RESEARCH ARTICLE

REVISED Molecular identification and phylogenetic analysis of GABA-producing lactic acid bacteria isolated from indigenous dadih of West Sumatera, Indonesia [version 3; peer review: 2 approved, 1 approved with reservations, 1 not approved]

Lili Anggraini [1], Yetti Marlida[2], Wizna Wizna[2], Jamsari Jamsari [3], Mirzah Mirzah[2], Frederick Adzitey[4], Nurul Huda [5,6]

[1]Graduate Program, Andalas University, Padang, West Sumatera, Indonesia
[2]Department of Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia
[3]Department of Plant Breeding, Faculty of Agriculture, Andalas University, Padang, West Sumatera, Indonesia
[4]Department of Veterinary Science, University for Development Studies, Temale, Ghana
[5]School of Food Industry, Universiti Sultan Zainal Abidin, Kuala Nerus, Terengganu, 21300, Malaysia
[6]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, 88400, Malaysia

v3 **First published:** 19 Oct 2018, **7**:1663 (https://doi.org/10.12688/f1000research.16224.1)
Second version: 06 Feb 2019, **7**:1663 (https://doi.org/10.12688/f1000research.16224.2)
Latest published: 17 Oct 2019, **7**:1663 (https://doi.org/10.12688/f1000research.16224.3)

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 γ-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**	**3**	**4**
version 3 (revision) 17 Oct 2019	✗ report			✓ report
version 2 (revision) 06 Feb 2019	✗ report	? report	✓ report	
version 1 19 Oct 2018				

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

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

3 **Sahilah Abd Mutalib**, Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia

4 **Usman Pato**, Riau University, Pekanbaru, Indonesia

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)

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.

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 3; peer review: 2 approved, 1 approved with reservations, 1 not approved]** F1000Research 2019, **7**:1663 (https://doi.org/10.12688/f1000research.16224.3)

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

REVISED **Amendments from Version 2**

Additional information on the reference of forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387R (5'-GGG CGG GGT GTA CAA GGC-3'). Updating the sentence of 1% agarose electrophoresis to 1 % (w/v) agarose electrophoresis. Mentioning the marker used 1 Kb Plus DNA ladder (ThermoFisher Scientific).

Any further responses from the reviewers can be found at the end of the article

Introduction

The non-proteinogenic amino acid γ-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 (Rizqiati *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 16 S 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 1387R (5'-GGG CGG GGT GTA CAA GGC-3'). of Laboratory of Medical Molecular Biology and Diagnostic, Indonesian Institute of Sciences. The reaction was carried out in a volume of 50 µl. The PCR mixture contained 22 µl of MQ, 25 µl DreamTaq Green DNA Polymerase (Thermo Fisher Scientific, USA), 1 µl of each forward and reverse primer (10 µM each, IDT synthesized) and 1 µl 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% (w/v) agarose electrophoresis in 1x TAE, 100 V for 30 minutes. The DNA bands formed from electrophoresis process was visualized using UV transluminator. The marker used was 1 Kb Plus DNA ladder (ThermoFisher Scientific).

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 SegMan 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.

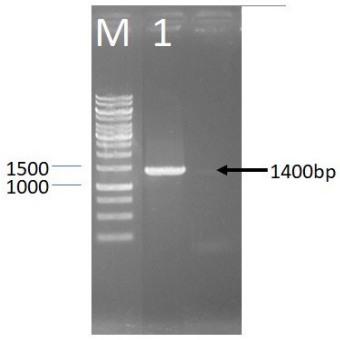

Figure 1. Agarose gel (1%) electrophoresis showing amplified 16S rRNA gene of LAB DS18. M, DNA marker; 1, PCR product of LAB DS18.

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 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 ≥200 (Figure 3).

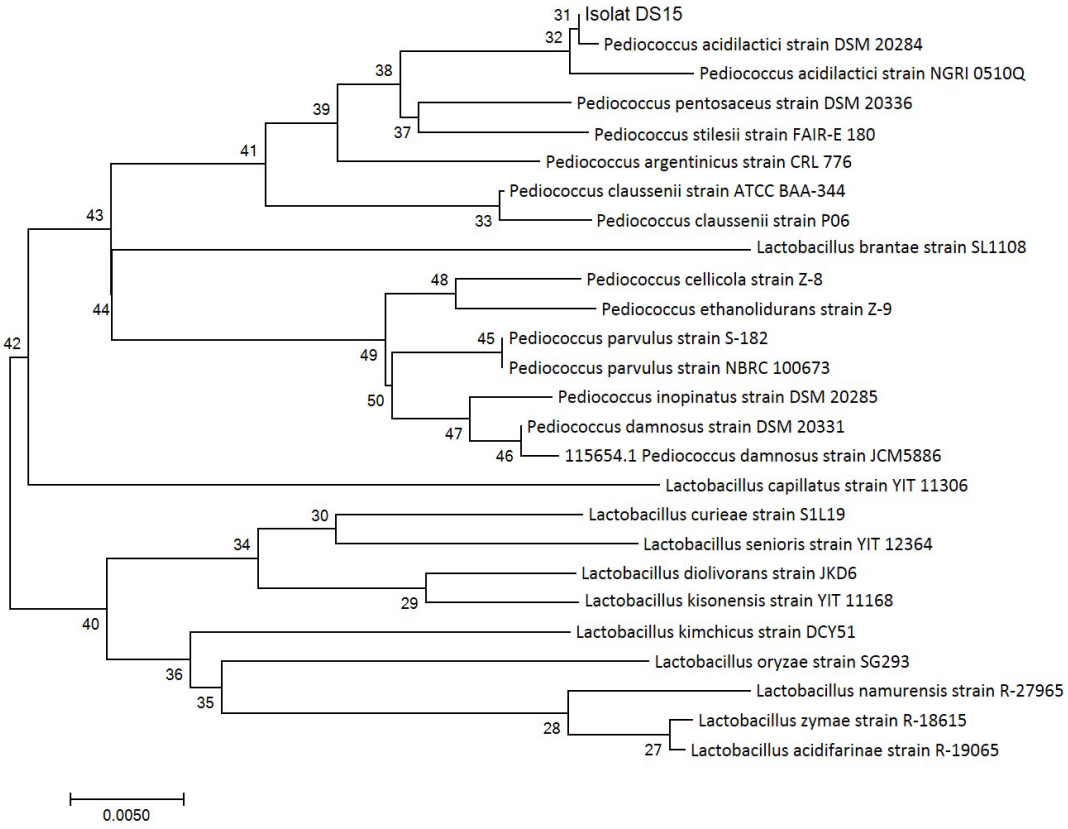

Figure 2. Phylogenetic tree of 16S rRNA gene of LAB DS18 using the neighbor-joining method.

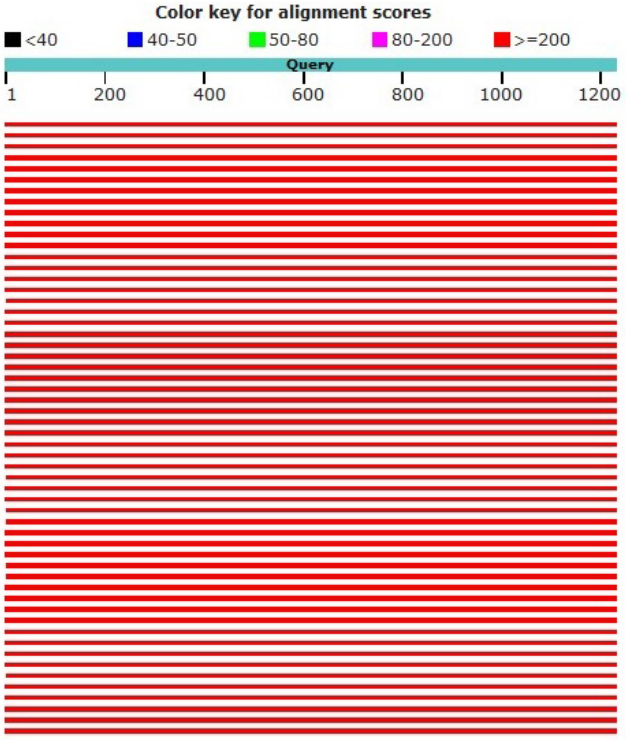

Figure 3. Graphic summary of DS18 and *Pediococcus acidilactici* strain DSM 20284.

From the results of this homology it can be concluded that the two sequences are the same and have an evolutionary relationship.

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 γ-aminobutyric acid-producing *leuconostoc* NC5 bacteria.** *Res J Med Sci.* 2009; **3**(4): 146–153.
Reference Source

Collado CM, Surono IS, Meriluoto 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, Baipai VK, Baek KH: **Production of gaba (γ - 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* mcc2034, 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 IdhD 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 23th 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.
Publisher Full Text

Nonaka S, Arai C, Takayama M, *et al.*: **Efficient increase of γ-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

Rizqiati 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 γ-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.
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 γ-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 (Funa-Sushi) 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

Open Peer Review

Current Peer Review Status: ✗ ? ✓ ✓

Version 3

Reviewer Report 12 March 2020

https://doi.org/10.5256/f1000research.22366.r56703

✓ **Usman Pato**
Faculty of Agriculture, Riau University, Pekanbaru, Indonesia

INTRODUCTION

1. In general, the introduction is relatively good but needs to be added by the results of research from Hosono et al 1989[1] and Wirawati et al., 2019[2] about micflora in dadih

2. In the introduction, the author needs to explain in more detail the role of GABA produced by LAB and other organisms

METHODS

1. An explanation should be added as to why only to choose the DS15 strain producing GABA in this study.

2. It is necessary to add the reference methods used in the 16S rRNA gene amplification analysis and electrophoresis process

RESULTS AND DISCUSSION

The results are well presented and discussed systematically because the authors used only one strain.

REFERENCES

The author needs to add references as a follow-up to suggestions for improvement in the introduction and method of this paper

The strength of this paper

The strength of study is the first research to report on GABA-producing LAB from dadih and local fermented milk products from Indonesia

The weakness of this paper

One of the weaknesses of this study is that the authors only used one LAB dadih isolate (strain DS15) so that no comparative data were produced and the discussion was relatively limited.

References

1. Hosono A, Wardojo R, Otani H: Microbial flora in dadih a traditional fermented milk in indonesia. *Life,*

Earth.
2. Wirawati CU, Sudarwanto MB, Lukman DW, Wientarsih I, et al.: Diversity of lactic acid bacteria in dadih produced by either back-slopping or spontaneous fermentation from two different regions of West Sumatra, Indonesia.*Vet World*. 2019; **12** (6): 823-829 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food Microbiology, Probiotic

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 November 2019

https://doi.org/10.5256/f1000research.22366.r55312

Qinglong Wu
Texas Children's Microbiome Center, Baylor College of Medicine, Houston, TX, USA

I did not see any improvements of scientific value that have been made in the revision.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 2

Reviewer Report 11 June 2019

https://doi.org/10.5256/f1000research.19627.r48482

Sahilah Abd Mutalib
Centre for Biotechnology and Functional Food, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia

1. Introduction - fairly good and can be improved
i. Dadih from Indonesia has- *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Lactococcus* - How did they identify the bacteria? Biochemical tests or using molecular approaches?
ii. Is there any data on dadih from Malaysia as well for comparison.

2. Methods - can be improved

2.1 Sample - Subtopic sample suggested to change - Bacterial strain
The month and year of the bacterium should be mentioned for ex: in June 2009. Why too long to continue the partial sequence 16s rRNA analysis?

2. 2 Isolation of bacterial genomic DNA
We used lysozyme-change to "Twenty(20) mg/ml of lysozyme was used to break down ...."
Please state where did you keep the genomic DNA. Example in -20^{o}C freezer or 4^{o}C refrigerator prior

analysis

2.3 16S rRNA gene amplification
Please state the **reference** after forward and reverse primers is mentioned.

2.4 Electrophoresis
1% change to 1% (w/v)
in 1x change to1x TAE, 100 V
The marker should be mentioned in this section, 1 Kb ladder? What kind of dye did you used? Red dye, syber green, ethidium bromide? State their brand as well

Results and discussion
Good - due to a single strain/isolate was studied, thus, the explanation is straight forward.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food microbiology, Halal Science, biomass degradation (EFB and POME)

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 14 Aug 2019

Nurul Huda, Universiti Malaysia Sabah, Malaysia, Malaysia

1. Introduction - fairly good and can be improved
i. Dadih from Indonesia has- *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Lactococcus* - How did they identify the bacteria? Biochemical tests or using molecular approaches?

They identify bacteria with a molecular approach using the 16SsRNA technique

ii. Is there any data on dadih from Malaysia as well for comparison.

No, we don't have data on dadih from Malaysia.

2. Methods - can be improved

2.1 Sample - Subtopic sample suggested to change - Bacterial strain
The month and year of the bacterium should be mentioned for ex: in June 2009. Why too long to continue the partial sequence 16s rRNA analysis?

Bacterial strains isolated in July 2017.
We did this isolation based on the method of Ali *et al.,* (2009), not isolates from the author.

2. 2 Isolation of bacterial genomic DNA
We used lysozyme-change to "Twenty (20) mg/ml of lysozyme was used to break down ...."
Please state where did you keep the genomic DNA. Example in -20 C freezer or 4 C refrigerator prior Analysis

We keep the genomic DNA in 4^{o}C refrigerator.

2.3 16S rRNA gene amplification
Please state the **reference** after forward and reverse primers is mentioned.

We got reference for forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387R (5'-GGG CGG GGT GTA CAA GGC-3') from Laboratory of Medical Molecular Biology and Diagnostic, Indonesian Institute of Sciences, Jakarta, Indonesia.

2.4 Electrophoresis
1% change to 1% (w/v)
Ok we will change
in 1x change to1x TAE, 100 V
Ok we will change
The marker should be mentioned in this section, 1 Kb ladder? What kind of dye did you used? Red dye, syber green, ethidium bromide? State their brand as well
We used 1 Kb Plus DNA Ladder (ThermoFisher Scientific)

Results and discussion
Good - due to a single strain/isolate was studied, thus, the explanation is straight forward.
Thank You.

Competing Interests: No competing interests were disclosed.

Reviewer Report 08 April 2019

https://doi.org/10.5256/f1000research.19627.r46293

?

Jagadish Mahanta
Regional Medical Research Centre, Indian Council of Medical Research (ICMR), Dibrugarh, Assam, India

Authors wanted to identify and characterize GABA producing LAB isolated from “Dadih”.

1. However, authors have taken a strain already isolated and identified in 2009. Authors have not mentioned anything about the gap in the previous research that necessitated undertaking the present exercise. Authors may clarify the issue.
2. Authors have done elaborate molecular testing and phylogenetic analysis of the bacteria taken from the stock. Authors should elaborate the achievement of this exercise.
3. As emphasized by the authors, they should elaborate, about characterization and GABA production potential of the strain
4. Authors should elaborate on the novelty of the study.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Apr 2019

Nurul Huda, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

Authors wanted to identify and characterize GABA producing LAB isolated from “Dadih”.

1. However, authors have taken a strain already isolated and identified in 2009. Authors have not

mentioned anything about the gap in the previous research that necessitated undertaking the present exercise. Authors may clarify the issue.

Exploration of isolates from dadih has been carried out, but no studies have used these isolates as GABA producers. In this study we obtained DS15 isolate from the isolation of various fermented foods which had the highest GABA production. We did this isolation based on the method of Ali *et al.,* (2019), not isolates from the author.

2. Authors have done elaborate molecular testing and phylogenetic analysis of the bacteria taken from the stock. Authors should elaborate the achievement of this exercise.

The result of BLAST at the NCBI GenBank site from the sequences showed that DS15 isolate were *Pediococcus acidilactici*. Based on the phylogenetic tree, DS15 has a 99% similarity or homology with *P. acidilactici* DSM 20284, with the difference of one base pair. The next closest species were *P. acidilactici* NGRI 0510Q, *P. pentosaceus* DSM 20336 and *P. argentinicus* CRL 776 with 98% similarity. *P. stilesi* FAIR-E 180 shows 98% similarity with 99% query coverage.

3. As emphasized by the authors, they should elaborate, about characterization and GABA production potential of the strain.

We have carried out quantitative screening on some of the isolates we obtained from dadih, and we found that DS15 isolates produced the highest amount of GABA. Data and discussion are used in another publications.

4. Authors should elaborate on the novelty of the study.

The novelty of this study was the use of bacterial isolates from dadih as a GABA producer.

Competing Interests: No competing interests were disclosed.

Reviewer Report 01 April 2019

https://doi.org/10.5256/f1000research.19627.r46425

Qinglong Wu
Texas Children's Microbiome Center, Baylor College of Medicine, Houston, TX, USA

The authors detailed the 16S rRNA gene-based to be a good lab protocol without demonstrating any scientific value. There is no experimental data to support the GABA production from isolate DS15. They have to present the GABA data in terms of GABA yield under defined fermentation conditions. Meanwhile, they have to demonstrate the pathway in isolate DS15 that is responsible for GABA biosynthesis and GABA export in this strain.

Secondly, the authors just use one isolate to achieve the claim "GABA producing LAB isolated from indigenous dadih was *Pediococcus acidilactici*". This is not a rigorous way.

Here are my questions:

1. What is the level of GABA in dadih?
2. How is GABA production capacity of DS15?
3. There is no massive bacterial isolation from dadih; neither no microbial community profiling for dadih, nor pathway identification of GABA production for microbial community of dadih; so one isolate from dadih does not mean anything.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food microbiology, microbiome science, microbial genomics, functional genomics, microbial GABA biosynthesis, biochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 10 Apr 2019

Nurul Huda, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

1. What is the level of GABA in dadih?

We don't count the amount of GABA on dadih. We did not count the amount of GABA produced in dadih. GABA is produced by lactic acid bacteria of dadih origin but not the dadih, so we don't count or determine GABA level of dadih

1. How is GABA production capacity of DS15?

GABA production capacity of DS15 was 49.365 mg/L

1. There is no massive bacterial isolation from dadih; neither no microbial community profiling for dadih, nor pathway identification of GABA production for microbial community of dadih; so, one isolate from dadih does not mean anything.

In this study, we isolated bacteria from various fermented food products (dadih, ikan budu, asam durian and tape singkong), determined their GABA producing ability and selected the isolate with the highest GABA production for further identification. The results of isolation and characterization are explained in other articles. The distribution of LAB isolates from the indigenous West Sumatera fermented food (dadih only) is;

1. Origin from Aiadingin area. Number of isolate 131; Number of LAB isolate 125; Number of GABA producing LAB isolate 23.
2. Origin from Sijunjung area. Number of isolate 166; Number of LAB isolate 93; Number of GABA producing LAB isolate 19.
3. Origin from Solok area. Number of isolate 100; Number of LAB isolate 96; Number of GABA producing LAB isolate 19.

In total, from 3 areas, number of isolate 397; number of LAB isolate 314; number of GABA producing LAB isolate 62.

Competing Interests: No competing interests were disclosed.