



## RESEARCH ARTICLE

# Molecular identification and phylogenetic analysis of GABA-producing lactic acid bacteria isolated from indigenous dadih of West Sumatera, Indonesia [version 1; peer review: awaiting peer review]

Lili Anggraini 1, Yetti Marlida<sup>2</sup>, Wizna Wizna<sup>2</sup>, Jamsari Jamsari 3, Mirzah Mirzah<sup>2</sup>, Frederick Adzitey<sup>4</sup>, Nurul Huda 5

<sup>1</sup>Graduate Program, Andalas University, Padang, West Sumatera, Indonesia

<sup>2</sup>Department of Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

<sup>3</sup>Department of Plant Breeding, Faculty of Agriculture, Andalas University, Padang, West Sumatera, Indonesia

<sup>4</sup>Department of Veterinary Science, University for Development Studies, Temale, Ghana

<sup>5</sup>School of Food Industry, Universiti Sultan Zainal Abidin, Kuala Nerus, Terengganu, 21300, Malaysia

**V1** First published: 19 Oct 2018, 7:1663 (  
<https://doi.org/10.12688/f1000research.16224.1>)

Latest published: 06 Feb 2019, 7:1663 (  
<https://doi.org/10.12688/f1000research.16224.2>)

## Abstract

**Background:** Dadih (fermented buffalo milk) is a traditional Indonesian food originating from West Sumatra province. The fermentation process is carried out by lactic acid bacteria (LAB), which are naturally present in buffalo milk. Lactic acid bacteria have been reported as one of potential producers of  $\gamma$ -aminobutyric acid (GABA). GABA acts as a neurotransmitter inhibitor of the central nervous system.

**Methods:** In this study, molecular identification and phylogenetic analysis of GABA producing LAB isolated from indigenous dadih of West Sumatera were determined. Identification of the GABA-producing LAB DS15 was based on conventional polymerase chain reaction. 16S rRNA gene sequence analysis was used to identify LAB DS15.

**Results:** PCR of the 16S rRNA gene sequence of LAB DS15 gave an approximately 1400 bp amplicon. Phylogenetic analysis showed that LAB DS15 was *Pediococcus acidilactici*, with high similarity of 99% at 100% query coverage to *Pediococcus acidilactici* strain DSM 20284.

**Conclusions:** It can be concluded that GABA producing LAB isolated from indigenous dadih was *Pediococcus acidilactici*.

## Keywords

indigenous dadih, GABA, LAB, 16S rRNA gene, phylogenetic analysis

## Open Peer Review

### Reviewer Status

#### Invited Reviewers

1 2

**REVISED**

version 2

published  
06 Feb 2019



report



report

version 1

published  
19 Oct 2018

1 **Qinglong Wu**, Baylor College of Medicine, Houston, USA

2 **Jagadish Mahanta**, Indian Council of Medical Research (ICMR), Dibrugarh, India

Any reports and responses or comments on the article can be found at the end of the article.

**Corresponding author:** Yetti Marlida ([yettimarlida@ansci.unand.ac.id](mailto:yettimarlida@ansci.unand.ac.id))

**Author roles:** **Anggraini L:** Investigation; **Marlida Y:** Supervision; **Wizna W:** Conceptualization; **Jamsari J:** Conceptualization; **Mirzah M:** Conceptualization; **Adzitey F:** Writing – Review & Editing; **Huda N:** Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This research was supported by Ministry of Research, Technology and Higher Education Republic of Indonesia through Master of Education Towards Doctoral Scholarship Program for Excellence Undergraduate and the support through World Class Professor Program Scheme-B No. 123.57/D2.3/KP/2018.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

**Copyright:** © 2018 Anggraini L *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Anggraini L, Marlida Y, Wizna W *et al.* **Molecular identification and phylogenetic analysis of GABA-producing lactic acid bacteria isolated from indigenous dadih of West Sumatera, Indonesia** [version 1; peer review: awaiting peer review] F1000Research 2018, 7:1663 (<https://doi.org/10.12688/f1000research.16224.1>)

**First published:** 19 Oct 2018, 7:1663 (<https://doi.org/10.12688/f1000research.16224.1>)

## Introduction

The non-proteinogenic amino acid  $\gamma$ -aminobutyric acid (GABA) is widely found in bacteria, animals, plants, and fungi (Dhakal *et al.*, 2012; Nonaka *et al.*, 2017). GABA acts as a neurotransmitter inhibitor of the central nervous system (Olsen & Li, 2012). It is formed by decarboxylation of L-glutamate, a reaction catalyzed by an enzyme that depends on the peridoxal phosphate of decarboxylated L-glutamate (Murray *et al.*, 2003). Lactic acid bacteria (LAB) have been reported as a potential producer of GABA (Seo *et al.*, 2013; Wu & Shah, 2017). LAB are generally regarded as safe and non-pathogenic microbes, and has been referred to as 'generally recognized as safe'. Therefore, GABA-producing LAB can be used directly in functional foods (Zhao *et al.*, 2017). Some LAB can be found in the dairy industry for the production of cheese, yogurt, and other fermented milk products (Yamada *et al.*, 2018).

Dadih (fermented buffalo milk) is an Indonesian traditional food originating from West Sumatra Province; it is an extremely popular dairy product in Bukittinggi, Padangpanjang, Solok, Lima Puluh Kota, and Tanah Datar, Indonesia (Surono, 2015). Dadih is made from buffalo milk which is fermented in bamboo for 24–48 hours. The fermentation process is carried out by LAB which are naturally present in buffalo milk (Rizqiaty *et al.*, 2015) and the environment (Wirawati *et al.*, 2017). Studies have found that, the LAB strains present in dadih are generally *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Lactococcus* (Collado *et al.*, 2007; Surono, 2003).

Extraction of DNA is a basic principle in molecular analysis and it is one of the success factors in DNA amplification that is used in the analysis of genetic characters (Mustafa *et al.*, 2016). Polymerase chain reaction (PCR) and phylogenetic analysis based on 16S rRNA gene sequences have been used for successful identification of isolates from various fermented food products (Malik *et al.*, 2015). These molecular approaches have allowed *Lactobacillus* species to be reliably identified (Henry *et al.*, 2015). This research was conducted to identify and to characterize GABA producing LAB isolated from indigenous dadih of West Sumatera based on 16S rRNA gene sequence analysis.

## Methods

### Sample

This study used lactic acid bacteria (LAB) DS15, a GABA-producing LAB isolated from dadih of West Sumatera origin. This bacterium was isolated previously according to the method described by Ali *et al.* (2009). The experiment was carried out at the Feed Technology Industry Laboratory, Faculty of Animal Science, Andalas University, West Sumatra, Indonesia. LAB DS15 was grown anaerobically in MRS medium (Merck, Darmstadt, Germany) at 30°C and stored for further analysis.

### Isolation of bacterial genomic DNA

Isolation of the total genome of LAB DS15 was done using Genomic DNA Mini Kit purchased from Invitrogen (PureLinkTM, USA) by following the manufacturer's instructions. We used Lysozyme (PureLinkTM, USA) at a concentration of

20 mg/ml to break down the bacterial cell wall to improve protein or nucleic acid extraction efficiency.

### 16S rRNA gene amplification

Genomic DNA of LAB DS15 was used for amplification of 16S rRNA gene. Amplification was done using forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 138R (5'-GGG CGG GGT GTA CAA GGC-3'). The reaction was carried out in a volume of 50  $\mu$ l. The PCR mixture contained 22  $\mu$ l of MQ, 25  $\mu$ l DreamTaq Green DNA Polymerase (Thermo Fisher Scientific, USA), 1  $\mu$ l of each forward and reverse primer (10  $\mu$ M each, IDT synthesized) and 1  $\mu$ l template. Amplification conditions were 5 minutes of preheating at 95°C, 30 seconds denaturation at 95°C, 30 seconds of primer annealing at 58°C, 1 minute extension step at 72°C and post cycling extension of 5 minutes at 72°C for 35 cycles. The reactions were carried out in a thermal cycler (Biometra's T-Personal Thermal Cycler, USA).

### Electrophoresis

PCR products were stored at 4°C for further examination using 1% agarose electrophoresis in 1X, 100 V TAE for 30 minutes. The DNA bands formed from electrophoresis process was visualized using UV transiluminator.

### Sequence alignment of the 16S rRNA gene

Sequencing of the 16S rRNA gene was performed at the Laboratory of Medical Molecular Biology and Diagnostic, Indonesian Institute of Sciences, Jakarta. Sequencing results were edited (contig and peak chromatogram verification) using the SeqMan™ II program. Analysis of 16S rRNA sequences of LAB DS15 was performed using NCBI BLAST. Multiple alignment was done using the ClustalX 2.1 program. BioEdit version 7.2.5 in edit mode to see the absence of an inverted sequence and align the sequence length. Kinship visualization was done using the combined phylogenetic tree of the MEGA 7.0.20 program with the Neighbor-Joining hood method (Saitou & Nei, 1987).

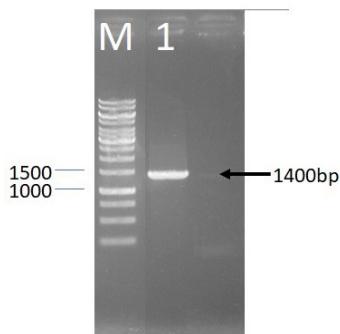
## Results and discussion

The identification of LAB DS15 to determine the strain was done based on 16S rRNA gene. The first step was amplification using PCR method, with the electrophoresis image shown in Supplementary File 1. The amplification process was carried out to obtain more copies of the 16S rRNA gene for the sequencing process. Analysis of sequencing results begun by aligning the base sequence of the 63F forward sequence and reverse 138R using the SeqMan program. PCR of the 16S rRNA gene of LAB DS15 gave an approximately 1400 bp amplicon (Figure 1).

Saitou & Nei (1987) indicated that the evolutionary history of organisms can be known using the neighbour-joining method. Organisms within the same taxa are normally clustered together in the phylogenetic tree and have better bootstrap values (Felsenstein, 1985). In this study, we drew a phylogenetic tree to scale and determined the evolutionary distances using the p-distance method. A total of 26 nucleotide sequences and

codon positions 1st + 2nd + and 3rd + noncoding were considered, using MEGA 7.0 as reported by Kumar *et al.* (2016) for evolutionary analyses.

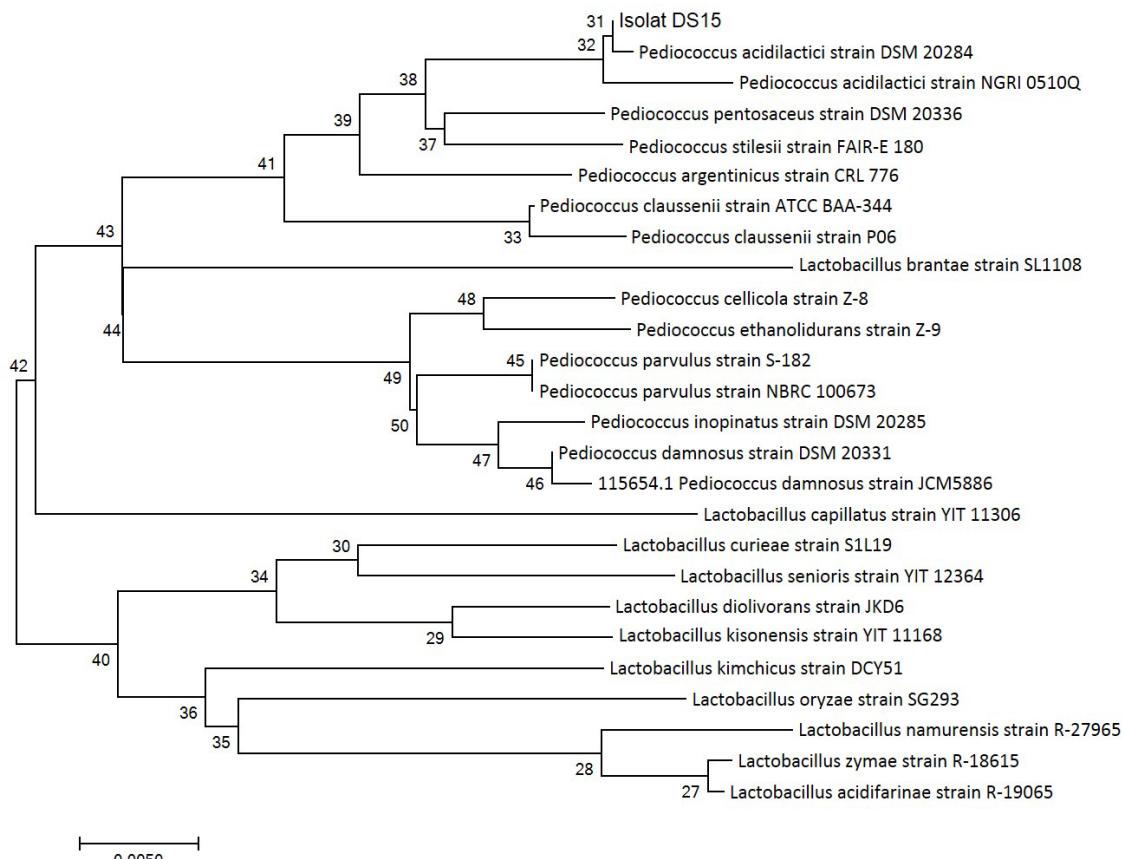
DNA sequencing results were analyzed using NCBI BLAST. According to Willey *et al.* (2009), 16S rRNA sequencing looks at the similarity of isolates to those already available in GenBank; this is one molecular detection method that is ideal enough to know the kinship relationship between bacteria because the 16S rRNA sequence is a gene found in all microbes



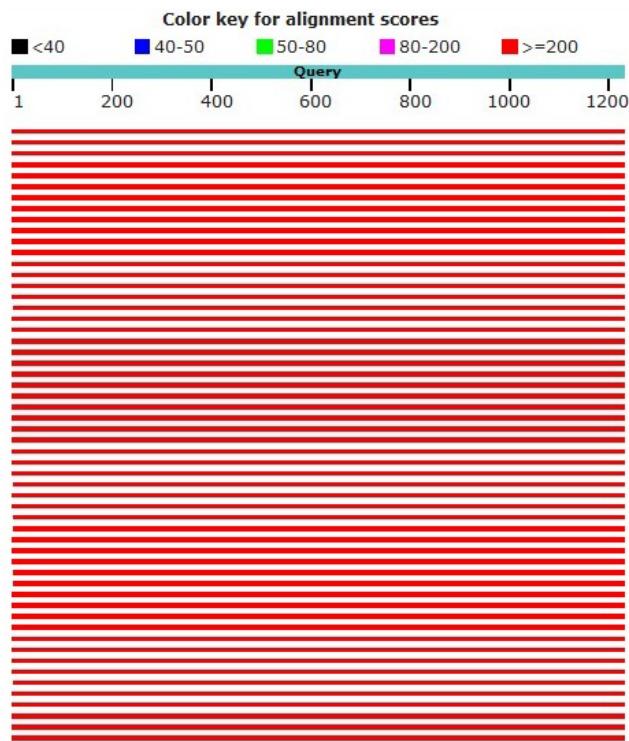
**Figure 1.** Agarose gel (1%) electrophoresis showing amplified 16S rRNA gene of LAB DS18. M, DNA marker; 1, PCR product of LAB DS18.

and is indispensable in maintain life. The 16S rRNA gene sequencing identified the LAB DS15 to belong to the genus *Pediococcus*, forming a well-defined cluster with *Pediococcus acidilactici*. This cluster was recovered in 100% of bootstrap analysis. *Pediococcus* spp. are widely described as probiotics (Porto *et al.*, 2017). Abbasiliasi *et al.* (2012) also found *Pediococcus acidilactici* in fermented milk products. *Pediococcus acidilactici* are important LAB which have been used as starter cultures in meat, vegetable and dairy fermentation causing characteristic flavor changes, improving hygiene and extending the shelf life of these products (Mora *et al.*, 1997; Porto *et al.*, 2017).

A phylogenetic tree (Figure 2) was constructed to determine the kinship relationship of LAB DS15. The phylogenetic tree is known to show a high consistency of the relationships between organisms. In this study, the isolate showed similarity of 99% at 100% query coverage to *Pediococcus acidilactici* strain DSM 20284. A value of 99% indicates that the isolate can be considered as the same species with *Pediococcus acidilactici* strain DSM 20284. The sequence of homology levels was high, as shown by the red color with a score of  $\geq 200$  (Figure 3). From the results of this homology it can be concluded that the two sequences are the same and have an evolutionary relationship.



**Figure 2.** Phylogenetic tree of 16S rRNA gene of LAB DS18 using the neighbor-joining method.



**Figure 3.** Graphic summary of DS18 and *Pediococcus acidilactici* strain DSM 20284.

The next closest species for which a sequence alignment of at least 100% query coverage was observed were *Pediococcus pentosaceus* strain DSM 20336, *Pediococcus acidilactici* strain NGRI 0510Q and *Pediococcus argentini* strain CRL 776 at 98% similarity to the DS15 isolate. *Pediococcus stilesi* strain FAIR-E 180 showed 98% similarity with 99% query coverage. An alignment query result of 100% indicates a significant alignment, which means the search sequence in this study was identical with the identified genus, even at the species level.

### Conclusion

The PCR of 16S rRNA gene sequence gave an approximately 1400 bp amplicon for LAB DS15, isolated from indigenous dadih of West Sumatera. Phylogenetic analysis showed that LAB DS15 was *Pediococcus acidilactici*, with 99% similarity to *Pediococcus acidilactici* strain DSM 20284.

### Data availability

*Pediococcus acidilactici* strain DS32 16S ribosomal RNA gene, partial sequence, obtained during this study. GenBank accession number MH938236: <http://identifiers.org/ncbigi/GI:1481059229>.

### Grant information

This research was supported by Ministry of Research, Technology and Higher Education Republic of Indonesia through Master of Education Towards Doctoral Scholarship Program for Excellence Undergraduate and the support through World Class Professor Program Scheme-B No. 123.57/D2.3/KP/2018.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

### Supplementary material

**Supplementary File 1.** Electrophoresis image of the PCR amplification product.

[Click here to access the data.](#)

## References

Abbasiliasi S, Tan JS, Ibrahim TA, *et al.*: **Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry.** *BMC Microbiol.* 2012; 12: 260. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Ali FWO, Abdulamir AS, Mohammed AS, *et al.*: **Novel, practical and cheap source for isolating beneficial  $\gamma$ -aminobutyric acid-producing leuconostoc NC5 bacteria.** *Res J Med Sci.* 2009; 3(4): 146–153. ISSN: 1993-6095 (Online). [Reference Source](#)

Collado CM, Surono IS, Meriliuto J, *et al.*: **Potential probiotic characteristics of *Lactobacillus* and *Enterococcus* strains isolated from traditional dadih fermented milk against pathogen intestinal colonization.** *J Food Prot.* 2007; 70(3): 700–705. [PubMed Abstract](#) | [Publisher Full Text](#)

Dhakal R, Baijal VK, Baeck KH: **Production of gaba ( $\gamma$ -Aminobutyric acid) by microorganisms: a review.** *Braz J Microbiol.* 2012; 43(4): 1230–41. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Felsenstein J: **Confidence Limits On Phylogenies: An Approach Using The Bootstrap.** *Evolution.* 1985; 39(4): 783–791. [PubMed Abstract](#) | [Publisher Full Text](#)

Henry DE, Halami PM, Prapulla SG: ***Lactobacillus plantarum* mccc2034, a novel isolate from traditional Indian lactic fermented preparation: molecular identification and evaluation of its *in vitro* probiotic potential.** *J Microbiol Biotechnol Food Sci.* 2015; 4(4): 328–331. [Publisher Full Text](#)

Kumar S, Stecher G, Tamura K: **MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.** *Mol Biol Evol.* 2016; 33(7): 1870–1874. [PubMed Abstract](#) | [Publisher Full Text](#)

Malik V, Devi U, Yadav RNS, *et al.*: **16s rRNA based phylogenetic analysis of *lactobacillus plantarum* isolated from various fermented food products of Assam.** *J Microbiol Biotechnol Food Sci.* 2015; 5(1): 20–22. [Publisher Full Text](#)

Mora D, Fortina MG, Parini C, *et al.*: **Identification of *Pediococcus acidilactici* and *Pediococcus pentosaceus* based on 16s rRNA and *ldhD* gene-targeted multiplex PCR analysis.** *FEMS Microbiol Lett.* 1997; 151(2): 231–236. [PubMed Abstract](#) | [Publisher Full Text](#)

Murray RK, Granner DK, Rodwell VW, *et al.*: **Harper's Illustrated Biochemistry 23<sup>rd</sup> edn.** McGraw-Hill Companies, Inc. USA. 2003.

Mustafa H, Rachmawati I, Udin Y: **Genomic DNA concentration and purity measurement of *Anopheles barbirostris*.** *Journal of Disease Vector.* 2016; 1: 7–10. E-ISSN 2354-8835. [Publisher Full Text](#)

Nonaka S, Arai C, Takayama M, *et al.*: **Efficient increase of  $\gamma$ -aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis.** *Sci Rep.* 2017; 7(1): 7057. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Olsen RW, Li GD: **Gaba.** In Brady ST, Siegel GJ, Albers LW, Price DL, (Editors). *Basic Neurochemistry (Eight edition): Principles of molecular, cellular, and medical neurobiology.* 2012; 367–376. [Publisher Full Text](#)

Porto MC, Kuniyoshi TM, Azevedo PO, *et al.*: ***Pediococcus* spp.: An important genus of lactic acid bacteria and pediocin producers.** *Biotechnol Adv.* 2017; 35(3): 361–374. [PubMed Abstract](#) | [Publisher Full Text](#)

Rizqiyati H, Sumantri C, Noor RR, *et al.*: **Isolation and identification of indigenous lactic acid bacteria from North Sumatra river buffalo milk.** *Indonesian Journal of Animal and Veterinary Sciences.* 2015; 20(2): 87–94. [Publisher Full Text](#)

Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol.* 1987; 4(4): 406–425. [PubMed Abstract](#) | [Publisher Full Text](#)

Seo MJ, Lee JY, Nam YD, *et al.*: **Production of  $\gamma$ -Aminobutyric Acid by *Lactobacillus brevis* 340G Isolated from Kimchi and Its Application to Skim Milk.** *Food Eng Prog.* 2013; 17(4): 418–423. [Publisher Full Text](#)

Surono IS: ***In vitro* probiotic properties of indigenous dadih lactic acid bacteria.** *Asian-Australas J Anim Sci.* 2003; 16(5): 726–731. [Publisher Full Text](#)

Surono IS: **Traditional Indonesian dairy foods.** *Asia Pac J Clin Nutr.* 2015; 24 Suppl 1: S26–S30. [PubMed Abstract](#) | [Publisher Full Text](#)

Willey JM, Sherwood LM, Woolverton CJ: **Prescott's Principles of Microbiology.** Boston: McGraw-Hill Higher Education. 2009; ISBN 9780073375236. [Reference Source](#)

Wirawati CU, Sudarwanto MB, Lukman DW, *et al.*: **Characteristic and development of cow's milk dadih as an alternate of buffalo's milk dadih.** *WARTAZOA Indonesian Bulletin of Animal and Veterinary Sciences.* 2017; 27(2): 95–103. [Publisher Full Text](#)

Wu Q, Shah NP: **High  $\gamma$ -aminobutyric acid production from lactic acid bacteria: Emphasis on *Lactobacillus brevis* as a functional dairy starter.** *Crit Rev Food Sci Nutr.* 2017; 57(17): 3661–3672. [PubMed Abstract](#) | [Publisher Full Text](#)

Yamada Y, Endou M, Morikawa S, *et al.*: **Lactic Acid Bacteria Isolated from Japanese Fermented Fish (Funazushi) Inhibit Mesangial Proliferative Glomerulonephritis by Alcohol Intake with Stress.** *J Nutr Metab.* 2018; 2018: 6491907. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Zhao A, Hu X, Wang X: **Metabolic engineering of *Escherichia coli* to produce gamma-aminobutyric acid using xylose.** *Appl Microbiol Biotechnol.* 2017; 101(9): 3587–3603. [PubMed Abstract](#) | [Publisher Full Text](#)

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact [research@f1000.com](mailto:research@f1000.com)

**F1000Research**