and not in human goods (Liu *et al*., 2023). Because raw materials for plants are rarely available and cultivation is costly, plant extraction techniques are not suitable for mass manufacturing. Most common extraction processes often lead to serious environmental problems thanks to the wastewater produced and the significant use of chemicals. Because of these processes, the recovered amino acids and proteins can lose their useful properties, seriously limiting the performance of the food industries (Ikeda and Takeno, 2020; Ye *et al*., 2022).

Common methods have many faults which is why new techniques in fermentation and synthetic biology are highly promising. Investigations have been carried out into using microbial cell factories and Escherichia coli to make amino acids, enabling a more sustainable and effective way. Such developments may circumvent the problems linked to current techniques by increasing yield, cutting environmental damage and allowing for the creation of stereoisomers. Synthetic biology means carefully producing microbes to better create amino acids (Wang *et al*., 2024). Using metabolic engineering or genetic approaches, researchers can support increased flow to desirable amino acids without increasing byproduct development. A good example is the use of metabolic engineering to help Escherichia coli make more aromatic amino acids by producing important enzymes in high amounts and blocking rival processes. With synthetic biology, it is now possible to make new and synthetic amino acids (Guo *et al*., 2024). Both β-hydroxyenduracididine and β-methylphenylalanine were successfully created in microbial cells using homemade synthetic operons and various heterologous routes. Because of these improvements, a wider range of amino acids can be accessed and proteins with individual properties can be made for biotechnology uses. With the help of synthetic biology, producing amino acids sustainably is possible by using less fossil fuel and less waste. To illustrate, cell-free biocatalysts can be used to make glycine and serine using carbon from CO_2, giving us a way to produce goods with a carbon negative method. It is possible that using clean energy and specific types of microbes in biotechnology can decrease production prices and still maintain strong yields (Chowdhury *et al*., 2024). Because of synthetic biology, handling the synthesis of amino acids in cells can be done with great precision. The creation of synthetic genetic circuits and biosensors has made genetic-code expansion and incorporating uncommon amino acids in proteins more efficient. This approach leads to better amino acid production after making it possible to adjust processes and watch them in real time. Using current synthetic biology, combined with CRISPR systems and artificial intelligence, has recently transformed amino acid production (Vanegas *et al*., 2017). Gene expression in *Corynebacterium glutamicum*, used widely in the industry for amino acid production, has been specifically tuned using CRISPRi/dCas9 methods. Using computer models and omics research has supported the advancement of promising strains and the finding of potential targets for metabolic engineering (Hao *et al*., 2024; Ye *et al*., 2022).

Key synthetic biology tools and strategies

Enhanced microbial strains and improved processes, facilitated by genetic engineering, currently enable the augmented production of amino acids. Techniques such as pathway optimization, CRISPR, and metabolic engineering enhance amino acid production in bacteria as shown in Figure 1. The integration of these techniques has enhanced production procedures in random mutagenesis. The production of amino acids through genetic engineering depends on many key processes.

Pathway optimization

In pathway optimization, scientists enhance metabolic pathways to increase the yield of desired chemicals. It has proven to be particularly successful in species such as *Escherichia coli* and *Corynebacterium glutamicum*, which are utilised in substantial quantities for amino acid production. Scientists utilise structured kinetic models to identify fluctuations in enzyme levels that facilitate the optimisation of cellular metabolism. Hatzimanikatis *et al*. demonstrated that altering the regulatory mechanisms of enzymes in metabolism might significantly increase the concentrations of xanthine monophosphate and guanosine monophosphate by up to 114 times the acceptable reference value. It demonstrates that employing optimal routes can enhance the production of amino acids (Hatzimanikatis *et al*., 1996; Ikeda and Takeno, 2020).

CRISPR technology

CRISPR technology has changed the science of genetic engineering since it makes it feasible to modify precise regions of an organism's DNA. It helps scientists modify specific genes related to amino acid production which boosts the productivity of the strains. SWITCH was developed for Saccharomyces cerevisiae and joins numerous CRISPR tools for direct genetic manipulation and for modifying metabolic pathways, leading to better amino acid synthesis over time. Also, *E. coli* strains have been modified using CRISPR, boosting the quantity of L-phenylalanine their

cells make by changing critical DNA components (Liu *et al.*, 2018; Vanegas *et al.*, 2017; Ye *et al.*, 2022).

Metabolic engineering

Metabolic engineering covers a larger range of approaches to change cellular processing for specific goals. Examples are introducing new pathways, getting rid of rivalling pathways and adjusting the levels where enzymes are produced. As an example, Liu *et al.* (2018) managed to make a lot of L-phenylalanine in *E. coli* by disabling the phosphotransferase system and altering specific transcription factors to increase the production of related enzymes, producing up to 72.9 g/L. Also, the application of metabolic engineering to S. cerevisiae improved the production of aromatic amino acids, enhancing the production of muconic acid and shikimic acid (Guo *et al.*, 2024; Liu *et al.*, 2018; Pastrana, 2017).

In conclusion, the use of tailored pathways, CRISPR and engineering technology in metabolism has greatly improved the production of amino acids. Such genetic engineering techniques increase how effective and productive amino acids are and open doors for developing ecologically sustainable ways of supplying them to a growing world. As more research is done, there is still much room for exciting improvements in microbial production systems.

Key Synthetic Biology Tools and Their Contribution

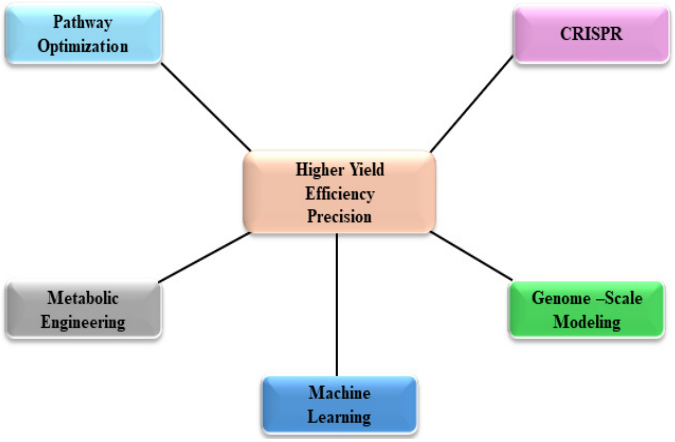

Figure 1: Key synthetic biology tools.

Host organisms and their modifications for amino acid production

Microbial fermentation for amino acid production is a popular topic in biotechnology and Escherichia coli, *Corynebacterium glutamicum* and yeast are main microbes used for the process. Every organism processes materials differently and this can be changed to support more amino acid production.

Escherichia coli

Several scientific research in molecular biology and biotechnology employ *E. coli* since it grows rapidly and we know a lot about its genetic structure. It has been substantially modified to synthesize several amino acids, including L-tryptophan and L-phenylalanine. The optimization of carbon metabolism and getting rid of obstacles in amino acid biosynthesis has been done via metabolic engineering. For example, when specific genes are inserted into *E. coli*, the quantity of amino acids the organism generates may grow higher. Some researchers have utilised *E. coli* to create p-amino-L-phenylalanine (L-PAPA), a form of amino acid not found in nature, by introducing foreign genes in the bacterium for increased production rates (Mohammadi *et al.*, 2018; Soo, 2022).

Corynebacterium glutamicum

Corynebacterium glutamicum is also a key contributor to amino acid synthesis and predominantly makes L-lysine and L-glutamate. A significant number of amino acids are produced by this organism when it develops in aerobic conditions (Ikeda and Takeno, 2020). Genetic alterations have been made to boost the efficiency of its processes, for example, the diaminopimelate route is vital for creating L-lysine. After introducing genes from *C. glutamicum* such as panD, into *E. coli*, both enzyme activity and amino acid production have dramatically gone up. Also, employing metabolic engineering, *C. glutamicum* may utilise glycerol to create amino acids owing to the creation of greater efficiency (Rittmann *et al.*, 2008).

Yeast

Saccharomyces cerevisiae yeast is being acknowledged more commonly for its capacity to synthesise amino acids because to its eukaryotic structure and success in business. Recently, utilising modular route rewiring, scientists have improved how yeast generates amino acids to concentrate metabolic energy on the things they want to create. Scientists may employ genetic engineering to enhance the number of amino acids produced by yeast by relaxing limitations and ensuring there are sufficient vital chemicals that enable yeast to be widely utilised in biotechnology (Stark *et al.*, 2024). The modified *E. coli*, *Corynebacterium glutamicum* and yeast employed in amino acid synthesis exhibit tremendous breakthroughs in metabolic engineering. They may be upgraded by modifying pathways and introducing heterologous genes, which increase their amino acid synthesis and assist numerous sectors, including medicines and food. Research advances in this area may make it simpler and more economical for microorganisms to help produce new goods (Boban *et al.*, 2024).

Computational tools for strain design in amino acid production

Microbial fermentation for generating amino acids is growing significantly since it offers benefits in the

food, pharmaceutical, and biotechnology industries. Restructuring strains to generate more amino acids has been greatly aided by the use of genome-scale modelling, machine learning, and computational techniques. It focuses on the numerous computational techniques that aid with strain design and how successfully they are implemented in metabolic engineering.

Genome-scale modeling (GSM)

GEMs are comprehensive representations of metabolic processes in organisms that researchers use to quantify and forecast various metabolic reactions. Genetic engineering models assist in identifying which genes should be introduced or eliminated to enhance the synthesis of desired chemicals, such as amino acids. Cautha *et al.* (2013) effectively enhanced tyrosine synthesis in Saccharomyces cerevisiae by constraint-based modelling. The study demonstrated that GEMs facilitated the selection of optimal gene deletions to enhance the synthesis of aromatic amino acids. Mishra *et al.* (2018) developed a comprehensive model of *Yarrowia lipolytica* to identify optimal genes for augmenting long-chain dicarboxylic acid synthesis. The technique they devised demonstrated that GEMs facilitate metabolic engineering, leading to increased product yields.

Machine learning approaches

Machine learning (ML) is now commonly employed in metabolic engineering in tandem with classic modelling approaches. ML systems can evaluate vast data to search out trends and make assumptions about how bacteria may operate which helps with strain creation. As an example, Tian and colleagues constructed the Presep model which employs machine learning to predict what proteins produced by Pichia pastoris (Tian *et al.*, 2013). Experiments demonstrated that findings from this model were accurate which speaks to the utility of ML for optimising protein synthesis in varied hosts. Zhang *et al.* (2019) have utilized a mix of modelling and machine learning to assist yeast enhance the quantity of tryptophan produced. They found a large boost in amino acid synthesis by integrating high-throughput data with predictive modelling, illustrating how powerful it is to apply both techniques together.

Integration of computational tools

Integrating genome-scale modelling with machine learning works quite well in the process of strain design. Oyetunde *et al.* (2019) devised a hybrid technique of looking at microbial bio-production performance combining data-driven approaches and GEMs. With this strategy, it was revealed what influenced the product's yields, revealing the connection between the numerous computational approaches applied. Using knowledge mining, feature extraction and machine learning, Czajka *et al.* (2021) were able to predict chemical titers in *Yarrowia lipolytica*. Their strategy made predictions more accurate by combining data from both literature and simulation, illustrating the benefit of utilising several sources of information in strain design.

CASE STUDIES

L-homoserine production in *Escherichia coli* using synthetic biology and metabolic engineering techniques

Sun *et al.* (2024) have conducted an in-depth study on the production of L-homoserine using Escherichia coli, employing techniques from synthetic biology and metabolic engineering. L-Homoserine is used in the synthesis of significant amino acids such as L-threonine and L-methionine, and it finds applications in several fields including agriculture, pharmaceuticals, and cosmetics. The scientists engineered many E. coli strains to enhance L-homoserine production by eliminating pathways for competing metabolites, augmenting the aspartate kinase gene, and optimising cofactor utilisation, namely NADPH. For instance, the deletion of the lysA, metA, and thrBC genes inhibited the complete processing of L-homoserine and altered the carbon allocation. Enhancements in titer were seen after the upregulation of genes that evade feedback mechanisms (e.g., thrAfbr, metLfbr) as well as rhtA, a gene implicated in metabolite transport. The most effective genetically modified strain produced 110.8 g/L of L-homoserine and yielded 0.62 g of L-homoserine per gram of glucose in a 2 L bioreactor, as shown in Figure 2. Modifications to the protein sequence enhanced carbon flow, the availability of essential chemicals, and the regeneration of NADPH. In synthetic biology, tools like as promoter engineering, gene knockouts, and cofactor balance management enhance the microorganism-driven production of L-homoserine, a primary objective in biomanufacturing.

Synthesis of L-threonine

L-threonine is an important amino acid utilised extensively in food, cosmetics, animal feed and pharmaceuticals. The thrABC operon serves a crucial function in controlling the biosynthesis of L-tryptophan. v. The findings revealed that higher expression of thrC than thrAB promoted cell growth and L-threonine synthesis; however, L-threonine production reduced when the thrC proportion was too high. The maximum L-threonine production was achieved when the expression intensity ratio of thrAB to thrC was 3:5. Secondly, a stationary phase promoter was also employed to dynamically modulate the expression of modified thrABC. This method increased cell proliferation and shortened the fermentation duration from 36 h to 24 h. Finally, the acetate metabolic overflow was minimised

by removing the ptsG gene, leading to a further rise in L-threonine synthesis. With these efforts, the final strain P2.1-2901ΔptsG achieved a titer of 40.06 g/L at 60 h of fermentation, which was 96.85% higher than the original control strain TH and the highest recorded titer in shake flasks. The maximum L-threonine output and productivity were attained in the described fed-batch fermentation, and L-threonine production is near the most significant litre (127.30 g/L).

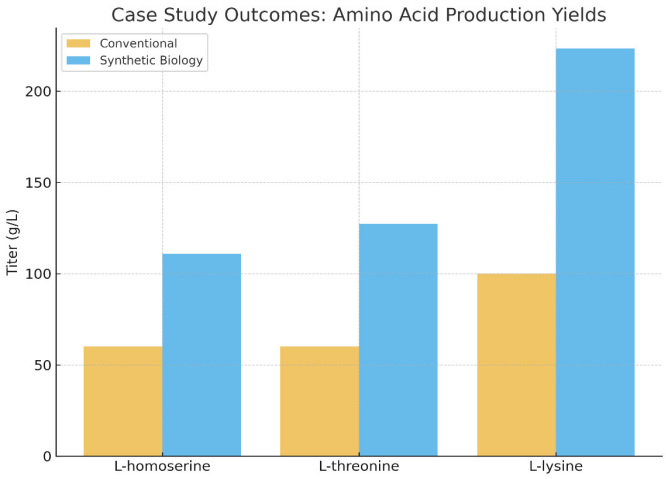

Figure 2: Amino acid production yield.

Trade-offs in typical batch cultures frequently reduced yields to less than 60g/L. Using biological engineering provides for greater control, allowing for larger yields and using less material.

L-lysine production

L-lysine is mostly produced using *Corynebacterium glutamicum* as the main host organism. An interesting study used systems metabolic engineering to develop an NADPH strain that regulates its own levels. By studying what changes happen to the DNA and chemically altering the process of making L-lysine molecules, scientists increased energy supply and efficient transportation. Since the promoter library was sensitive to the intracellular level of L-lysine, dynamic regulation of the NADPH pool was possible. This made it possible for the engineered strain to reach an L-lysine titer of 223.4 ± 6.5 g/L when fermented during fed-batch experiments (Liu *et al.*, 2023).

The yield of conventional fermentation (for instance, using random mutagenesis strains) was around 100 g/L; however, the procedure was ineffective and required several processing steps. Titer levels were virtually doubled, and substantially more energy became accessible owing to synthetic biology.

Challenges and future directions

Many restrictions currently prohibit widespread commercial utilisation of amino acid production via synthetic biology. When products of the synthetic process overwhelm host cells, a serious prospective issue occurs termed metabolic load. As a consequence, such strains frequently develop less effectively, generate less material and have poor stability. For instance, while designing Escherichia coli for L-homoserine, increasing the number of thrA, metL and rhtA genes boosted yields a lot but this required careful planning to prevent hurting the cells and retain their productivity. Options like modular pathways, altering promoters and integrating genes into the genome are being examined to minimise the stress and preserve optimal metabolic activity.

An additional key concern is toxicity in products, notably for amino acids that are not normal in proteins. An intracellular L-homoserine level over 13 mM will slow down or halt the development and metabolism of *E. coli*. For this reason, researchers have devised efflux systems employing transporters such as rhtA and eamA to remove the product from the cell and preserve homeostasis. Toxicity of comparable proportions is found when L-tryptophan is overproduced which may result in damage to membranes and issues with synthesizing proteins. Improving how microorganisms respond with stress by ALE and stress pathway modifications may overcome these problems.

A key challenge is the necessity for systems to adjust as things expand. Many times, the results obtained in a laboratory are not the same as in big commercial fermenters, as problematic elements like oxygen transport, pH and nutrients are involved. In such large-scale fermentations of L-lysine, a shortage of oxygen may cause the bacteria generate less useful L-lysine and more by-products. Better bioreactor designs with oxygen vectors or better mixing techniques function better, although they make the process more complicated and more expensive. Further process controls which include real-time monitoring and new techniques of feeding the components, are required to ensure uniformity at a wide scale.

Fermentations that are extended are fraught with complications owing to genomic instability. Taking away or modifying plasmids and changing designed pathways might result in less product being generated. Thus, adding genes that synthesise essential products, establishing synthetic auxotrophies and inserting toxin-antitoxin modules are employed to maintain the insect's features stable across time. They reduce the use of antibiotics and allow manufacturers to maintain high titers throughout repeated runs. In addition to these issues, several of the processes in the synthesis of amino acids may be limited by a shortage of NADPH and other cofactors. The production of L-homoserine from aspartate requires plenty of NADPH to steer the intermediates. Enhancing pntAB which encodes a pyridine nucleotide transhydrogenase,

has been done to enhance NADPH production and bring about greater yields. Optimising these biosynthetic systems involve making alterations in metabolic networks and incorporating enzymes that need a range of cofactors.

Advances in this area are contributing to the production of additional amino acids for a wider range of uses. By employing cell-free biosynthesis, AI and ncAA (non-canonical amino acids), scientists are currently developing novel techniques to boost conventional amino acid production. A recent advance is the dependence on cell-free systems, where biosynthetic processes are occurring outside live cells with the aid of separated enzymes and components. Because of these systems, users do not have to maintain cells alive, deal with their potential responses or handle different reaction components. Cell-free systems play a role in quick prototyping of routes for amino acid synthesis and modifying enzyme levels for optimum conversion. In particular, constructing multi-enzyme complexes in the lab has been utilised to synthesise L-tryptophan and its derivatives cell-free, making the design-build-test cycle significantly quicker. Also, cell-free methods may handle unconventional substrates or cofactors better than whole-cell approaches.

AI is having a huge effect on how leaf food routes are produced. Because of developments in machine learning and computational biology, optimising and predicting biosynthetic pathways has never been quicker and more accurate. Deploying deep learning-based enzyme labelling, looking at what products and reactants are most harmful and forecasting flow of materials across portions of the metabolic network assist to uncover novel enzymes, expose locations that cause toxicity and make ideas for improvement. To manufacture amino acids, AI has helped create enzymes that do not modify their activity owing to feedback, perfect codon uses and redistribute metabolic fluxes for a higher yield. Working with data enables academics to look at many more options than could be evaluated by traditional trials. Similarly, introducing non-canonical amino acids (ncAAs) to biological processes means enhancing what amino acids can accomplish in a particular system. Because of orthogonal tRNA–synthetase pairings and modified ribosomes, peptides and proteins in cells may be produced using ncAAs. Usually, novel amino acids feature distinctive groups (eg. azides, alkynes, fluorophores) that may make them unusual by permitting bio-orthogonality, metal binding or redox processes. Biosynthesising ncAAs, such as L-homophenylalanine and L-azidohomoalanine, enhances their use in creating improved materials, generating novel pharmaceuticals, and bioengineering.In addition, Vibrio natriegens which has a faster growth rate than *E. coli* and synthetic minimum cells are being studied for the manufacture of amino acids. The new hosts are able to develop fast, have unique methods of producing energy and are simple to manage, which makes them suitable for synthesising uncommon amino acids.

CONCLUSION

The production of amino acids may now be improved with higher yields, more efficiency, and less environmental impact thanks to the use of synthetic biology. By modifying the behaviours of bacteria, changing their genomes and carefully adding cofactors, people in science have succeeded in creating record quantities of L-lysine, L-threonine, L-tryptophan and L-homoserine. These technologies rely less on petrochemical processes and purification and instead, they make it feasible to utilise glucose from renewable sources which is excellent for the earth. Synthetic biology offers a vast amount of promise that has not yet been achieved. Using cell-free biosynthesis, artificial intelligence for metabolic engineering and introducing new kinds of amino acids are boosting possibilities in both biology and industry. They make it feasible to synthesise novel biomolecules faster, enhance those used in biotechnology and produce compounds not readily created with the conventional techniques. In the expanding usage of industrial biotechnology, synthetic biology may swiftly create novel techniques to generate amino acids and related biochemicals. Its capacity to develop platforms that can be modified, grown and are favourable to the environment brings it to the front of the bioeconomy. The progress of synthetic biology implies that both increased global demand for amino acids will be satisfied and a change from old fossil-based processes to newer biological-based ones will occur in chemical manufacture.

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NOVELTY STATEMENT

This review presents a compilation of the current trends in synthetic biology, metabolic engineering, CRISPR technologies, and computational technologies to enhance amino acid synthesis. It shows actual cases of how such technologies have dramatically enhanced microbial productivity and provided fresh opportunities in sustainable production. In contrast to the previous reviews, this article also describes the emerging trends, including AI-directed strain design and cell-free production systems. Altogether, it provides a clear and more recent point of view to make researchers and industry shift to more efficient and environmental friendly production of amino acids.

AUTHOR'S CONTRIBUTION

MS: Literature review, writing original draft, data interpretation and initial editing. MHS: Writing review and editing, conceptualization supervision, and final manuscript revision.

Ethical considerations

This review paper does not involve any studies with human participants or animals performed by any of the authors. Therefore, no ethical approval or informed consent was required.

Generative AI and AI-assisted technology statement

Generative artificial intelligence (AI) tools were not used by the authors to scientifically produce, analyze, or interpret the content of this manuscript. The only AI-aided software (grammar and spell-checking, etc.) was used to enhance language clarity. The scientific concepts, interpretations, and conclusions are solely the work of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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