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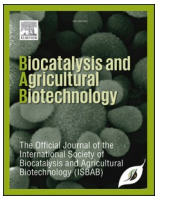

Investigation of glutamic acid production capacity of *Stenotrophomonas* sp. strain CG2 isolated from soil

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ABSTRACT

Glutamic acid is a widely used amino acid in the food and pharmaceutical industries due to its role as a flavor enhancer and a metabolic precursor. This study aimed to identify glutamic acid-producing bacterial strains from soil samples collected across different regions. Among 262 isolates screened, *Stenotrophomonas* sp. strain CG2 exhibited the highest production capacity and was identified through 16S rRNA sequencing. Fermentation parameters including pH, temperature, incubation time, and agitation speed were optimized using the Plackett–Burman design, leading to a maximum yield of 3.76 ± 0.65 g/L under optimized conditions (pH 7.0, 30 °C, 200 rpm, 84 h), compared to 2.72 g/L in unoptimized TSB medium. The produced glutamic acid was purified using ion-exchange resin, yielding a recovery efficiency of 53.48 ± 3.28 %, and its identity was confirmed by FT-IR, RAMAN, and LC-MS/MS analysis.

This study is among the first to systematically explore Stenotrophomonas spp. for glutamic acid biosynthesis under optimized fermentation conditions. The results provide insight into the strain's specific responses to nutrient composition, revealing its potential for future biotechnological applications. By expanding the microbial landscape of amino acid producers, this work offers a foundation for using CG2 in sustainable bioproduction processes, particularly those leveraging food or agro-industrial waste streams.

1. Introduction

The increasing global population has led to a growing demand for proteins and amino acids, which play essential roles in numerous biological functions and industrial applications (Ali et al., 2011; Adhikari et al., 2025). Among the L-amino acids, L-glutamic acid is particularly significant due to its widespread use as a flavor enhancer, feed supplement, pharmaceutical ingredient, and precursor in peptide synthesis and agricultural chemicals. Following antibiotics, amino acids represent one of the most important classes of fermentation-based bioproducts, with L-glutamic acid being the first amino acid produced commercially (Shyamkumar et al., 2014; Kolawole et al., 2011; Sun et al., 2024).

L-glutamic acid was first identified by the German chemist Karl Heinrich Leopold Ritthausen in 1866 and has since become the foundation of a multibillion-dollar industry. Although it can be synthesized chemically, microbial fermentation has become the preferred method for production due to its higher stereoselectivity and environmental sustainability. Chemical synthesis routes often

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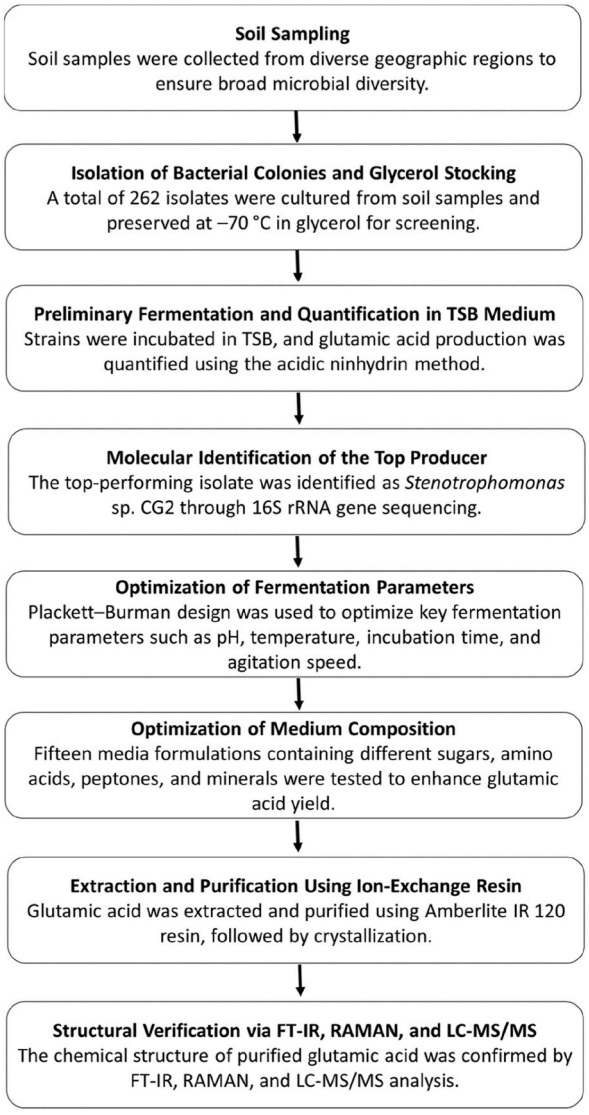

Fig. 1. FiSchematic workflow of the experimental study illustrating the main stages: (i) soil sample collection, (ii) bacterial isolation and screening for L-glutamic acid production, (iii) molecular identification (16S rRNA sequencing), (iv) fermentation and process optimization, (v) glutamic acid extraction and purification using ion-exchange resin, and (vi) chemical characterization via FT-IR, RAMAN, and LC-MS/MS techniques.

result in racemic mixtures that reduce product purity, making them unsuitable for large-scale applications (Zareian et al., 2012; Birnbaum and Demain, 1969).

Beyond its industrial importance, L-glutamic acid serves as a key flavor enhancer in the food industry—most notably as monosodium glutamate (MSG)—and has seen global adoption due to its umami taste (Harada-Padermo et al., 2020; Jyothi et al., 2005). In addition, L-glutamic acid functions as an excitatory neurotransmitter in the central nervous system, contributes to gastrointestinal health, and plays important roles in growth and metabolism in both infants and the elderly (Hamdi et al., 2024; Korytko, 2024; Burrin and Stoll, 2009; Yamamoto et al., 2009; Li et al., 2024).

The microbial production of L-glutamic acid is highly dependent on strain selection, medium composition, and fermentation parameters such as pH, temperature, agitation speed, and incubation duration. Extensive research has focused on optimizing production in strains such as *Corynebacterium glutamicum* and *Brevibacterium flavum*, which dominate current industrial processes due to their high yield and metabolic robustness (Shakoori et al., 2012; Dahiya et al., 2021; Liu et al., 2024). In addition, metabolic engineering approaches have further improved glutamic acid titers in these conventional strains (Cao et al., 2018; Zhang et al., 2022; Shangguan et al., 2023).In contrast to these well-studied species, this study investigates the glutamic acid production potential of Stenotrophomonas sp., a genus not traditionally associated with amino acid biosynthesis. Despite its known roles in environmental and biotechnological contexts, systematic studies exploring its fermentative capabilities for glutamic acid production remain extremely limited.

This work is among the first to isolate, identify, and optimize a Stenotrophomonas strain (CG2) for glutamic acid biosynthesis under defined fermentation conditions. The strain was selected from a pool of 262 soil-derived bacterial isolates, and its nutrient utilization profile was analyzed in relation to carbon, nitrogen, and mineral source preferences. By characterizing this underexplored genus, the study expands the known microbial diversity of glutamic acid producers and offers foundational insights for the development of sustainable bioprocesses using unconventional strains. Moreover, the findings suggest that food and agricultural wastes containing CG2-preferred nutrients could be valorized as cost-effective fermentation substrates.

The aim of this study was to isolate and characterize glutamic acid-producing bacterial strains from soil samples collected from different locations. The most efficient strain was identified using 16S rRNA sequencing, and fermentation conditions were optimized to enhance glutamic acid yield. Analytical validation of the purified product was performed using spectroscopic and chromatographic techniques to confirm its identity and purity.

2. Materials and methods

2.1. Materials and experimental workflow

The experimental study was carried out through a series of sequential stages including bacterial isolation, fermentation optimization, glutamic acid extraction, and chemical characterization (Fig. 1). The schematic workflow below summarizes the overall process and provides a visual guide to the methodological framework employed in this research.

The following chemicals and media components were used during the experimental procedures: glutamic acid standard (Merck), ninhydrin reagent, glucose, fructose, lactose, and various microbiological growth media including Tryptic Soy Agar, Nutrient Agar, Plate Count Agar, and Violet Red Bile Glucose Agar. Ion-exchange resin (Amberlite IR 120) was used for purification studies. All chemicals were of analytical grade and were obtained from a local distributor of Sigma-Aldrich Chemie GmbH, Germany.

2.2. Sample collection

Bacterial colonies were isolated from various natural sources, including soil and dairy products. Soil samples were collected from seven distinct regions in Turkey, each representing different climatic conditions and altitude variations.

2.3. Isolation of bacterial colonies

To isolate bacterial colonies, 2 g of soil sample was placed into a sterile 5 mL tube containing 2 mL of isotonic solution (1.5 % NaCl) and vortexed. Then, 0.1 mL of the mixture was transferred to agar media in Petri plates using a sterile bent glass spreader. The plates were incubated at 37 °C for 24 h. After incubation, single colonies were selected, transferred into vials containing glycerol, and stored at −70 °C for further analysis (Prashanthi et al., 2021; Zhang et al., 2021).

2.4. Cultivation and quantification of glutamic acid

The isolated bacterial strains were inoculated into conical flasks containing 100 mL of Tryptic Soy Broth (TSB). The flasks were incubated at 37 °C in an orbital shaker (Daihan-IS20R, South Korea) at 125 rpm for 48 h. After incubation, 10 mL of the fermented broth was centrifuged at 4100 rpm for 7 min, and the supernatant was filtered through a 0.45 μm membrane filter to separate the cells (Ali et al., 2011; Shakoori et al., 2012; Wang et al., 2023). The filtered supernatant was stored at 4 °C for further analysis.

The acidic ninhydrin method, as described by Gul et al. (2012), was employed to determine glutamic acid concentration quantitatively. For this purpose, 50 μL of the supernatant was transferred into a 5 mL screw-capped Pyrex tube, followed by 550 μL of ninhydrin reagent. The tubes were incubated at 100 °C in a water bath for 1 h. After cooling to room temperature, 1.6 mL of glacial acetic acid was added. Absorbance was measured at 365 nm using a UV spectrophotometer (Metas UV-5100, China). To construct a calibration curve, standard glutamic acid solutions (0.25, 0.5, 1, and 2.5 g/L) were prepared, and the same procedure was applied to these standards (Stauβ et al., 2024; Verni et al., 2024).

2.5. Molecular identification of glutamic acid-producing isolates

The highest glutamic acid-producing strain was identified using 16S rRNA nucleotide sequencing (ribotyping). Genomic DNA was extracted following a previously described method (Carozzi et al., 1991), and the 16S rRNA gene was amplified by PCR using RS-1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RS-3 (5′-AAGGAGGTGATCCAGCCGCA-3′) primers (Edwards et al., 1989). The PCR protocol was applied as reported earlier (Richter and Rosselló-Móra, 2009). Sequencing was conducted using the ABI Prism 377 DNA Sequencer (Applied Biosystems, USA). The obtained 639 bp 16S rRNA gene sequence was compared to known sequences in the BLAST GenBank NIH database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the most homologous strain, considering Average Nucleotide Identity (ANI) values (Chun et al., 2018). The sequence was found to share 97 % identity with Stenotrophomonas sp. (MK424600.1) and was designated as Stenotrophomonas sp. strain CG2. The 16S rRNA gene sequence of strain CG2 was submitted to the NCBI GenBank database under the accession number OM341624. Although the strain has not yet been deposited in a WFCC-recognized culture collection, arrangements are currently underway for its formal deposition to facilitate future accessibility and reproducibility of research.

Table 1
Incubation parameters tested in Plackett–Burman experimental design for fermentation optimization. Each condition was tested in triplicate (n = 3).

Run	pH	Time (h)	Shaking speed (rpm)	Temperature (°C)	Run	pH	Time (h)	Shaking speed (rpm)	Temperature (°C)
1	7.5	48	75	37.5	17	7.5	24	125	37.5
2	7.5	48	100	37.5	18	7.5	36	125	37.5
3	7.5	48	125	37.5	19	7.5	48	125	37.5
4	7.5	48	150	37.5	20	7.5	60	125	37.5
5	7.5	48	175	37.5	21	7.5	72	125	37.5
6	7.5	48	200	37.5	22	7.5	84	125	37.5
7	7.5	48	225	37.5	23	7.5	96	125	37.5
8	7.5	48	250	37.5	24	7.5	108	125	37.5
9	7.5	48	125	10	25	5.5	48	125	37.5
10	7.5	48	125	15	26	6	48	125	37.5
11	7.5	48	125	20	27	6.5	48	125	37.5
12	7.5	48	125	25	28	7	48	125	37.5
13	7.5	48	125	30	29	7.5	48	125	37.5
14	7.5	48	125	35	30	8	48	125	37.5
15	7.5	48	125	40	31	8.5	48	125	37.5
16	7.5	48	125	45					

2.6. Optimization of glutamic acid production conditions

To optimize glutamic acid production yields, incubation protocols were carried out under various growth conditions. The Plackett–Burman design (Ding et al., 2023; Devesa-Rey et al., 2023) was employed to determine the optimal pH, incubation time, temperature, and agitation speed (RPM) (Table 1). Additionally, the effects of growth medium composition, including carbohydrates, amino acids, nitrogen sources, and minerals, on glutamic acid production were assessed (Table 2).

For this purpose, the designated ingredients were added into 250 mL conical flasks, and the flasks were incubated at 37 °C in an orbital shaker at 125 rpm for 48 h (Yan et al., 2021). After each incubation period, glutamic acid concentration was measured using a UV spectrophotometer via the acidic ninhydrin method. In total, 31 experimental runs were conducted, with the center point replicated three times to assess the repeatability of the results. For statistical analysis, glutamic acid concentration was considered as the primary response variable.

2.7. Extraction of glutamic acid

This method was preferred due to its suitability for small-scale laboratory conditions and its effectiveness in achieving reproducible recovery results in analytical studies. Ion-exchange resin (Amberlite IR 120) was used for the extraction and purification of glutamic acid. The resins were initially soaked in 95 % ethanol to remove impurities, then washed with distilled water, followed by three cycles of washing with 4 N HCl. Subsequently, the resins were rinsed again with distilled water and alternately treated with 2 N NaOH to ensure complete purification.

For the adsorption process, 50 g of washed resin was added into 1 L of fermented broth in a 5 L flask, allowing the resin to absorb glutamic acid molecules. The pH of the broth was adjusted to 1.8–2.0 using 1 N HCl to promote ion exchange between glutamic acid and the resin. The flask was then shaken for 5 h at 42 °C and 120 rpm in a shaking water bath (Daihan-IS20R, South Korea). After this period, glutamic acid-loaded resins were separated by filtration. The glutamic acid concentration in the liquid phase was measured using a UV spectrophotometer following the acidic ninhydrin method.

For the elution process, the pH was increased to 3.8–4.0 by treating the broth with urea and sodium hydroxide, which facilitated the release of glutamic acid from the resin. The liquid phase was then acidified to pH 3.2 (the isoelectric point of glutamic acid) by adding 1 N HCl. The solution was left to stand at 20 °C for 48 h, allowing glutamic acid crystals to form. Finally, the eluent was evaporated, and dry solid crystals of glutamic acid were obtained (Nampoothiri and Pandey, 1999).

2.8. Chemical analysis of glutamic acid

The chemical identity and purity of the extracted glutamic acid were confirmed through FT-IR spectroscopy, RAMAN spectroscopy, and LC-MS/MS analysis. Analytical-grade glutamic acid (≥99 %, Sigma-Aldrich) was used as the reference standard in all analytical methods.

Fourier-transform infrared (FT-IR) spectroscopy was carried out using a Bruker VERTEX 70 spectrometer within the range of 4000–400 cm^{-1}. Approximately 2 mg of dried glutamic acid powder was homogenized with spectroscopic-grade potassium bromide (KBr) and pressed into a 13 mm pellet using a manual hydraulic press. Each spectrum was recorded at a resolution of 4 cm^{-1} with 32 scans averaged per sample. The resulting spectra were analyzed by comparing the absorption bands with reference data to confirm the presence of functional groups such as –COOH and $–NH_2$ (Dhamelincourt et al., 1991).

RAMAN spectroscopy was performed using a WITec Alpha 300R confocal Raman microscope equipped with a 532 nm Nd:YAG laser. About 2–3 mg of glutamic acid powder was placed on a quartz slide, and spectral data were acquired under a laser power of 10 mW, with an integration time of 10 s and three accumulations per sample. The spectral range was set between 200 and 2000 cm^{-1}. The

Table 2
Compositions of different growth media used in glutamic acid production trials. Media formulations were tested in triplicate (n = 3).

Ingredients (g/L)	R	A1	A2	B1	B2	B3	B4	C1	C2	C3	D1	D2	D3	E1	E2	E3	E4
Glucose (D+)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lactose	–	–	–	–	–	–	–	–	2.5	–	–	–	–	–	–	–	–
Fructose	–	–	–	–	–	–	–	–	–	2.5	–	–	–	–	–	–	–
Urea	–	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–
Peptone	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
Casein Peptone	17	17	17	17	17	20	17	17	17	17	17	17	17	17	17	17	17
Soya Peptone	3	3	3	6	3	3	3	3	3	3	3	3	3	3	3	3	3
Beef Extract	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
Malt Extract	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
Yeast Extract	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–
NaCl	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
KH_2PO_4	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	–	–	–
K_2HPO_4	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5
$FeSO_4$ $7H_2O$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.25	–	–
$MgSO_4$ $7H_2O$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.25	–
Methionine	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Serine	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 3
Validation of glutamic acid production by top 5 isolates. Values represent mean ± SD (n = 5). Groups sharing the same letter are not significantly different (Tukey's test, $p < 0.05$).

Isolate Code	1 (g/L)	2 (g/L)	3 (g/L)	4 (g/L)	5 (g/L)	Avarage L-Glutamic acid Productuon
TK.1.4.1	1.36	1.40	1.27	1.34	1.45	1.36 ± 0.07^{A}
TK.3.1.1	1.30	1.25	1.30	1.06	1.21	1.22 ± 0.10^{AB}
TK.2.5.1	1.10	1.14	0.99	1.16	0.90	1.09 ± 0.18^{B}
TI.3.2.1	0.99	1.25	1.01	0.89	1.32	1.06 ± 0.11^{B}
TK.4.2.1	1.28	1.09	0.89	1.25	1.15	1.13 ± 0.16^{AB}

characteristic vibrational bands observed were compared with known Raman spectra of standard glutamic acid to confirm molecular identity (Peica et al., 2007).

LC-MS/MS analysis was conducted using an Agilent 1260 Infinity II liquid chromatography system coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was achieved on a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm), maintained at 25 °C. The mobile phases consisted of 0.1 % formic acid in water (A) and methanol (B), and gradient elution was performed from 5 % to 80 % B over 10 min at a flow rate of 0.3 mL/min. A 5 μL injection volume was used. The mass spectrometer was operated in positive electrospray ionization (ESI+) mode, and multiple reaction monitoring (MRM) was employed for detection of glutamic acid. Quantification was based on a five-point external calibration curve ranging from 0.01 to 10 μg/mL, with excellent linearity ($R^2 > 0.998$) (Purwaha et al., 2014).

2.9. Statistical analysis

All analyses were performed in triplicate, and the mean values along with standard deviations were calculated. Significant differences between groups were determined using one-way ANOVA, followed by Tukey's post hoc test at a 95 % confidence interval ($p < 0.05$). Statistical analyses were conducted using Minitab 17 (Minitab Ltd, Coventry, UK).

3. Result and discussion

3.1. Selection and validation of glutamic acid-producing isolates

Microbial diversity in the collected soil samples provided a range of bacterial isolates with potential glutamic acid production capacity. A total of 262 bacterial colonies were isolated and screened for L-glutamic acid production. Five different agar media (Tryptic Soy Agar, Peptone-supplemented Nutrient Agar, Nutrient Agar, Plate Count Agar, and Violet Red Bile Glucose Agar) were used for isolation, while Tryptic Soy Broth (TSB) was used for subsequent glutamic acid production screening. Among the 262 isolates, five strains exhibited significantly higher glutamic acid production and were selected for further evaluation (Table 3). To ensure repeatability, each strain was cultured five times under identical conditions, and glutamic acid concentrations were quantified. The highest glutamic acid production was observed in strain TK.1.4.1 (1.36 ± 0.07 g/L), followed by TK.3.1.1 (1.22 ± 0.10 g/L). Statistical analysis (one-way ANOVA, $p < 0.05$) indicated that the glutamic acid production of TK.1.4.1 was significantly higher than the other strains. Tukey's post hoc test revealed that TK.1.4.1 belonged to group A, while TK.3.1.1 and TK.4.2.1 shared group AB, confirming statistical differences in production levels. Compared to previous studies, the observed glutamic acid production levels in this study were within the range reported for wild-type bacterial strains. For instance, a maximum of 11.4 g/L glutamic acid production from environmental isolates was reported (Shakoori et al., 2012), while 5.2 g/L was obtained from Bacillus cereus (Chattopadhyay et al., 1978).However, the production capacity of the newly isolated strains in this study suggests that further metabolic engineering could enhance their industrial applicability.

3.2. Identification of the best producing strain

To identify the highest glutamic acid-producing strain, 16S rRNA gene sequencing was performed on selected isolates. The amplified 16S rRNA gene sequences were uploaded to the National Center for Biotechnology Information (NCBI) BLAST database to determine their genetic similarity to known bacterial species. The BLAST query results revealed that the best-performing strain exhibited 99 % sequence identity with *Stenotrophomonas* sp., and was therefore designated as *Stenotrophomonas* sp. strain CG2.

Although several studies have reported diverse metabolic roles for *Stenotrophomonas* spp., their application in fermentative L-glutamic acid production remains underexplored. To the best of our knowledge, this is among the first studies to systematically investigate the glutamic acid biosynthesis capacity of a *Stenotrophomonas* strain under defined fermentation conditions.

However, it has been documented that *Pseudomonas reptilivora*, which is genetically related to *Stenotrophomonas*, enhances glutamic acid production by 13 % when co-cultured with *Corynebacterium glutamicum* (Shyamkumar et al., 2014).

This taxonomically informed observation suggests that certain *Stenotrophomonas* strains may harbor native metabolic traits favorable for amino acid biosynthesis. Therefore, even though CG2 is not a taxonomically novel strain, its biotechnological application in amino acid fermentation represents a novel functional context.

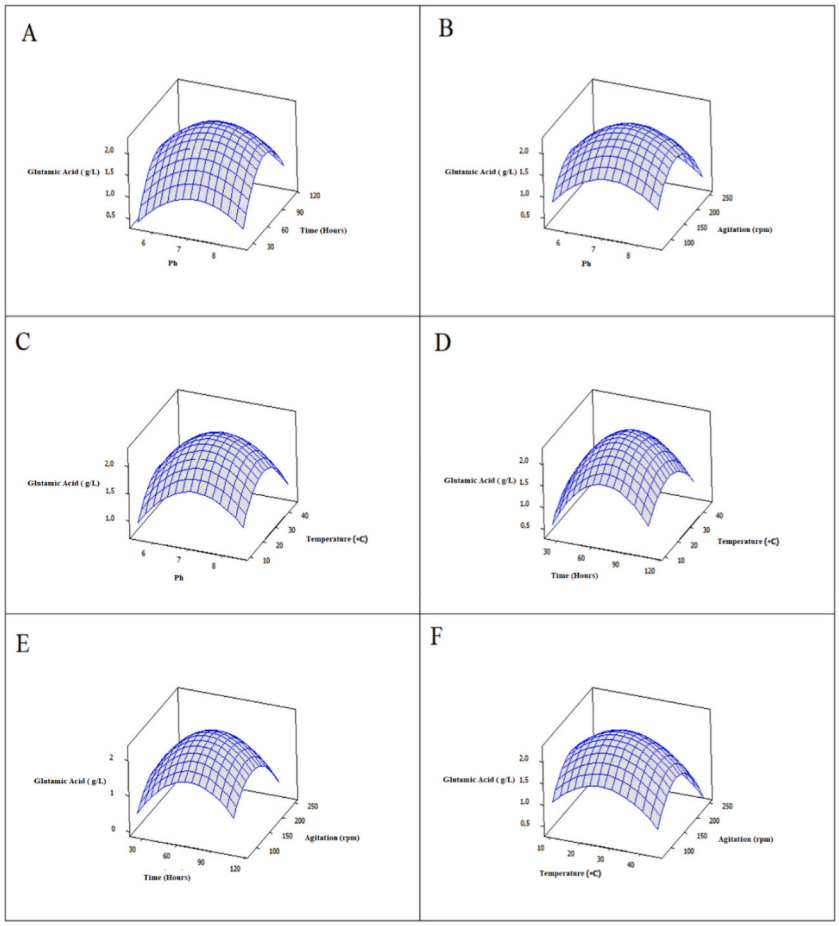

Fig. 2. Three-dimensional surface plots showing the interaction effects of selected fermentation parameters (A: pH-time, B: pH-agitation speed, etc.) on glutamic acid production by Stenotrophomonas sp. strain CG2. Data represent mean ± SD (n = 3).

This finding suggests that *Stenotrophomonas* sp. CG2 may have potential metabolic advantages for glutamic acid biosynthesis, warranting further investigation into its genetic and physiological traits.

3.3. Influence of fermentation parameters and growth medium composition on glutamic acid production

The impact of incubation conditions on glutamic acid production was assessed by varying temperature, pH, incubation time, and agitation speed in Tryptic Soy Broth (TSB). A total of 31 experimental runs were conducted to systematically analyze the effects of these parameters. The results indicated that increasing incubation time significantly enhanced glutamic acid production ($p < 0.05$), whereas a pH above 7.0 resulted in a considerable reduction in yield. A similar trend was observed in the interaction between temperature and pH, where higher temperatures combined with alkaline conditions further suppressed production efficiency. The highest glutamic acid production was obtained when the agitation speed was maintained between 175 and 225 rpm, and the pH was in the range of 6.5–7.5. Temperature was another key factor influencing production, with the optimal range determined as 25–35 °C. The combination of prolonged incubation time and moderate temperature favored higher yields, with maximum production observed at 84 h. Under the optimized conditions of 84 h, pH 7.0, 200 rpm, and 30 °C, the highest glutamic acid concentration was recorded as 2.72 g/L in TSB medium.

Further analysis of the interactive effects of incubation parameters on glutamic acid production was conducted using 3D response surface plots (Fig. 2). These plots revealed that increasing incubation time generally led to higher production yields, particularly when pH was maintained between 6.5 and 7.5 (Fig. 2A). Similarly, the combination of agitation speed and pH showed that the optimal range for agitation was 175–225 rpm, beyond which production efficiency declined (Fig. 2B). Temperature was found to have a critical role, with the highest yields observed at 25–35 °C (Fig. 2C), while excessive heat (above 40 °C) led to reduced productivity. The synergistic effects of incubation time and temperature demonstrated that extended incubation (up to 84 h) at moderate temperatures enhanced production, whereas shorter fermentation periods or higher temperatures significantly reduced yield (Fig. 2D). Additionally, agitation speed had a strong effect on production when combined with incubation time and temperature, with optimal conditions centered around 200 rpm and 30 °C (Figs. 2E and 1F). These findings underscore the necessity of precise control of incubation conditions to

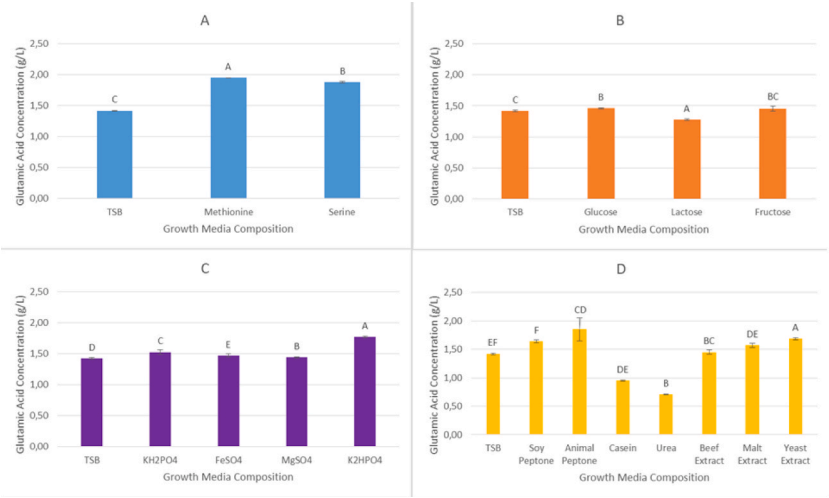

Fig. 3. Effects of various media components (amino acids, carbon sources, chemicals, and protein sources) on glutamic acid production by Stenotrophomonas sp. strain CG2. Data represent mean ± SD (n = 3). Different letters indicate statistically significant differences (Tukey's test, $p < 0.05$).

maximize glutamic acid biosynthesis in *Stenotrophomonas* sp. strain *CG2*.

To further optimize glutamic acid production, different growth media compositions were tested by supplementing TSB with amino acids, carbohydrates, nitrogen sources, and minerals. The addition of serine and methionine at 2 g/L each resulted in a 32 % and 37 % increase in glutamic acid production, respectively, when compared to the control, as observed in Fig. 3A. Among the tested carbon sources, glucose and fructose yielded the highest production levels, with an approximate 2.5 % increase over the control, while lactose supplementation resulted in a 10 % reduction in glutamic acid concentration (Fig. 3B). This suggests that glucose and fructose serve as more efficient energy sources for glutamic acid biosynthesis, whereas lactose may introduce metabolic inefficiencies or unfavorable regulatory effects on the microorganism's metabolism.

The effect of various mineral sources was also evaluated, showing that monopotassium phosphate (KH_2PO_4) at 0.5 g/L led to a 7 % increase, while dipotassium phosphate (K_2HPO_4) at 2.5 g/L significantly enhanced production by 25 % (Fig. 3C). Additionally, the inclusion of 0.25 g/L iron sulfate ($FeSO_4$) increased yield by 3 %, while the same concentration of magnesium sulfate ($MgSO_4$) resulted in only a 1 % improvement. These results indicate that phosphorus supplementation plays a key role in boosting microbial glutamic acid production, likely due to its involvement in ATP synthesis and metabolic regulation.

Different nitrogen sources were also examined to assess their effect on glutamic acid biosynthesis (Fig. 3D). Urea at 8 g/L significantly reduced production by 33 %, likely due to its inhibitory effects on bacterial metabolism at high concentrations. Conversely, yeast extract, beef extract, and malt extract contributed to increases of 18 %, 2 %, and 10 %, respectively. The highest production improvement was observed when animal peptone was used as a nitrogen source, leading to a 20 % increase, whereas soybean peptone improved production by 15 % compared to animal peptone. Interestingly, casein peptone had a slight negative effect, reducing glutamic acid yield by 2 %, suggesting that the composition and digestibility of nitrogen sources play a critical role in optimizing microbial fermentation efficiency.

Based on these findings, the optimized growth medium formulation for maximum glutamic acid production was determined to include glucose as the primary carbon source, along with animal peptone, casein peptone, malt extract, sodium chloride, dipotassium phosphate, monopotassium phosphate, and iron sulfate. Under these optimized conditions, the highest recorded glutamic acid concentration was 3.76 ± 0.065 g/L, marking a significant enhancement in production compared to the non-optimized medium.

These results are consistent with previous findings. For instance, a glutamic acid production of 11.4 g/L was achieved through medium and process optimization (Shakoori et al., 2012), while 5.2 g/L was obtained from Bacillus cereus (Chattopadhyay et al., 1978). Reported yields in other studies have ranged from 0.27 to 2.09 g/L (Adeogun et al., 2017), and a maximum of 92.53 g/L has been obtained from soil-isolated microorganisms (Kebede and Abate, 2016). Compared to these findings, Stenotrophomonas sp. strain CG2 exhibits promising potential for microbial glutamic acid production, although its yield remains lower than some of the previously reported values.

The results of this study demonstrate that incubation conditions and medium composition significantly impact glutamic acid biosynthesis in *Stenotrophomonas* sp. strain CG2. The observed variations in production efficiency highlight the importance of optimizing nitrogen and carbon sources, as well as maintaining suitable incubation conditions to enhance microbial fermentation efficiency. The response surface analysis confirmed that pH, temperature, incubation time, and agitation speed play crucial roles in achieving higher yields, with the best production obtained under moderate pH (7.0), a temperature of 30 °C, and a controlled agitation speed of 200 rpm. While the initial trials using Tryptic Soy Broth (TSB) medium yielded a maximum glutamic acid concentration of 2.72 g/L, the optimization of fermentation conditions resulted in a significantly enhanced production of 3.76 ± 0.65 g/L. Given the relatively moderate yield compared to industrial strains, further research should focus on metabolic engineering, adaptive laboratory

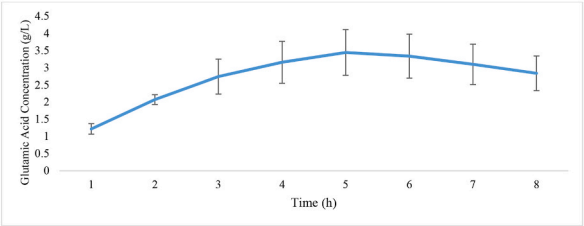

Fig. 4. Time-dependent recovery of glutamic acid using ion exchange resin. Values represent mean ± SD (n = 3).

evolution, and process-scale optimization to improve the commercial feasibility of *Stenotrophomonas* sp. strain CG2 in glutamic acid production. Unlike previous studies focused on traditional glutamic acid producers such as *Corynebacterium glutamicum* or *Bacillus* species, this work systematically evaluates the response of *Stenotrophomonas* CG2 to different fermentation conditions and media components for the first time, providing insights that may facilitate the valorization of nutrient-rich agro-industrial wastes in future studies.

Although the glutamic acid yield obtained from Stenotrophomonas sp. strain CG2 remains lower than those typically reported for extensively engineered strains of Corynebacterium glutamicum, it is important to consider the nature of the strain used in this study. CG2 is a wild-type soil isolate with no prior genetic modifications or adaptive evolution. As such, its performance reflects its native metabolic capacity, offering a realistic baseline for future improvement. In contrast, industrial strains of C. glutamicum have been refined through decades of genetic optimization to reach high production levels under narrowly controlled conditions. By investigating a non-traditional genus, this study expands the microbial repertoire for glutamic acid production and opens up new avenues for sustainable bioprocess development—particularly those aimed at valorizing low-cost or alternative substrates. Further studies may enhance CG2's biosynthetic potential through metabolic engineering or co-culture strategies while preserving its unique environmental adaptability.

When compared to industrially established production strains such as Corynebacterium glutamicum, Stenotrophomonas sp. strain CG2 exhibits a significantly lower glutamic acid yield under similar fermentation conditions. This difference is largely attributable to the fact that C. glutamicum has undergone decades of metabolic engineering, adaptive laboratory evolution, and process optimization. In contrast, CG2 has demonstrated strong environmental responsiveness—particularly in its tolerance to varying nitrogen sources, pH levels, and temperatures—indicating a flexible metabolic architecture. These features suggest that CG2 may serve as a promising host for targeted metabolic engineering applications. In this context, strategies such as the upregulation of glutamate dehydrogenase (GDH) or glutamate synthase (GOGAT) pathways, elimination of feedback inhibition, or redirection of carbon flux toward α-ketoglutarate could be employed (Becker and Wittmann, 2012). Additionally, genome-scale metabolic modeling and transcriptomic analyses may play a key role in identifying regulatory bottlenecks (Park et al., 2007). Such systems biology approaches have been successfully applied in other non-conventional microorganisms to enhance glutamate production and could similarly help unlock CG2's latent potential in bio-based glutamic acid synthesis. Furthermore, CG2's adaptive responses to various carbon and nitrogen sources make it a promising microbial platform for sustainable bioprocesses aimed at valorizing food and agro-industrial residues. Its tolerance to complex and variable raw materials, in particular, may offer significant advantages in converting such substrates into value-added compounds like glutamic acid.

3.4. Molecular characterization of purified glutamic acid

The fermented broth contained various impurities, including bacterial cells, macromolecules, pigments, inorganic substances, and organic compounds. Therefore, a purification process was essential to efficiently isolate glutamic acid. The purification steps involved filtration, centrifugation, and ion exchange resin (Amberlite IR 120) treatment to remove unwanted components. The time-dependent interaction profile between glutamic acid and the ion-exchange resin in the fermentation medium is shown in Fig. 4. The results indicate that glutamic acid adsorption onto the resin gradually increased over time, reaching its maximum interaction at the 5th hour, after which no further significant increase was observed. This suggests that equilibrium between the resin and the glutamic acid in the fermentation medium was achieved at this point. In addition to identifying a promising new candidate strain, this work uniquely explores the biotechnological feasibility of *Stenotrophomonas* sp. CG2 for L-glutamate fermentation, filling a gap in current microbial biotechnology literature.

Although ion-exchange resin chromatography may not be the most economical or scalable method for glutamic acid purification in industrial applications, it was purposefully selected for this study due to its high selectivity and reproducibility. The technique enables efficient separation of amino acids from fermentation media without requiring complex crystallization systems or large solvent volumes. In early-stage microbial screening studies, where experimental control and data reliability are prioritized over process economics, this method ensures accurate quantification and structural verification. While continuous crystallization or solvent extraction may offer higher recovery efficiency in large-scale settings, they are often unsuitable for low-volume laboratory processes. Therefore, despite its well-recognized limitations, the ion-exchange resin method provided a practical and analytically sound solution for the objectives of this work.

Following the purification process, glutamic acid was precipitated by adjusting the pH to its isoelectric point (pH 3.9), causing the

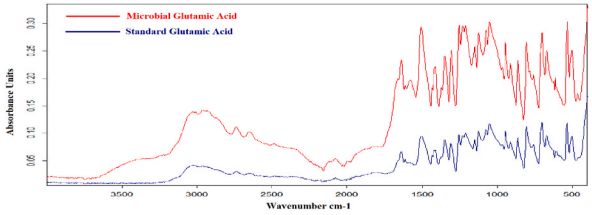

Fig. 5. FT-IR spectra of extracted and reference glutamic acid. Spectra obtained from dried samples. Characteristic peaks of COOH (1504 cm^{-1}), NH_2 (1637 cm^{-1}), CH_2 (1350 cm^{-1}), and O–H (1230 cm^{-1}) groups are highlighted. Representative spectrum shown.

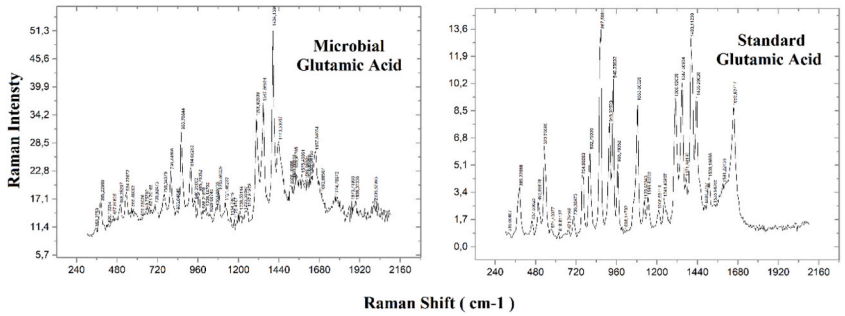

Fig. 6. Raman spectra of extracted and reference glutamic acid samples. Spectral profiles represent average of duplicate runs.

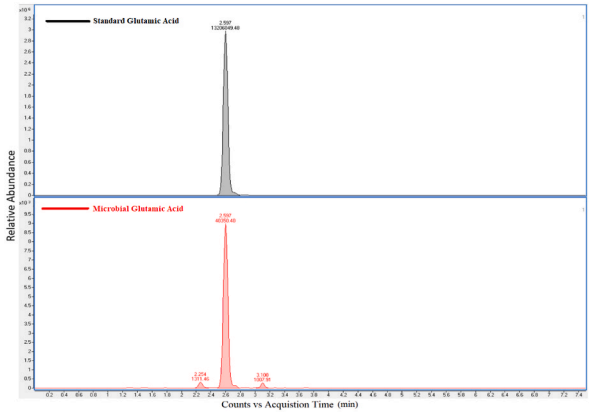

Fig. 7. LC-MS/MS chromatograms of extracted and standard glutamic acid under identical conditions. Peaks correspond to glutamic acid retention time. Analysis performed in triplicate.

glutamic acid molecules to become insoluble and facilitating their recovery through precipitation. Subsequent cooling of the eluent further enhanced precipitation, allowing for efficient crystallization of the glutamic acid. The overall recovery efficiency was determined to be 53.48 ± 3.28 %, indicating a moderate yield from the purification process.

To confirm the purity of the extracted glutamic acid, FT-IR and RAMAN spectroscopic analyses were conducted and compared to a standard glutamic acid reference. The spectral profiles from FT-IR and RAMAN spectrometry (Figs. 5 and 6) demonstrated strong similarities between the extracted and standard glutamic acid, confirming the chemical integrity of the recovered product.

Infrared spectra analysis revealed that the characteristic peaks of functional groups in the extracted glutamic acid were consistent with those of the standard. In the 600–1200 cm^{-1} range, the fingerprint (skeletal vibration) region of the glutamic acid molecule was observed. Notably, the peak at 1504 cm^{-1} corresponded to the carboxyl (-COOH) group, while the peak at 1637 cm^{-1} was attributed to the amine (-NH_2) group. The 1350 cm^{-1} peak was identified as belonging to CH_2 vibrations, whereas the O-H interaction in the carboxyl group was represented by the peak at 1230 cm^{-1} (Lanzilotta and Mcquillan, 2000).

The RAMAN spectrum analysis of microbial and standard glutamic acid (Fig. 6) further confirmed structural integrity. The peak at 1124 cm^{-1} was associated with NH_3, while the peak at 1083 cm^{-1} corresponded to the interaction between the carbon skeleton and NH_3 (Dhamelincourt et al., 1991). The strong 1400 cm^{-1} peak was attributed to the vibrational interactions between the carbon atom in the molecular skeleton and the oxygen atoms bonded via a double bond. Additionally, the characteristic peaks at 867 cm^{-1} and 965

cm^{-1} represented O-H and O interactions(Kabischt and Klose, 1978). Peaks in the 1125–1347 cm^{-1} region were identified as CH_2 vibrational movements (Peica et al., 2007).

For further validation, LC-MS/MS analysis was performed, and the obtained spectra exhibited peak patterns similar to those of the standard glutamic acid sample (Fig. 7). These results confirm that the extracted glutamic acid was structurally and chemically identical to the standard reference, validating the efficiency of the extraction and purification method.

Despite the well-known limitations of ion-exchange chromatography in terms of cost and scalability, its use in this study was a deliberate and contextually appropriate choice. The method provided high selectivity, reproducibility, and analytical clarity, which are essential in early-stage microbial screening and metabolite verification. These attributes are particularly valuable when evaluating the biosynthetic capacity of underexplored strains, where structural validation and process control take precedence over industrial feasibility. Beyond methodological validation, this study contributes to the diversification of microbial platforms for amino acid production by systematically investigating Stenotrophomonas sp. strain CG2—a genus rarely associated with glutamic acid biosynthesis. Although CG2 does not match traditional strains such as Corynebacterium glutamicum in production efficiency, it demonstrates promising characteristics including environmental resilience, metabolic versatility, and adaptability to alternative nutrient sources. These features may offer complementary advantages to those of conventional production strains, particularly in fermentation systems focused on sustainability or operational flexibility. Therefore, this study not only highlights the glutamic acid production potential of CG2, but also fills a notable methodological gap in the literature by providing one of the few examples where the purified product is rigorously validated using advanced analytical tools such as FT-IR, RAMAN, and LC-MS/MS. This comprehensive purification and characterization strategy strengthens the positioning of CG2 as a non-conventional microbial resource and offers a reliable reference point for future bioprocess development efforts.

4. Conclusion

This study identified Stenotrophomonas sp. strain CG2 as a promising microbial candidate for glutamic acid production, selected from 262 soil isolates. Under optimized fermentation conditions, it achieved a yield of 3.76 ± 0.065 g/L. Medium optimization revealed that specific carbon, nitrogen, and mineral sources significantly enhanced productivity, suggesting the potential use of food and agro-industrial wastes as low-cost substrates. Although glutamic acid was recovered at a moderate efficiency (53.48 ± 3.28 %) using ion-exchange resin, this method was suitable for small-scale laboratory validation.

Importantly, this is among the first studies to systematically evaluate glutamic acid biosynthesis by a Stenotrophomonas strain. The findings contribute new process-level insights into the metabolic behavior of a non-conventional genus and broaden the microbial repertoire for amino acid production. Future efforts should explore scale-up strategies and genetic modifications to enhance the strain's industrial applicability.

CRediT authorship contribution statement

Cihat Guner: Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources. **Ertan Ermis:** Visualization, Validation, Supervision, Project administration, Methodology. **Kubra Ozkan Guner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation.

Declaration of competing interest

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

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Data availability

The data that has been used is confidential.

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