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Safety Regulations of Edible Ingredients Derived from *Corynebacterium glutamicum*

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Review Article

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Abstract

Corynebacterium glutamicum has been researched and developed as a strain that produces various edible ingredients, starting with glutamic acid. Due to environmental pollution and food security issues, studies on using *C. glutamicum* as a single-cell protein (SCP) are emerging. For the past 20 years, cases valid as safe among edible ingredients derived from *C. glutamicum* by the European Food Safety Authority (EFSA) or U.S. Food and Drug Administration (FDA) were L-glutamic acid, L-lysine, L-arginine, L-valine, L-isoleucine, L-histidine, L-threonine, L-methionine, L-tryptophan, L-glutamine, 2′-fucosyllactose, corn syrup fermentation product, and D-psicose 3-epimerase. According to validation, it is recommended that the final product should be free of viable cells and recombinant DNA of the production strain. Although there is a possibility that viable cells may be present in the final product, it can be considered safe if the strain qualifies for Qualified Presumption of Safety (QPS) or Generally Recognized as Safe (GRAS). Even if there is a possibility that recombinant DNA may be present in the final product, it can be considered safe as long as it is not a gene of concern for antibiotic resistance, toxicity, or pathogenicity. This review provides insights for future safety validation of edible ingredients derived from *C. glutamicum*, including SCPs.

Keywords: *Corynebacterium glutamicum*; Edible Ingredient; Single Cell Protein; EFSA; FDA

Introduction

Microorganisms can be applied to biological processes in various industries such as food, pharmaceuticals, fine chemicals, cosmetics, energy, and new materials. Microbiological processes with industrial applications are advantageous in many respects over general chemical processes, indicating the importance of industrial microorganisms in applied microbiology [1]. *Corynebacterium glutamicum* is a Gram-positive bacterium discovered in 1957 by Japanese researchers while screening organisms that secrete L-glutamic acid [2]. When added to food, glutamate improves taste and sweetness and balances the taste [3]. Research and development for the mass production of glutamic acid allowed *C. glutamicum* to become one of the essential microorganisms in the food industry.

C. glutamicum was also excellent at producing amino acids other than L-glutamic acid. Molecular biology and genetic engineering advances have made *C. glutamicum* a more effective cell factory. In recent years, *C. glutamicum* has produced amino acids in units of 1 million tons per year in the world market. In particular, the annual L-glutamic acid and L-lysine production volume amounts to 2.2-2.5 million tons [4,5]. Advances in genetic engineering technology have also significantly contributed to research on producing other

edible ingredients by *C. glutamicum* [6,7]. Later, research was conducted to produce various biochemical substances in the pharmaceutical and cosmetics industries [8,9]. In this way, *C. glutamicum* has been used in industry for over 60 years and is recognized as a food-grade microorganism. Over the past ten years, studies on the metabolic engineering approach of *C. glutamicum* and the bioconversion of various substrates and edible ingredient synthesis through metabolic engineering have been summarized extensively and in detail in several review articles [4-17]. However, few reviews have been on using *C. glutamicum* single-cell protein (SCP) as an edible ingredient. Furthermore, from a safety perspective, no information specifically addresses the fermentation of *C. glutamicum* and its edible components.

This review summarizes *C. glutamicum*-SCP studies for alternative protein development and highlights its efficacy. Furthermore, it outlines the safety regulatory policies of influential public institutions and provides details of the institution's review of the safety of edible ingredients derived from *C. glutamicum*. This review aims to serve as a reference for innovations in utilizing *C. glutamicum* as a safe edible microorganism and for the safety screening of various edible ingredients derived from *C. glutamicum*.

Efficacy of *C. glutamicum*-SCP as an Edible Ingredient

Population growth has increased meat production, resulting in land and drinking water shortages and global warming. Additionally, the efficiency of converting feed to meat products cannot keep up with the growing demand for protein. Therefore, there is a need for alternative protein sources that are highly sustainable without harmful effects. For that purpose, SCP, obtained from microorganisms and algae cells using various wastes as substrates, is an alternative protein with very high potential. SCP's research is extensively documented [18-21]. However, information and cases about *C. glutamicum*-SCP are still rare. The following describes the efficacy of the *C. glutamicum*-SCP diet in several animals.

Similar performance, nutrient digestibility, and intestinal morphology were obtained when 2.5% of the feed of weaned pigs was replaced with Prosin and Protide (two kinds of *C. glutamicum*-SCP) [22]. A diet supplemented with 2.1% *C. glutamicum* cell mass improves the growth performance of nursery pigs. It also reduces malondialdehyde without affecting nutrient digestibility, intestinal morphology, and the microflora of the jejunal mucosa [23]. Dog treats containing 0.2% heme-SCP showed beneficial changes in gut microbiota regardless of administration method [24]. Mice that consumed a diet containing 0.05-0.5% of heme-SCP had a leaner body type than those that did not. Dissection of the mice showed that mice fed heme-SCP showed a decrease in subcutaneous fat and increased muscle mass. Additionally, the heme-SCP diet showed expansion of intestinal flora related to anti-obesity [25]. Diets containing 10-30% *C. glutamicum*-SCP instead of soy, poultry, and fishmeal showed lower growth performance and alkaline protease and aminopeptidase activities in mullet than in diets without SCP. Unlike mammals such as pigs, dogs, and rats, the reason for the negative results is presumed to be insufficient protein utilization. It has also been proposed that incomplete SCP cell wall lysis results in specific organization of the gray mullet's digestive system (lack of acid stomach digestion) and failure to develop functional gizzards (absence) [26]. For *C. glutamicum*-SCP to become an effective edible ingredient for all species, additional research and development, such as optimization of the manufacturing process and dietary method, is expected to be necessary.

Regulatory Policies of Public Institutions for Edible Ingredients

The European Food Safety Authority (EFSA) operates the Qualified Presumption of Safety (QPS) process. QPS status results from a preliminary assessment that addresses safety concerns for humans, animals, and the environment. During this process, experts evaluate the microorganism's taxonomic identity, associated body of knowledge, and potential safety issues. EFSA conducts QPS assessments of microorganisms used in feed additives, food additives, food enzymes, food flavorings, and novel food and plant protection products ("regulated products") before they are approved for use on the European market (https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps). EFSA had proposed in 2007 that QPS status would only apply to *C. glutamicum* if used for amino acid production purposes [27]. EFSA has since received requests to extend the QPS status of *C. glutamicum* to other uses. Based on the references accumulated so far, it was confirmed that *C. glutamicum* does not pose any risk associated with toxic metabolites in the fermentation broth. Therefore, EFSA extended the QPS status of *C. glutamicum* to other production purposes in 2018 [28]. The update to the list of classified QPS-recommended microbial agents notified to EFSA by March 2023 did not identify any new safety concerns for *C. glutamicum*, and therefore, its QPS status remained unchanged [29].

The U.S. The Food and Drug Administration (FDA) operates the Generally Recognized as Safe (GRAS) program. GRAS status is an abbreviation for the phrase Generally Recognized as Safe. Substances to be added to food must receive premarket approval from the FDA unless qualified experts generally recognize them as safe under the conditions of intended use. It determines whether a substance added to food is generally recognized as safe based on scientific data, expert opinion, and records of its use in food before

1958. Substances on the GRAS list are considered safe for intended use in food without additional approval (https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras).

Validation of Amino Acids Derived from *C. glutamicum*

The following information describes amino acids derived from the fermentation of specific *C. glutamicum* strains that EFSA and FDA have validated over the past 20 years.

L-glutamic Acid

L-glutamic acid produced by fermentation of *C. glutamicum* and its sodium salt form, monosodium L-glutamate (MSG), has no unique taste in itself but has been evaluated to be effective when used as nutritional additives or flavoring compounds [30-32].

No viable cells of the producing strain were detected in the final form of MSG-monohydrate produced by fermentation of the non-genetically modified strain KCCM 80188. Therefore, it was confirmed that no safety issues related to the production strain occurred. Ultimately, the product was concluded safe for the target species, consumers, and the environment [30].

KCCM 80187 is a genetically modified strain to produce MSG-monohydrate. However, no genes of concern for antibiotic resistance, virulence, and pathogenicity were identified in the whole genome sequence data. No antibiotics were used in the fermentation process, and no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, it was confirmed that no safety issues related to production strains and genetic modification occurred. In conclusion, the product was considered safe for the target species, consumers, and the environment [31].

No genes encoding antibiotic resistance and virulence determinants were identified in the whole genome sequence data of NITE BP-01681, the genetically modified strain used to produce L-glutamic acid and MSG-monohydrate. In addition, no antibiotics were used in the fermentation process, and no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, it was confirmed safe from problems caused by production strains and genetic modification. The product was not considered to pose any safety concerns other than the risk of inhalation [32].

L-lysine

L-lysine is an essential amino acid for all animal species. L-lysine and its salts produced through the fermentation of *C. glutamicum* are effective in improving animal growth by optimizing the amino acid profile of feed [33-50].

In the report validating L-lysine sulphate produced by fermentation of the non-genetically modified strain DSM 14764, information related to the detection of viable cells of the producing strain was not specified in the final product. However, based on available data, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the product was safe for the species, consumers, and the environment [33].

CGMCC 3704 is a genetically modified strain to produce L-lysine sulphate. However, the genetic modification of the production strains has been insufficiently characterized, and uncertainty remains regarding the possible absence/presence of recombinant DNA containing antibiotic-resistance genes in the final product. Therefore, the FEEDAP panel could not conclude the safety of the products produced by fermentation of this strain [34].

L-lysine (base), L-lysine HCl, and L-lysine sulphate were produced by fermentation of genetically modified strains NRRL B-50547, KCCM 11117P, and KCTC 12307BP and non-genetically modified strains KCCM-10227, DSM 24990, and DSM 16615. In the first validation, the final product produced by fermentation of NRRL B-50547 contained a recombinant antibacterial resistance gene. The final product produced by fermentation of KCCM 11117P had viable cells of the producing strain and the possibility of recombinant DNA. For KCTC 12307BP, data on identity, the safety of genetic modification, and the possibility of antimicrobial resistance are incomplete. In addition, the issue of the possibility of the presence of viable cells and recombinant DNA of the production strain in the final product produced by fermentation of the strain was not resolved. KCCM-10227, DSM 24990, and DSM 16615 are non-genetically modified strains but were determined to be resistant to certain antibiotics. Nevertheless, data on the absence of DNA from the production strain in the final product was not provided or was insufficient. Therefore, even though antibiotics were not used in any fermentation process, the FEEDAP panel could not conclude the safety of the products produced from the fermentation of the above six strains [35]. In the second validation, viable cells and recombinant DNA of the producing strain were not detected in the final product of NRRL B-50547 and KCCM 11117P fermentation. In the final product produced by fermentation of KCTC 12307BP, uncertainty regarding the presence/absence of viable cells and recombinant DNA of the production strain remained. However, the strain was suitable for the QPS approach for safety assessment, no safety issues occurred due to genetic modification, and the introduced antibiotic resistance gene did not remain in the genome of the production strain.

In a reevaluation of the antibiotic susceptibility of KCCM-10227 and DSM 24990, it was confirmed that there were no concerns related to infectious antibiotic resistance, so it was confirmed that there was no longer any possibility of DNA being present in the final product. In addition, it was confirmed that viable cells of the production strain were not detected. The application for validation of DSM 16615 has been withdrawn. In conclusion, the FEEDAP panel considered that the products produced by fermentation of the above five strains were safe for target species, consumers, and the environment [36].

NRRL B-50775 is a genetically modified strain to produce L-lysine HCl and L-lysine (base). Although the strain's genome contains genes considered dangerous when present in the product, viable cells and recombinant DNA of the production strain were not detected in the final product. Therefore, it was confirmed that no safety issues related to production strains and genetic modification occurred. Ultimately, the product was concluded safe for the target species, consumers, and the environment [37].

In the primary validation of CCTCC M 2015595 for producing L-lysine HCl and L-lysine sulphate, no information was provided on the origin and history of the strain, including whether it was genetically modified. In other words, assessing whether sequences of concern that will remain in the production strain have been introduced is impossible. In addition, there was a possibility that the viability and DNA of the producing strain remained in the final product. Although no antibiotic resistance genes existed in the strain's genome, the FEEDAP Panel could not conclude about the product's safety based on the above evidence [38]. Even in the second validation, the origin and history of the strain, including whether it was genetically modified, were unclear However, the whole genome sequence data confirmed that there were no genes of concern for toxins or toxicity in addition to antibiotic resistance. Crucially, since no viable cells of the production strain and its DNA were detected in the final product, it was confirmed that no safety issues related to the production strain and genetic modification occurred. Based on this, the FEEDAP panel evaluated the product as safe for the target species and the environment. Still, no conclusion on the additive's safety for users could be drawn due to the absence of data [39].

No viable cells of the producing strain were detected in the final form of L-lysine HCl and L-lysine (base) produced by fermentation of the non-genetically modified strain KCCM 10227. Therefore, it was confirmed that no safety issues related to the production strain occurred. Although mildly irritating to the eyes, the product was considered safe for target species, consumers, and the environment [40].

NRRL-B-67439 and NRRL-B-67535 are genetically modified strains to produce L-lysine HCl and L-lysine (base). However, no antibiotic-resistance genes were identified in the whole genome sequence data. No antibiotics were used in the fermentation process, and no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, it was confirmed that no safety issues related to production strains and genetic modification occurred. The product was considered safe for target species, consumers, and the environment, except that inhalation toxicity was inconclusive due to the absence of data [41].

CGMCC 7.266, which produces L-lysine HCl and L-lysine sulphate, is a genetically modified strain, but no antibiotic-resistance genes have been identified. In the absence of data, the FEEDAP panel could not conclude about the product's potential for inhalation toxicity, skin or eye irritation, or dermal sensitivity. However, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the product was considered safe for the target species, consumers, and the environment [42].

DSM 32932 is a genetically modified strain to produce L-lysine HCl. However, no antibiotic-resistance genes were identified, and no antibiotics were used in the fermentation process. In addition, since no viable cells and recombinant DNA of the production strain were detected in the final product, it was confirmed that no safety issues related to the production strain and genetic modification occurred. Although it is an eye irritant, the product is considered safe for target species, consumers, and the environment [43].

KCCM 80216 and KCTC 12307BP are genetically modified strains to produce L-lysine HCl and L-lysine (base). However, no genes of concern for antibiotic resistance were identified in the whole genome sequence data, and no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, it was confirmed that no safety issues related to production strains and genetic modification occurred. Ultimately, the product was considered safe for the target species, consumers, and the environment [44,45].

In the report validating L-lysine sulphate produced by fermentation of the KFCC 11043 strain, information on genetic modification was not specified. However, no genes of concern for antibiotic resistance were identified in whole genome sequence data. Additionally, since no viable cells of the production strain were detected in the final product, it was confirmed that no safety issues related to the production strain occurred. Therefore, the product was considered safe for the target species, consumers, and the environment [46].

CGMCC 14498 is a genetically modified strain to produce L-lysine HCl and L-lysine sulphate. However, no antibiotic-resistance genes were identified in the whole genome sequence data. Due to the absence of data, the FEEDAP panel could not conclude about the product's potential for inhalation toxicity, skin or eye irritation, or dermal sensitivity. However, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the product was considered safe for the target species, consumers, and the environment [47].

KCCM 80183 is a genetically modified strain to produce L-lysine (base) and L-lysine HCl. However, no genes of concern for antibiotic resistance, virulence, and pathogenicity were identified in the whole genome sequence data. In addition, since no viable cells and recombinant DNA of the production strain were detected in the final product, it was confirmed that no safety issues related to the production strain and genetic modification occurred. Although both products are toxic by inhalation and L-lysine HCl mildly irritates the eyes, they were ultimately concluded safe for target species, consumers, and the environment [48].

No viable cells of the producing strain were detected in the final form of L-lysine sulphate produced by fermentation of the non-genetically modified strain KCCM 80227. Therefore, it was confirmed that no safety issues related to the production strain occurred. Ultimately, the product was concluded to be safe for target species, consumers, and the environment [49].

CGMCC 17927, which produces L-lysine HCl and L-lysine sulphate, is a genetically modified strain, but no genes of concern for antibiotic resistance have been identified. Furthermore, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the product was considered safe for the target species, consumers, and the environment [50].

L-arginine

L-arginine is classified as a non-essential amino acid for adults and most mammalian species but is essential for mammalian neonates, strict carnivores, birds, fish, and reptiles. In addition to its role as a component of protein synthesis, arginine has a variety of functions in metabolism. L-arginine produced by the fermentation of *C. glutamicum* is an effective nutritional source for all species. This product can be used as an arginine replacement in dietary protein [51-57].

In the report validating L-arginine produced by fermentation of the non-genetically modified strain ATCC-13870, information related to the detection of viable cells of the producing strain was not specified in the final product. However, based on available data, FEEDAP concluded that the product was safe for the target species, consumers, and the environment [51].

In the final form of L-arginine produced by fermentation of the non-genetically modified strain KCTC 10423BP, viable cells and DNA of the producing strain were undetected. Therefore, it was confirmed that no safety issues related to the production strain occurred. Ultimately, the product was concluded to be safe for the target species, consumers, and the environment [52].

KCCM 80099 is a genetically modified strain to produce L-arginine. Although the origin and history of the strain and the genetic modification process have been adequately described, uncertainty remains regarding the possible presence of antimicrobial resistance genes in the genome. However, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Except for the fact that the product is corrosive to the eyes and skin, it was confirmed to be safe for the target species, consumers, and the environment when consumed appropriately under the indicated conditions [53].

No viable cells of the producing strain were detected in the final form of L-arginine produced by fermentation of the non-genetically modified strain KCCM 10741P. Therefore, it was confirmed that no safety issues related to the production strain occurred. Although the product is considered corrosive to the skin or eyes and poses a risk of inhalation, it was confirmed to be safe for the species, consumers, and the environment when consumed appropriately under the indicated conditions [54].

Although KCCM 80182, which is used to produce L-arginine, is a genetically modified strain, it was confirmed that there was no genetic modification related to antibiotic resistance. In addition, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Except for the fact that the product is corrosive to the skin or eyes, it was confirmed to be safe for the target species, consumers, and the environment when consumed appropriately under the indicated conditions [55].

No viable cells of the producing strain were detected in the final form of L-arginine produced by fermentation of the

non-genetically modified strain NITE SD 00285. Therefore, it was confirmed that no safety issues related to the production strain occurred. Although the product is considered corrosive to the skin or eyes and poses a risk of inhalation, it was confirmed to be safe for target species, consumers, and the environment at approved dosages [56].

No viable cells of the producing strain were detected in the final form of L-arginine produced by fermentation of the non-genetically modified strain CGMCC 20516. Therefore, it was confirmed that no safety issues related to the production strain occurred. The FEEDAP panel assessed the product as safe for the target species and the environment but could not conclude the additive's safety for users due to the absence of data [57].

L-valine

L-valine is an essential amino acid that may be limited under certain feeding conditions. L-valine is effective as a supplemental amino acid to maintain or restore the proper balance of amino acids for animal nutrition. Protein supplementation has become more critical since reduced animal protein diets were introduced in livestock farming for economic and environmental reasons. Accordingly, L-valine has become one of the crucial amino acids for fattening livestock in vegetable feed formulations [58-64].

KCCM 80058 is a genetically modified strain to produce L-valine. In the report validating this, information confirming the possibility of the strain possessing antibiotic-resistance genes was not specified. However, since viable cells and recombinant DNA of the production strain were not detected in the final product, it was confirmed that no safety issues related to the production strain and genetic modification occurred. In conclusion, the FEEDAP panel assessed the product as safe for target species, consumers, and the environment [58]. KCCM 80058 was also notified as AGRN 35 in the Current Animal Food GRAS Notices Inventory. However, the FDA noted discrepancies between the original submission and the amendment regarding the description of genetic modifications. They also identified deficiencies in the biogenic amine analysis provided in the amendment. Therefore, the FDA proposed suspending the evaluation of AGRN 35 and requesting that a new GRAS notification be resubmitted (https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory).

DSM 25202 is a genetically modified strain to produce L-valine. In the report validating this, information confirming the possibility of the strain possessing antibiotic-resistance genes was not specified. In the absence of data, the FEEDAP panel considered the product to be potentially harmful if inhaled, an irritant to the skin, eyes, and mucous membranes, or a potential dermal sensitization. However, since viable cells and recombinant DNA of the production strain were not detected in the final product, it was confirmed that no safety issues related to the production strain and genetic modification occurred. The product was considered safe for target species, consumers, and the environment [59].

No viable cells of the producing strain were detected in the final form of L-valine produced by fermentation of the non-genetically modified strain KCCM 11201P. Therefore, it was confirmed that no safety issues related to the production strain occurred. In conclusion, the FEEDAP panel considered the product safe for target species, consumers, and the environment [60].

In the validation request for CGMCC 11675 to produce L-valine, it was unclear whether it was genetically modified, and no information was provided on the origin and history of the strain. The report did not specify information regarding the detection of viable cells and DNA of the producing strain in the final product. In conclusion, due to uncertainty about the potential for genetic modification, the FEEDAP panel could not conclude whether the product was safe for the target species, consumers, and the environment [61].

L-valine produced by fermentation of non-genetically modified strains CGMCC7.358, CGMCC 7.366, and CGMCC 18932 did not allow conclusions to be made regarding the potential for inhalation toxicity, skin or eye irritation, or dermal sensitization due to a lack of data. However, since viable cells of the production strain were not detected in the final product, it was confirmed that no safety issues related to the production strain occurred. Therefore, when consumed appropriately under the presented conditions, the FEEDAP panel considered the product safe for target species, consumers, and the environment [62-64].

KCCM 80240 is a genetically modified strain to produce L-valine. However, according to the AGRN 48 document published in the Current Animal Food GRAS Notices Inventory, genetic modification has minimal potential ramifications on the host organism's cellular metabolism. Additionally, no viable cells of the production strain were detected in the final product. Based on the above evidence, along with other published information, the FDA has concluded that there is no doubt that the product resulting from the fermentation of this strain has GRAS status as a source of L-valine in livestock and poultry diets (https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory).

L-isoleucine

As an essential amino acid, L-isoleucine produced by the fermentation of *C. glutamicum* is considered an effective feed flavoring and nutritional additive for animal species [65-67].

No viable cells of the producing strain were detected in the final form of L-isoleucine produced by fermentation of the non-genetically modified strain KCCM 80189. Therefore, it was confirmed that no safety issues related to the production strain occurred. Ultimately, the product was concluded to be safe for the target species, consumers, and the environment [65].

KCCM 80185 is a genetically modified strain to produce L-isoleucine. Although viable cells of the production strain were not detected in the final product, recombinant DNA could be present. However, genes of concern, such as antibiotic resistance, virulence, and pathogenicity, were not identified in the whole genome sequence data of the producing strain. Therefore, no safety issues would arise even if recombinant DNA were possibly present in the final product. In conclusion, the FEEDAP panel considered the product to be safe for target species, consumers, and the environment [66]. The second validation concluded that no recombinant DNA was present in the final product [67].

L-histidine

As an essential amino acid, L-histidine produced by fermentation of *C. glutamicum* is considered an effective feed flavoring and nutritional additive for animal species [68,69].

KCCM 80172 is a genetically modified strain to produce L-histidine HCl monohydrate. However, the strain's identity has been established, it is sensitive to relevant antimicrobial agents, and no safety concerns are associated with genetic modification. Furthermore, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Although mildly irritating to the eyes, the product was confirmed safe for the target species, consumers, and the environment when consumed appropriately under the indicated conditions [68].

L-histidine HCl monohydrate produced by fermentation of the non-genetically modified strain KCCM 80179 mildly irritates the eyes. However, no viable cells of the producing strain were detected in the final product. Therefore, it was confirmed that no safety issues related to the production strain occurred. In conclusion, the FEEDAP panel considered the product safe for target species, consumers, and the environment when consumed appropriately under the presented conditions [69].

L-threonine

As an essential amino acid, L-threonine produced by fermentation of *C. glutamicum* is considered an effective feed flavoring and nutritional additive for animal species [70,71].

The report validating L-threonine produced by fermentation of the KCCM 80117 strain did not specify information on genetic modification of the producing strain and the results of live cell and DNA detection in the final product. However, due to the antibiotic susceptibility test, the strain satisfied QPS eligibility, and the final product was confirmed to be of high purity (>99%). Therefore, the FEEDAP panel concluded that the product is safe for target species, consumers, and the environment [70].

KCCM 80118 is a genetically modified strain to produce L-threonine. Although specific related information was blinded in the validation report, it was ultimately confirmed that no safety issues related to genetic modification existed. In addition, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the product was considered safe for the target species, consumers, and the environment [71].

KCCM 80178 is a genetically modified strain to produce L-histidine. However, the AGRN 34 document published in the Current Animal Food GRAS Notices Inventory clearly states the absence of antimicrobial resistance markers in the genetic engineering process and confirms that the genetic modification has minimal potential ramifications on the host organism's cellular metabolism. Additionally, no viable cells of the production strain were detected in the final product. Based on the above evidence and other published information, the FDA has concluded that there is no doubt that the product resulting from the fermentation of this strain has GRAS status for its intended use (https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory).

L-methionine

Methionine is an essential amino acid for all animal species. L-methionine produced by fermentation of *C. glutamicum* is considered an effective feed flavoring and nutritional additive for animal species [72,73].

KCCM 80184 is a genetically modified strain to produce L-methionine. Although specific information related to

genetic modification is blinded in the validation report, it is evaluated to be safe regarding genetic modification of the production strain. In addition, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the FEEDAP panel assessed the product as safe for target species, consumers, and the environment [72].

KCCM 80245 is a genetically modified strain to produce L-methionine. However, no genes of concern for antibiotic resistance, virulence, and pathogenicity were found in the whole genome sequence data. No antibiotics were used in the fermentation process, and no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, it was confirmed that no safety issues related to production strains and genetic modification occurred. In conclusion, the product was considered safe for the target species, consumers, and the environment [73].

L-tryptophan

As an essential amino acid, L-tryptophan produced by fermentation of *C. glutamicum* is considered an effective amino acid source for animal species. KCCM 80176 for producing L-tryptophan is a genetically modified strain, but it has been proven to have no antibiotic resistance genes. In addition, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. In conclusion, the FEEDAP panel assessed the product as safe for target species, consumers, and the environment [74].

L-glutamine

Glutamine is approved for use in foods for nutritional purposes, flavoring, cosmetics, and veterinary products. Although L-glutamine is a non-essential amino acid, it can act as a conditionally essential amino acid, primarily in growing animals. It has several specific effects, including improving intestinal development and immune responses. L-glutamine produced by the fermentation of *C. glutamicum* is considered an effective nutritional and flavour additive for animal species. NITE BP-02524, which produces L-glutamine, is a genetically modified strain, but no antibiotic-resistance genes of concern were observed. In addition, it was confirmed that no safety issues related to the production strain and genetic modification occurred because no viable cells and recombinant DNA of the production strain were detected in the final product. Therefore, the FEEDAP panel assessed the product as safe for the target species, consumers, and the environment [75].

Validation of Other Edible Ingredients Relevant to *C. glutamicum*

The following information describes other edible ingredients and food enzyme derived from the fermentation of specific *C. glutamicum* strains that EFSA and FDA have validated over the past 20 years.

2′-fructosyllactose (2′ FL)

2′-FL has already been approved to be used in a number of foods produced through chemical synthesis or fermentation using derived strains of *Escherichia coli* K-12 DH1 or *E. coli* BL21 (DE3). Moreover, the expanded use of 2′ FL mixtures for supplementation of infant food has recently been evaluated by EFSA with positive results. *C. glutamicum* APC199 (KCTC 13735BP) is a genetically modified strain to produce 2′ FL. However, the final product did not detect ten target genes introduced during genetic modification, including four antibacterial resistance genes identified in whole genome sequence analysis. Additionally, viable cells of the production strain were not detected. Ultimately, the FEEDAP Panel concluded that the consumption of novel foods comprised of the product is safe for the proposed uses and levels of use [76]. APC199 (KCTC 13735BP) has also been notified as GRAS Notice Inventory GRN 932. The document also implies that the production strain of the final product is not included and that there is no genotoxicity. Taken together with other published information, the FDA has concluded that there is no doubt that the product resulting from the fermentation of this strain has GRAS status for its intended use (https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=932&sort=GRN_No&order=DESC&startrow=1&type=basic&search=932).

C. glutamicum Corn Syrup Fermentation

GRN 792, notified in the GRAS Notice Inventory, describes *C. glutamicum* corn syrup fermentation produced by fermenting corn glucose syrup with the non-genetically modified strain ATCC 13032. The corn syrup fermentation product is used as a flavouring agent in gravies and sauces, herb and spice mixtures, seasonings, seasonings, mayonnaise-like products, meat and fish analogy, and soups and broths. The final product does not contain viable cells of the producing strain and is not considered to cause mutations or chromosomal abnormalities. Taken together with other published information, the FDA has concluded that there is no doubt that the product resulting from the fermentation of this strain has GRAS status for its intended use (https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=

GRASNotices&id=792&sort=GRN_No&order=DESC&startrow=1&type=basic&search=792).

D-psicose 3-epimerase

Although D-psicose 3-epimerase is not an edible ingredient, it is a food enzyme that catalyzes the epimerization of D-fructose at the C3 position to produce D-psicose (also known as D-allulose). *C. glutamicum* which produces this enzyme is a genetically modified strain into which the plasmid pFIS-1-DPE-2.8 has been introduced. The plasmid is considered hazardous even if it is a food enzyme preparation consisting of the non-viable FIS002 due to the presence of a copy of a resistance gene for an antimicrobial of human importance (blinded). However, evidence shows that under certain intended use conditions, D-psicose production with this food enzyme removes total organic solids, and the final form of D-psicose is free of recombinant DNA. The FEEDAP Panel concluded that food enzyme preparations containing D-psicose 3-epimerase produced with FIS002 would not pose a risk under applicable conditions [77].

Conclusion and Prospect

C. glutamicum has been studied as a cell factory producing amino acids and other edible ingredients. Due to environmental pollution and food security issues, research is emerging to apply *C. glutamicum* as an edible ingredient beyond its role as a producer. The *C. glutamicum*-SCP diet showed efficacy in supporting growth, improving intestinal flora, and anti-obesity in mammals such as pigs, dogs, and rats. However, amino acids have remained dominant among the edible ingredients from *C. glutamicum* that have been valid as safe by EFSA or the FDA. According to validation cases, the final product should be free from viable cells of the producing strain and recombinant DNA. Even if viable cells may be present in the final product, it can be considered safe if the strain is suitable for QPS or GRAS. Additionally, even if recombinant DNA may be present in the final product, it can be considered safe if it is not a gene of concern for antibiotic resistance, toxicity, or pathogenicity. Recently, based on the data accumulated so far, the production of other edible ingredients has also been considered safe as it is considered that there is no risk related to toxic metabolites in *C. glutamicum* fermentation broth. Since the primary material of *C. glutamicum*-SCP is crude protein, it can be easily mass-produced even with non-genetically modified strains suitable for QPS or GRAS. In the future, if it is proven that there are no toxic substances among the SCP components and the manufacturing process is optimized to demonstrate efficacy for all animal species, it is expected to be safe as a future alternative food.

Credit Authorship Contribution Statement

Sehyeon Park: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Writing-Original Draft, Writing-Review & Editing. Taeyeon Kim: Investigation, Validation. Soyeon Lee: Resources. Pil Kim: Conceptualization, Formal analysis, Writing-Review & Editing, Funding acquisition, Project administration.

Conflict of Interest Statement

The authors declare no conflict of interest. The funders had no role in the design of the study and the interpretation of data.

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