EAS Journal of Biotechnology and Genetics
Abbreviated Key Title: EAS J Biotechnol Genet
ISSN: 2663-189X (Print) & ISSN: 2663-7286 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-8 | Issue-3 | Jul-Sep-2026 | DOI: https://doi.org/10.36349/easjbg.2026.v08i03.002

Original Research Article

Mining, Cloning, and Bioinformatic Analysis of Amidase Genes from Three Brewing Microorganisms

Jun Liu[1], Han Wang[1], Yingchun Zhao[1], Hejia Wen[1], Lijuan Yang[1,2]*

[1]School of Food and Liquor Engineering, Sichuan University of Science & Engineering, Yinbin, China
[2]Brewing Science and Technology Key Laboratory of Sichuan Province, Sichuan University of Science & Engineering, Yibin, China

Article History
Received: 11.06.2026
Accepted: 29.07.2026
Published: 03.08.2026

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Ethyl carbamate (EC), a Group 2A carcinogen, is unavoidably formed during fermentation and poses a persistent safety concern in alcoholic beverages and fermented foods. Although enzymatic degradation offers a mild and substrate-specific strategy, EC hydrolases suitable for brewing environments remain scarce, and heterologous expression of known candidates often results in inactive inclusion bodies. In this study, we mined amidase genes from three brewing-associated microorganisms—*Bacillus velezensis*, *Clavispora lusitaniae*, and *Saccharomyces cerevisiae*—based on the conserved GGSSGG motif of the AS-family amidases. Three full-length genes, *amiE* (1458 bp), *amdA* (1632 bp), and *amd08* (1665 bp), were successfully cloned into pET-28a vectors. Prokaryotic expression revealed that AmiE (52.63 kDa) and AmdA (60.76 kDa) were predominantly deposited as insoluble inclusion bodies, while Amd08 (61.39 kDa) was not detected due to apparent gene silencing. To circumvent these expression barriers, we performed comprehensive bioinformatic analyses. All three proteins were predicted as stable, hydrophilic molecules with theoretical pI values of 5.12–5.47 and negative GRAVY indices. Secondary structures were dominated by α-helices and random coils, and homology modeling confirmed the presence of intact GGSSGG motifs and Lys-Ser-Ser catalytic triads in each protein. Multidimensional model validation further supported their stereochemical reliability. Collectively, this study enriches the genetic reservoir of brewing-origin EC hydrolases and provides essential molecular and structural foundations for future solubility engineering and enzyme preparation development.

Keywords: Ethyl carbamate, EC Hydrolase, Gene Mining, Heterologous Expression, Bioinformatic Analysis.

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

1 INTRODUCTION

Ethyl carbamate (EC, urethane) has a molecular formula of $C_3H_7NO_2$ and a relative molecular mass of 89.09 [1]. As a natural by-product of fermentation [2]. EC is generated from the reaction between nitrogen-containing precursors such as urea and citrulline and ethanol [3, 4], and widely exists in Baijiu, Huangjiu, vinegar, soy sauce and other fermented foods [5]. Classified as a Group 2A probable carcinogen by the International Agency for Research on Cancer, long-term cumulative intake will continuously damage human liver [4], kidney and nervous systems, making it a long-standing food safety problem to be solved in the brewing industry [6, 7]. Most existing EC control methods are indirect regulation measures, including fermentation process adjustment, raw material precursor removal [8], low-yield strain screening [9], and chemical inhibitor addition [10], which can only reduce EC production instead of completely degrading EC formed in finished products, with prominent application limitations. Although commercial acid urease can degrade urea precursors [11], it cannot directly decompose EC, and its activation relies on heavy metal ions, bringing hidden risks of food residue. EC hydrolase that can specifically cleave EC to produce non-toxic small molecules serves as a degradation tool more suitable for food systems, yet the development and application of such enzymes are confronted with core bottlenecks. Although a small number of soluble EC hydrolases have been reported, these enzymes generally exhibit poor thermal stability and low ethanol tolerance, and their activity decays rapidly in high-alcohol and weakly acidic actual brewing systems, with unsatisfactory storage and reusability,

***Corresponding Author:** Lijuan Yang
School of Food and Liquor Engineering, Sichuan University of Science & Engineering, Yinbin, China

making them difficult for industrial promotion [5-12]. Therefore, mining novel EC hydrolase genes from various brewing microorganisms and predicting their structural potential adapted to fermentation environments via bioinformatics carries important theoretical and application values.

At present, there are three mainstream strategies for functional gene mining: the first is homologous cloning based on known conserved protein sequences [13], which can rapidly screen candidate genes relying on public database conserved motifs with simple operation and short cycle; the second is metagenomic library screening [14], which can obtain genes from uncultivated microorganisms but requires heavy construction and screening workload with high costs; the third is strain transcriptome differential analysis [15], which is suitable for degradation pathway analysis yet involves cumbersome pre-induction culture. Targeted cloning relying on the shared GGSSGG catalytic segment of AS-family amidases can rapidly obtain a large number of potential degradation genes to expand the existing enzyme gene bank.

Daqu acts as an enrichment carrier of brewing microorganisms, containing abundant bacteria and yeast strains, and serves as excellent material for mining novel EC-degrading enzymes. In this study, *Bacillus velezensis* isolated from Daqu and preserved in the laboratory with verified EC degrading capacity, together with preserved *Clavispora lusitaniae* and industrial *Saccharomyces cerevisiae*, were selected as three representative brewing strains. Targeted cloning of amidase coding genes was conducted based on the GGSSGG conserved sequence. The pET-28a vector was uniformly adopted for prokaryotic cloning and induced expression. Bioinformatic sequence analysis was taken as supplementary evidence to predict degradation potential from protein physicochemical properties, spatial structures and conserved catalytic sites, so as to expand the EC hydrolase gene bank and provide theoretical and material support for follow-up research.

2 MATERIALS AND METHODS

2.1 Strains, Plasmids and Reagents

Test strains: *Bacillus velezensis* isolated from Daqu of a distillery in Yibin; *Clavispora lusitaniae* and *Saccharomyces cerevisiae* preserved long-term in our laboratory. Cloning host *E. coli* DH5α and expression host *E. coli* BL21(DE3) were purchased from TransGen Biotech. Prokaryotic vector pET-28a(+) was obtained from Miaoling Biotechnology. Restriction endonucleases BamH Ⅰ, Xho Ⅰ and T4 DNA Ligase were supplied by TaKaRa. Bacterial/fungal genomic DNA extraction kits, gel recovery kits and plasmid extraction kits were products of Tiangen Biotech. Ethyl carbamate standard was bought from Macklin Biochemical. Other inorganic and organic reagents were of analytical grade.

2.2 Medium Preparation

LB medium (for bacteria culture): Tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, natural pH. Solid medium was supplemented with 15 g/L agar and sterilized at 121 °C for 20 min for the cultivation of *Bacillus velezensis* and *E. coli*.

YPD medium (for yeast culture): Glucose 20 g/L, peptone 20 g/L, yeast extract 10 g/L, natural pH. Solid medium contained 20 g/L agar and was sterilized at 115 °C for 25 min for the passaging of *Clavispora lusitaniae* and *Saccharomyces cerevisiae*. Kanamycin-resistant LB medium was supplemented with kanamycin at a final concentration of 50 μg/mL for the screening and scale-up of recombinant strains.

2.3 Main Instruments

Gradient PCR instrument, ultra-micro nucleic acid spectrophotometer, agarose gel electrophoresis system, gel imaging system, high-speed refrigerated centrifuge, ultrasonic cell disruptor, thermostatted shaking incubator, complete SDS-PAGE equipment.

2.4 Nucleic Acid and Protein Electrophoresis

2.4.1 Agarose Nucleic Electrophoresis

1.0% agarose gel was prepared by dissolving 1.0 g agar powder in 100 mL 1×TAE buffer via heating. After cooling, nucleic acid dye was added and the mixture was poured into gel trays for solidification. DNA samples were mixed with 6× DNA loading buffer and loaded alongside DNA Marker (100–500 bp). Electrophoresis was carried out at a constant voltage of 120 V for 20 min, and gel images were captured by the imaging system.

2.4.2 SDS-PAGE Protein Electrophoresis

The separating gel (12%) and stacking gel (5%) were prepared [16, 17]. Recombinant bacteria were induced by IPTG, washed with pre-cooled PBS, and disrupted in an ice bath via sonication. The lysate was centrifuged at 7000 r/min and 4 °C for 15 min to collect whole-cell, supernatant and precipitate fractions. Protein samples were mixed with 2× SDS loading buffer, denatured in boiling water for 5 min, and subjected to electrophoresis. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 and decolorized with glacial acetic acid-ethanol mixture to observe target protein distribution.

2.5 Candidate Gene Screening

Genomic annotation protein sequences of *Bacillus velezensis* (CP000560.2), *Clavispora lusitaniae* (GCA_009498135.1) and *Saccharomyces cerevisiae* (CAKOBR010000005) were retrieved from NCBI GenBank and Pfam databases. The GGSSGG catalytic hexapeptide and Lys-Ser-Ser catalytic triad were set as core screening indicators. Truncated open reading frames lacking complete catalytic domains were eliminated. The

preliminary candidate sequences were aligned with functionally verified EC hydrolases, and three target genes with intact conserved catalytic elements were selected for subsequent cloning experiments.

2.6 Strain Activation and Genomic DNA Extraction

Cryopreserved strains were streaked for single colonies: *Bacillus velezensis* on LB plates, two yeast strains on YPD plates. Single colonies were picked and cultured in corresponding liquid media until logarithmic phase, followed by centrifugation to collect bacterial pellets. Bacterial genomic DNA was extracted using bacterial kits, fungal genomic DNA via fungal kits. The integrity of genomic DNA was detected by 1% agarose electrophoresis, and qualified DNA samples were stored at −20 °C.

2.7 Gene Cloning and Recombinant Vector Construction

Specific primers containing BamH Ⅰ and Xho Ⅰ restriction sites were designed according to full-length gene sequences. PCR amplification was performed using corresponding genomic DNA as templates. PCR products were electrophoretically verified, recovered and purified. Purified gene fragments and pET-28a vector were double-digested synchronously, recovered and ligated with T4 DNA Ligase at 16 °C overnight. Ligation products were transformed into DH5α competent cells, and kanamycin-resistant plates were used to screen positive single colonies. Positive plasmids identified by colony PCR and double digestion were sent for full-length sequencing to confirm no base deletion or frameshift mutation.

2.8 Induced Expression and Solubility Detection of Recombinant Proteins

Sequencing-verified recombinant plasmids were transformed into BL21(DE3) competent cells. Positive single colonies were cultured in kanamycin-containing LB medium overnight to prepare seed liquid. Seed cultures were inoculated into fresh LB medium at a volume ratio of 2% and cultured to OD_{600} = 0.6–0.8, followed by IPTG induction at a final concentration of 1 mmol/L for 4 h at 37 °C. After induction, bacteria were collected and washed twice with pre-cooled PBS, then disrupted by sonication in ice bath. The lysate was centrifuged at 7000 r/min and 4 °C for 15 min to collect whole-cell, supernatant and precipitate protein samples for SDS-PAGE detection. Empty pET-28a transformants were set as negative controls to eliminate vector interference.

2.9 Bioinformatic Analysis Methods

The complete amino acid sequences obtained from sequencing were analyzed via multiple online tools. ExPASy ProtParam was used to calculate amino acid quantity, molecular weight, pI, instability index, aliphatic index and GRAVY value for comparison with reported EC hydrolases [18]. SOPMA was adopted to predict protein secondary structures and count the proportion of α-helices, extended strands, β-turns and random coils. ProtScale was used to draw full-length hydrophobicity curves and analyze hydrophilic and hydrophobic distribution of amino acid residues [18, 19]. SWISS-MODEL was applied for homologous modeling to select templates with optimal homology and coverage to build monomer spatial structures [20]. The SAVES platform integrating Ramachandran plot, Verify3D, ERRAT and PROCHECK[21, 22] was used to comprehensively evaluate model stereochemical qualityand analyze the spatial arrangement of GGSSGG motif and Lys-Ser-Ser catalytic triad to predict EC-degrading capacity [12-24].

3 RESULTS AND ANALYSIS

3.1 Screening and Multiple Sequence Alignment of Target Genes

AS-family amidase homologous sequences were retrieved from NCBI GenBank and Pfam databases for screening in the whole genomes of test strains. Screening criteria: complete coding sequence, intact GGSSGG conserved segment, eliminate truncated and mutated sequences lacking catalytic motifs. Finally, three genes *amiE* (1458 bp), *amdA* (1632 bp) and *amd08* (1665 bp) were obtained. The amino acid sequences encoded by the three genes were aligned with EC hydrolases with verified degradation activity (Fig 1). The catalytic core regions of the three candidate proteins shared high homology, all contained the intact AS-family signature GGSSGG hexapeptide, and key conserved residues such as LKD and GSIR were highly consistent to jointly form the Lys-Ser-Ser catalytic triad. The complete consistency of core catalytic elements with reported efficient EC hydrolases proved that *amiE*, *amdA* and *amd08* possessed the molecular basis for EC degradation, and could be taken as key targets for subsequent cloning and structural analysis.

3.2 PCR Amplification and Identification of Recombinant Plasmids

Genomic DNA of three strains was used as template for specific PCR amplification. Electrophoresis showed single bands without smearing, and fragment lengths were 1458 bp (*amiE*), 1632 bp (*amdA*), 1665 bp, consistent with theoretical values (Fig 2A). After double digestion of purified fragments and pET-28a vector, two distinct bands of vector and target gene could be observed without self-ligation bands of empty vector. Full-length sequencing confirmed intact open reading frames without mutation or frameshift, proving reliable vector construction (Fig 2B).

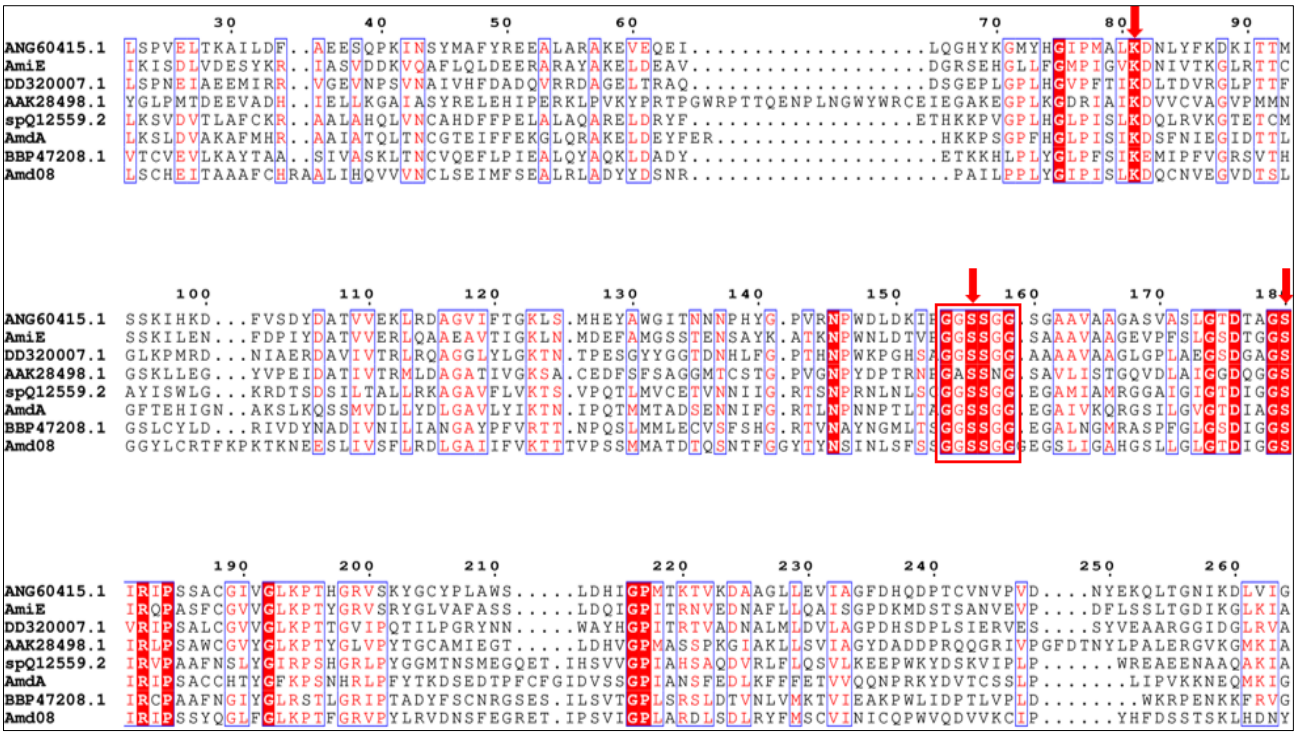

Fig. 1: Multiple sequence alignment of candidate amidases.Red boxes mark the conserved GGSSGG catalytic hexapeptide; red arrows indicate key residues forming the Lys-Ser-Ser catalytic triad.

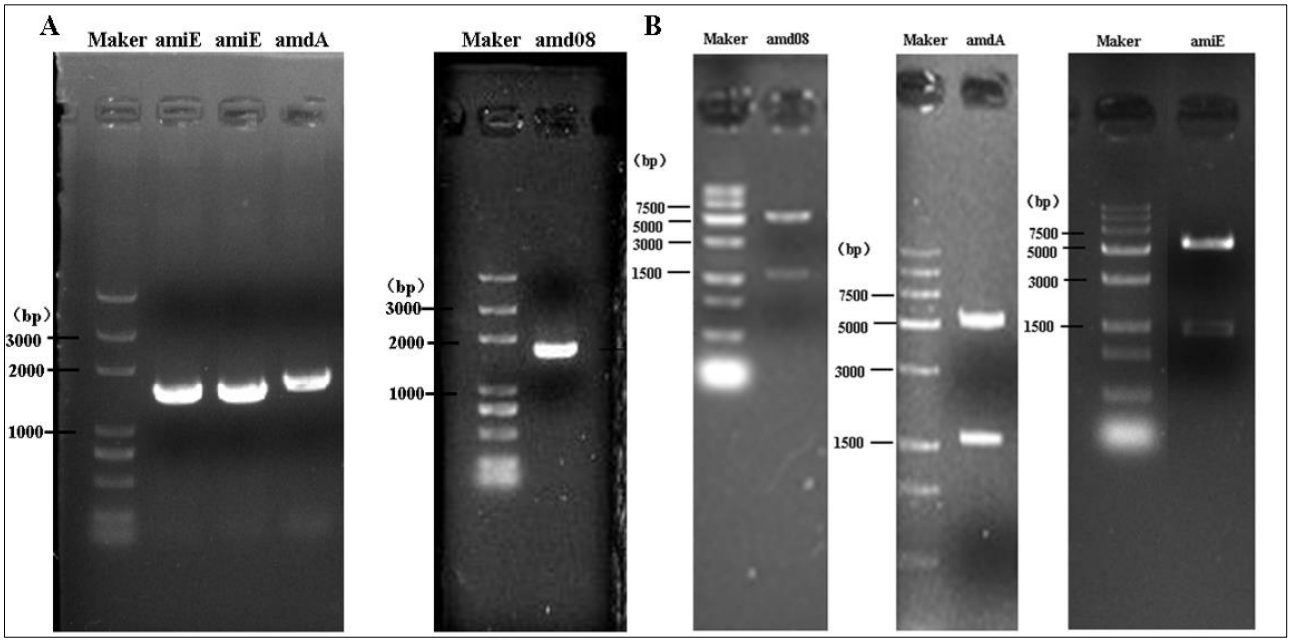

Fig. 2: PCR amplification and double restriction enzyme digestion identification of target genes. M: DNA Marker (100~5000 bp); A: PCR products of three genes; B: double digestion products of corresponding recombinant plasmids.

3.3 SDS-PAGE Analysis of Recombinant Protein Solubility

Recombinant strains were induced by IPTG, disrupted and separated into fractions for SDS-PAGE detection (Fig 3). Clear bands matching 52.63 kDa and 60.76 kDa could be observed in whole-cell samples of *amiE* and *amdA*, yet no corresponding bands existed in supernatant, and all target proteins accumulated as precipitates. No specific bands were detected in whole cell, supernatant and precipitate of *amd08* strain, indicating gene silencing under this induction condition. This result conforms to existing research that AS-family amidases are prone to misfolding during prokaryotic expression and cannot yield active proteins, so bioinformatic analysis was adopted to supplement functional verification of the three genes.

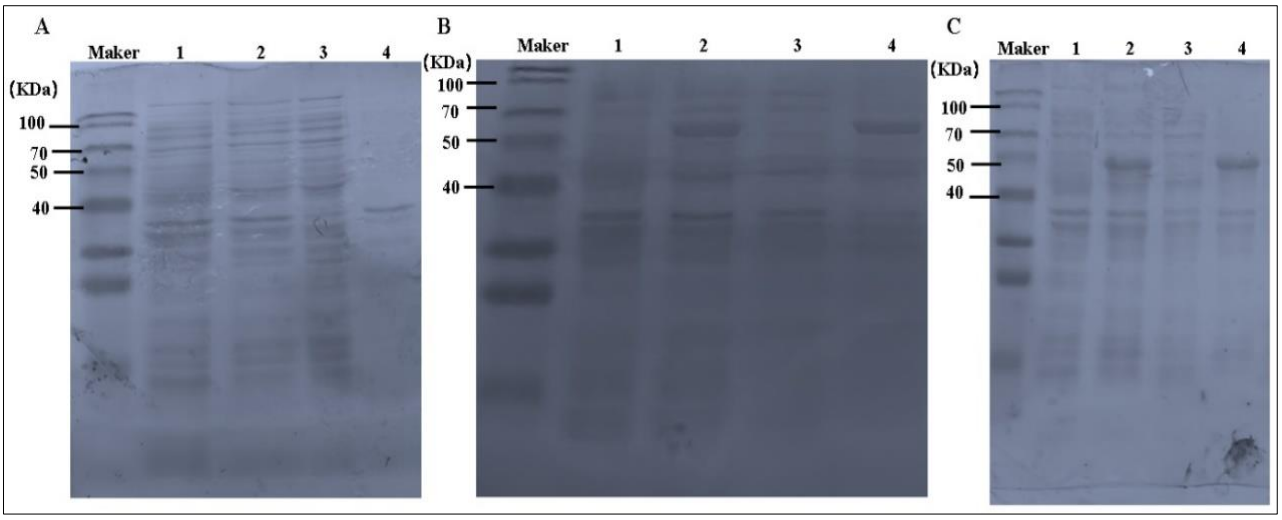

Fig. 3: SDS-PAGE detection results of three recombinant amidase proteins. M. Protein molecular weight Marker (12–140 kDa); (A) Amd08 recombinant protein; (B) AmdA recombinant protein; (C) AmiE recombinant protein; 1: Whole cell of engineering bacteria carrying empty pET-28a without induction; 2: Whole cell after IPTG induction and disruption; 3: Supernatant after cell disruption and centrifugation; 4: Precipitate after cell disruption and centrifugation.

3.4 Bioinformatic Analysis of Candidate Proteins

3.4.1 Analysis of Physicochemical Properties

Key physicochemical parameters of AmiE, AmdA and Amd08 were quantified and compared with reported EC hydrolases (Table 1). The GRAVY values of all three proteins were negative, indicating hydrophilic properties typical of intracellular microbial hydrolases. Their pI values were weakly acidic, matching the matrix of fermented foods, and instability indices were within reasonable ranges, showing good in vitro structural stability. The distribution of acidic and basic residues was balanced, and all physicochemical indicators were highly consistent with functional EC hydrolases, preliminarily proving their basic amidase properties.

Table 1: Comparison of physicochemical properties between candidate amidases and reported EC hydrolase-related proteins

Index	**AmiE**	**Amd08**	**AmdA**	***Rhodococcus equi* strain TB-60**	***Lysinibacillus fusiformis* SCO2**	***Candida parapsilosis***	***Aspergillus oryzae***	***Agrobacterium tumefacens* d_3**
Amino acids/aa	485	549	554	472	472	551	545	517
Molecular weight/kDa	52.63	61.39	60.76	50.70	51.48	61.74	60.10	55.89
pI	5.33	5.47	5.12	5.03	5.39	5.71	6.23	5.34
Asp+Glu	66	66	64	58	58	67	66	61
Arg+Lys	54	56	41	38	45	60	60	48
Instability index	33.53	34.21	36.22	34.70	40.70	40.20	34.66	28.78
Aliphatic index	86.91	90.13	85.34	83.35	94.85	87.97	88.81	81.35
GRAVY value	-0.213	-0.199	-0.207	-0.213	-0.146	-0.256	-0.240	-0.184

3.4.2 Secondary Structure and Hydrophobicity Analysis

The secondary structure composition and global hydrophobic distribution of the three amidases were shown in Figure 4 and Figure 5. Secondary structure prediction showed that α-helices and random coils were the main structural components of all three proteins, with low proportions of extended strands and β-turns. α-helices evenly distributed to maintain protein skeleton, while abundant random coils on the surface and active centers provided flexibility for substrate binding, consistent with AS-family hydrolases (Fig 4).

Hydrophobicity profiles showed no continuous long hydrophobic fragments, consistent with negative GRAVY values. Massive hydrophilic regions covered protein surfaces to support intracellular folding, while local weak hydrophobic segments inside maintained spatial skeletons, conforming to the characteristics of soluble intracellular hydrolases (Fig 5).

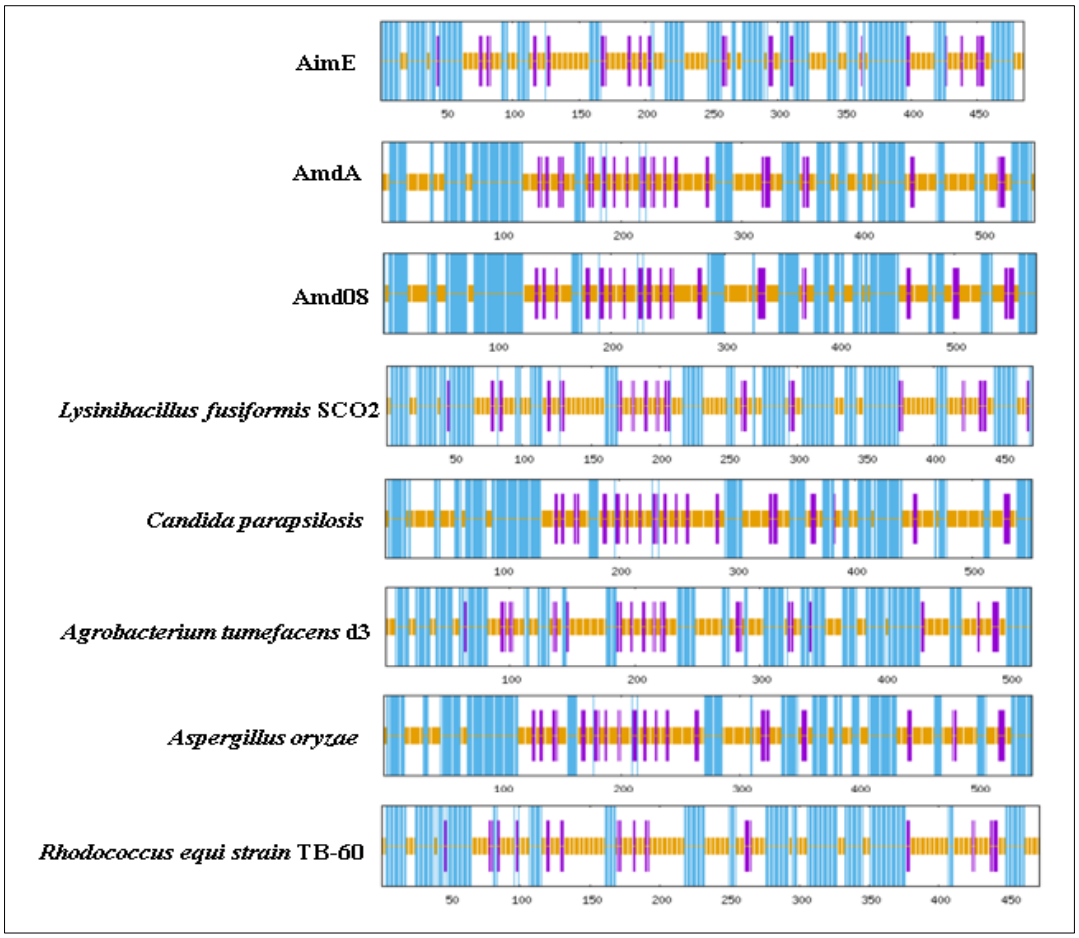

Fig. 4: Secondary structure prediction of candidate amidase proteins. Different color lines represent secondary structural elements: blue: α-helix; orange: extended strand; purple: β-turn; blank area: random coil.

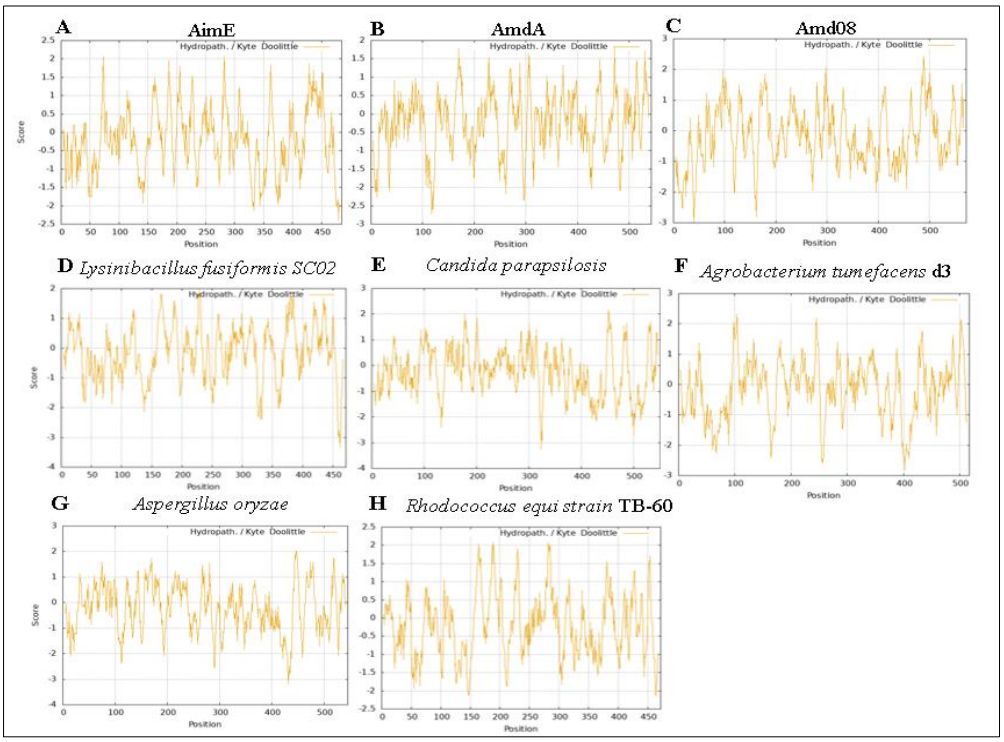

Fig. 5: Hydrophobicity distribution prediction of candidate amidase proteins. X-axis:amino acid sequence position; Y-axis: hydrophobic value; value > 0 = hydrophobic region, value < 0 = hydrophilic region.

3.4.3 Three-Dimensional Homology Modeling and Model Evaluation

Three-dimensional models of the three proteins were constructed via SWISS-MODEL (Figure 6). AmiE took glutamyl-tRNA amidotransferase as template, identity 87.01%, GMQE=0.97, full coverage of residues 1–485, forming monomer structure. Lys79, Ser154 and Ser178 clustered spatially to form catalytic triad, which coordinated with GGSSGG hexapeptide to form catalytic pocket. Amd08 adopted yeast AMD2 AlphaFold template with identity 98.91%, GMQE=0.94, complete catalytic triad. AmdA shared 100% template identity, GMQE=0.93, regular spatial arrangement of catalytic domains. All three models had high template homology and intact catalytic sites.

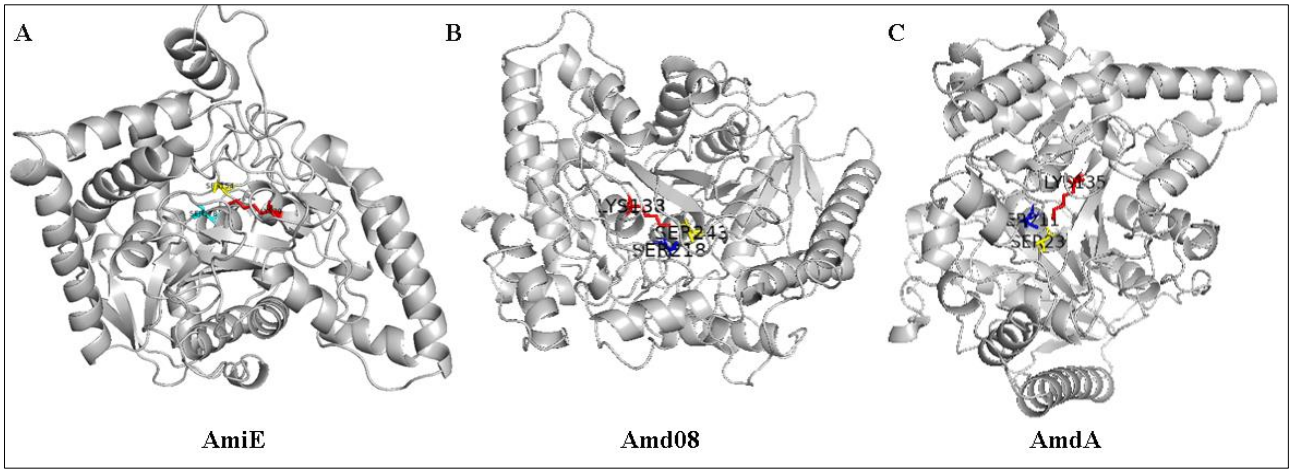

Fig. 6: Three-dimensional homologous structural models of three candidate amidases. A, B, C respectively stand for AmiE, Amd08 and AmdA; the core catalytic area is concentrated in the center of each protein model.

Multi-index stereochemical evaluation including Ramachandran plot, Verify3D and ERRAT was carried out to assess model reliability (Fig 7). For AmiE model, QMEANDisCo Global = 0.84 ± 0.05, ERRAT factor = 94.28, 91.2% residues fell in favored region, 8.6% in allowed region, 0.2% in generously allowed region without disallowed residues. Amd08 model had 90.0% residues in favored zone, merely 0.2% disallowed residues and zero bad atomic contacts. AmdA's ERRAT reached 96.16% with all residues distributed in favored and allowed regions. Although AmdA's Verify3D score was slightly lower than the recommended threshold, the model was still credible after comprehensive evaluation of multiple indicators. All three models owned compact folding and intact catalytic domains, which could be used for molecular docking and site-directed mutation research.

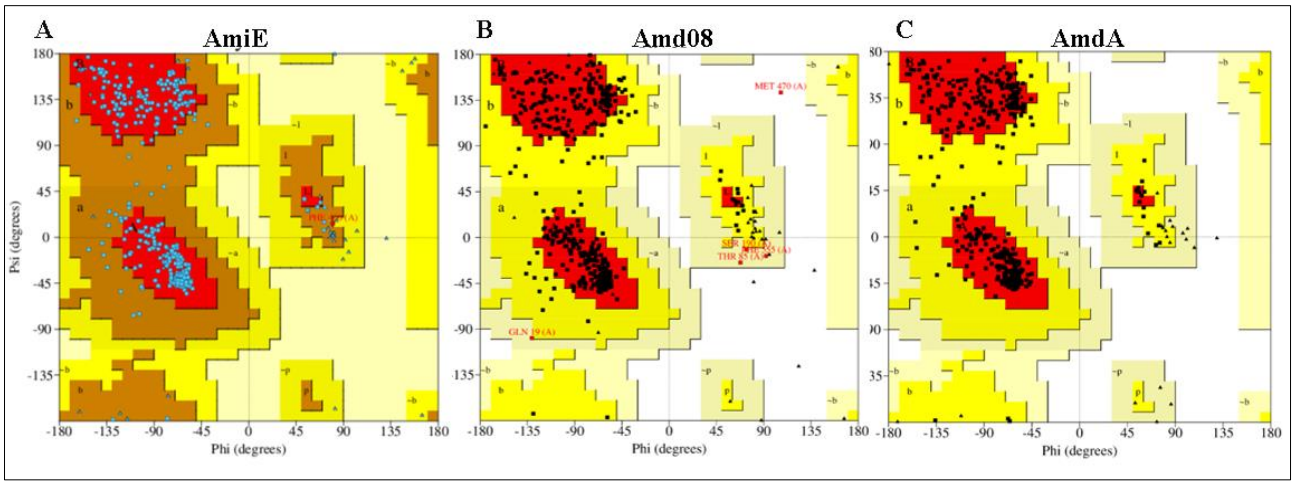

Fig. 7: Quality evaluation of three-dimensional models for candidate amidases. A. Ramachandran plot; B. Verify3D quality scoring chart; C. ERRAT structural quality statistical chart, which are used to comprehensively evaluate the stereochemical rationality and folding reliability of protein models.

4 CONCLUSIONS AND PROSPECTS

Targeting the bottlenecks of scarce brewing-adapted EC hydrolase genes and defective heterologous expression, this study cloned *amiE* (1458 bp), *amdA* (1632 bp) and *amd08* (1665 bp) from *Bacillus velezensis*, *Clavispora lusitaniae* and *Saccharomyces cerevisiae*, and constructed pET-28a recombinant vectors for

prokaryotic expression. Prokaryotic expression results showed that AmiE (52.63 kDa) and AmdA (60.76 kDa) expressed as insoluble inclusion bodies without active proteins, while Amd08 (61.39 kDa) exhibited gene silencing with no target protein detected. Systematic bioinformatic analysis demonstrated that the three proteins had pI values of 5.12~5.47 and negative GRAVY values with stable conformation. Their secondary structures contained flexible random coils. Homology modeling confirmed intact GGSSGG hexapeptide and Lys-Ser-Ser catalytic triad, and multi-dimensional evaluation verified rational stereochemistry. This study expands the resource bank of brewing-derived EC hydrolases and makes up the shortage of functional genes and expression defects, providing molecular materials and theoretical support for subsequent protein modification and functional verification.

Based on current experimental results and existing bottlenecks, follow-up research can be carried out on protein soluble transformation, enzymatic characterization and industrial application. Codon optimization, soluble tag fusion and gradient induction optimization can be adopted to solve the silencing of *amd08* and inclusion formation of AmiE and AmdA, so as to obtain high-purity active recombinant enzymes and systematically test kinetic parameters, pH and ethanol tolerance. Based on resolved three-dimensional catalytic structures, site-directed mutagenesis can be conducted to improve enzyme activity and solubility. Modified amidases can be applied in real fermented food substrates to test EC degradation efficiency and evaluate industrial potential, providing theoretical and technical support for green prevention and control of EC in fermented beverages.

Acknowledgements

This work was supported by the Postgraduate Innovation Fund of Sichuan University of Science & Engineering (Grant No. Y2024193). We thank Majorbio Bio-pharm Technology Co., Ltd. for technical support in transcriptome sequencing and data analysis. We also express sincere gratitude to the supervisors, teachers and colleagues in our research group for their valuable suggestions and assistance during the experiment and manuscript preparation, and to the university's laboratory platform for providing experimental instruments and research conditions.

CRediT Authorship Contribution Statement

Jun Liu: Methodology, Investigation, Data curation, Validation, Resources. Han Wang: Methodology, Investigation, Data curation, Formal analysis, Writing– original draft. Yingchun Zhao: Methodology, Investigation, Data curation, Validation. Hejia Wen: Software, Validation, Visualization. Lijuan Yang: Conceptualization, Project administration, Supervision, Funding acquisition, Writing– original draft, Writing– review & editing.

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.

Data Availability: Data will be made available on request.

REFERENCES

1. Li J J, e.a., *Isolation, purification and enzymatic properties of ethyl carbamate hydrolase.* Journal of Food and Biotechnology, 2014. 33(12): p. 1239-1245.
2. Wang, C., M. Wang, and M.-p. Zhang, *Ethyl carbamate in Chinese liquor (Baijiu): Presence, analysis, formation, and control.* Applied Microbiology and Biotechnology, 2021. 105(11): p. 4383-4395.
3. Chen, Y., et al., *Elimination of ethyl carbamate in fermented foods.* Food Bioscience, 2022. 47: p. 101725.
4. Baan, R., et al., *Carcinogenicity of alcoholic beverages.* Lancet Oncol, 2007. 8(4): p. 292-3.
5. Shalamitskiy, M.Y., et al., *Ethyl Carbamate in Fermented Food Products: Sources of Appearance, Hazards and Methods for Reducing Its Content.* Foods, 2023. 12(20).
6. Zhang X, e.a., *Bioinformatic analysis of ethyl carbamate hydrolase family.* Chinese Journal of Bioinformatics, 2022. 20(1): p. 64-74.
7. Cameán, A., et al., *Differentiation of Spanish brandies according to their metal content.* Talanta, 2001. 54(1): p. 53-59.
8. Liu, Q., et al., *Biocatalytic detoxification of ethyl carbamate in fermented beverages using a novel high-ethanol-tolerant urethanase from Acidisoma silvae.* Lwt, 2025. 225.
9. Ding X, e.a., *Screening of strains degrading ethyl carbamate in strong-flavor Baijiu and study on enzyme-producing characteristics.* Food and Fermentation Industries, 2018. 44(7): p. 29-36.
10. Sakano, K., et al., *Metabolism of carcinogenic urethane to nitric oxide is involved in oxidative DNA damage.* Free Radical Biology and Medicine, 2002. 33(5): p. 703-714.
11. Qi, S., et al., *Research progress on ethyl carbamate in food.* Journal of Food Science, Nutrition and Health, 2025. 4(2): p. 6-12.
12. Dong, N., et al., *Genetic Engineering Production of Ethyl Carbamate Hydrolase and Its Application in Degrading Ethyl Carbamate in Chinese Liquor.* Foods, 2022. 11(7).
13. Liang Y B, L.B.C., Li Y Q, *Mining novel α-L-rhamnosidase based on metagenomics.* Chinese Journal of Biochemistry and Molecular Biology, 2017. 33(1): p. 66-72.
14. Qian Y L, e.a., *Advances in microbial analysis of atmospheric particulate matter.* Asian Journal of Ecotoxicology, 2019. 14(2): p. 53-62.

15. Fei Y, e.a., *Transcriptome study of petunia calyx and young leaves.* Acta Horticulturae Sinica, 2015. 42(12): p. 2519-2525.
16. Yao X R N, e.a., *Breeding of yeast strains producing ethyl carbamate degrading enzyme and optimization of enzyme production conditions.* China Condiment, 2018. 43(1): p. 43-47.
17. H, Y.Q., *Optimization of fermentation conditions and expression purification of key enzymes of Bacillus subtilis producing pulcherriminic acid*. 2021, Sichuan University of Science & Engineering.
18. Gasteiger, E., et al., *Protein identification and analysis tools on the ExPASy server*, in *The proteomics protocols handbook*. 2005, Springer. p. 571-607.
19. Geourjon, C. and G. Deleage, *SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments.* Bioinformatics, 1995. 11(6): p. 681-684.
20. Waterhouse, A., et al., *SWISS-MODEL: homology modelling of protein structures and complexes.* Nucleic acids research, 2018. 46(W1): p. W296-W303.
21. Rahman, A., et al., *Targeting Essential Hypothetical Proteins of Pseudomonas aeruginosa PAO1 for Mining of Novel Therapeutics: An In Silico Approach.* Biomed Res Int, 2023. 2023: p. 1787485.
22. Mazumder, L., et al., *Structural and Functional Annotation and Molecular Docking Analysis of a Hypothetical Protein from Neisseria gonorrhoeae: An In-Silico Approach.* Biomed Res Int, 2022. 2022: p. 4302625.
23. Kumar, H. and P. Kim, *Artificial intelligence in fusion protein three-dimensional structure prediction: Review and perspective.* Clin Transl Med, 2024. 14(8): p. e1789.
24. Mazumder, L., et al., *In-silico characterization and structure-based functional annotation of a hypothetical protein from Campylobacter jejuni involved in propionate catabolism.* Genomics Inform, 2021. 19(4): p. e43.

Cite This Article: Jun Liu, Han Wang, Yingchun Zhao, Hejia Wen, Lijuan Yang (2026). Mining, Cloning, and Bioinformatic Analysis of Amidase Genes from Three Brewing Microorganisms. *EAS J Biotechnol Genet, 8*(3), 50-58.