



UNVEILING THE GENETIC BASIS OF BACTERIOICIN PRODUCTION FROM *Enterococcus faecium* ATCC 19434

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ABSTRACT

The prevalence of resistance to traditional antimicrobials is increasing rapidly, leading to a significant rise in annual deaths from antibiotic-resistant illnesses. In 2020, the global death toll from such diseases was approximately 500,000. If current trends continue, this figure could exceed 10 million by 2050, with associated economic damages surpassing 100 trillion USD. These alarming statistics highlight the urgent need for alternative antimicrobials. Bacteriocins are considered among the most promising options. *Enterococcus faecium* ATCC 19434 is known to produce a potent bacteriocin effective against *Listeria monocytogenes* and *Staphylococcus aureus*, though its specific bacteriocin and genetic properties have not been fully explored. This study aimed to identify the gene responsible for bacteriocin production in *Enterococcus faecium* ATCC 19434. DNA was extracted from the bacterium, and polymerase chain reaction (PCR) was conducted. The PCR products were analyzed through agarose gel electrophoresis, sequenced, and subjected to homology searches using the BLASTN and BLASTX tools from the National Center for Biotechnology Information (NCBI). Results revealed that *Enterococcus faecium* ATCC 19434 contains a gene encoding enterocin B. Additionally, it was found to harbor the gene for enterocin A immunity protein production. This discovery paves the way for future genetic modification of *Enterococcus faecium* ATCC 19434 to enhance enterocin B synthesis, facilitating its commercial application.

Keywords: Bacteriocin, Enterocin B, Enterocin A immunity protein, Genetic characterization, *Enterococcus faecium*

INTRODUCTION

In the pre-antimicrobial period, infectious diseases accounted for a whopping one-third of global annual deaths. The discovery of antimicrobial agents was a massive breakthrough which engendered significant drop in global mortality due to infectious diseases. Antimicrobials are not only used in treatment of infections but are also used in prevention of post-operative infections (Hocking et al., 2021).

However, overuse and misuse of conventional antimicrobials fostered development of resistance by pathogens. Within the last 20 years, avalanche of reports on the rise of antibiotic resistance in different parts of the world have been made (Crysler & Streu, 2022; Kaczor et al., 2021). Moreover, some pathogens have developed multidrug resistance which exacerbates the conundrum (Jernigan et al., 2020). Consequently, infectious diseases that were once treatable have become difficult to treat. As of 2020, global annual deaths due to antimicrobial-resistant diseases stands at 500 000. Based on the current trend, this number is expected to rise to over 10 million by 2050 (Hasan et al., 2020). If urgent action is not taken, infectious diseases will have the health impact it had during the pre-antimicrobial era. More so, by 2050 over 100 trillion USD would be lost due to antimicrobial resistant infections (Denku et al., 2022). Against this backdrop, the world health organization craves the indulgence of researchers in developing novel antimicrobials.

Bacteriocins are globally considered plausible alternatives to conventional antimicrobials (Simons et al., 2020). These ribosomally synthesized peptides are generally regarded as safe and act against their microbial targets using novel mechanisms that evade development of resistance. Additionally, their thermal, pH and chemical stability favor

their deployment as replacement for conventional antimicrobials (Wayah et al., 2022; Wayah & Philip, 2018). Bacteriocins are widely divided into two classes: class I (which are post-translationally modified to form lanthionine or methyl lanthionine bridges), and class II (which are not subjected post-translational processing). Class II bacteriocins are unmodified, small and thermally-stable peptides. They are further subdivided into various subclasses which include class IIa (pediocin-like bacteriocins), class IIb (comprising of two different peptides, each having reduced activity against their microbial target but when combined, act synergistically against their microbial target), class IIc (cyclic bacteriocins) and class IId (non-pediocin-like, one-peptide bacteriocins) (Cotter et al., 2005). In other classification schemes, large and thermally-sensitive bacteriocins were placed in class III (Alvarez-Sieiro et al., 2016) while bacteriocins with carbohydrate or lipid groups were placed in class IV (Kaškonienė et al., 2017; Klaenhammer, 1993).

Enterococcus faecium is a lactic acid bacterium. Bacteriocin production has been observed among some strains of *Enterococcus faecium* such as *Enterococcus faecium* ATCC 19434 (Javed et al., 2011). This bacteriocin is highly potent against major human pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* suggesting its future application in the treatment of diseases associated with these pathogens. However, the specific type of bacteriocin produced by *Enterococcus faecium* ATCC 19434 has not been investigated. Moreover, the genetic organization of the operon for bacteriocin production has not been ascertained. Consequently, the objective of this investigation was to identify the gene that is responsible for the production of bacteriocin in *Enterococcus faecium* ATCC 19434.

MATERIALS AND METHODS

DNA Extraction

Total DNA was extracted from *Enterococcus faecium* ATCC 19434 using MO BIO microbial DNA isolation kit (Valencia) according to manufactures' instructions. To a 2 ml collection tube 1.8 ml of *Enterococcus faecium* ATCC 19434 culture was added. The tube was centrifuged at 10,000 x g for 30 seconds at room temperature. Thereafter, the supernatant was decanted and the tube was spined at 10,000 x g for 30 seconds at room temperature. A pipette tip was used to completely remove remnant supernatant. The resulting cell pellet was resuspended in 300µl of MicroBead solution and vortexed lightly. The content was transferred to a MicroBead tube and 50 µl of solution MD1 was added. MO BIO vortex adapter tube holder was used to horizontally secure the tubes and maximum vortexing was done for 10 minutes. The resulting supernatant was moved to a clean 2 ml collection tube thereafter, 100 µl of solution MD2 was added, vortexed for 5 seconds and incubated at 4 °C for 5 minutes. Centrifugation of the tube was carried out at 10,000 x g for 1 minute at room temperature and the supernatant was transferred to a clean 2 ml collection tube. To 900 µl of the supernatant, solution MD3 was added and vortexed for 5 seconds afterwards, the spin filter was loaded with 700 µl of the supernatant and centrifugation was performed at 10,000 x g for 30 seconds at room temperature. After discarding the flow-through, the remaining supernatant was added to the spin filter, and centrifuged at 10,000 x g for 30 seconds at room temperature.

Thereafter, 300 µl of solution MD4 was added and centrifuged at 10,000 x g for 30 seconds at room temperature and the flow-through was discarded. The tube was centrifuged at 10,000 x g for 1 minute at room temperature. The spin filter was carefully placed in a new 2 ml collection tube and 50 µl of solution MD5 was added to the center of the white filter membrane. The tube was centrifuged at room temperature for 30 seconds at 10,000 x g thereafter, the spin filter was discarded. The resulting DNA was stored at -40 °C for downstream applications.

PCR Detection of Enterocin Genes in *Enterococcus faecium* ATCC 19434

PCR was carried out using enterocin-specific primers (Table 1). PCR for enterocin GM-1, enterocin A, enterocin B, enterocin P and enterocin L50A and B required an initial denaturation step (95 °C for 5 minutes) followed by 30 cycles of further denaturation at 95 °C for 1 minute, annealing at 56 °C for 1 minute and extension at 72 °C for 1 minute. Afterwards, a final extension at 72 °C for 3 minutes. With respect to enterocin Q, hiracin JM79 and enterocin CRL35, number of cycles and annealing temperature were adjusted to 35 and 60 °C respectively. Gel electrophoresis of PCR products was carried out using 2.5 % agarose. PCR products were sequenced and homology searches was conducted using blast n and blast x of the NCBI database (Kang & Lee, 2005; Saavedra et al., 2004; Sánchez et al., 2008).

Table 1: PCR primers used in this study

Primer	Sequence	Reference
Enterocin GM-1 F	5'-GCTACGCGTTCATATGGTAATGGTG-3'	(Kang & Lee, 2005)
Enterocin GM-1 R	5'-ATGTCCCATACCTGCCAAACCAGAAGC-3'	
Enterocin A F	5'-GAGATTTATCTCCATAATCT-3'	(Kang & Lee, 2005)
Enterocin A R	5'-GTACCACTCATAGTGGAA-3'	
Enterocin B F	5'-GAAAATGATCACAGAATGCCTA-3'	(Kang & Lee, 2005)
Enterocin B R	5'-GTTGCATTTAGAGTATACATTTG-3'	
Enterocin P F	5'-TTATGCGCGTTATTGTGTG-3'	(Kang & Lee, 2005)
Enterocin P R	5'-CATAACTCAAAGTCCCGACC-3'	
Enterocin L50A and B F	5'-TCCCTACAGTCTCCCTTCC-3'	(Kang & Lee, 2005)
Enterocin L50A and B R	5'-TCTAGCGTTAAGCCGAATG-3'	
Enterocin Q F	5'-AACAAAGAAAATTGCGGCTG-3'	(Kang & Lee, 2005)
Enterocin Q R	5'-AGGGCTACTTGGATAGTACAC-3'	
Hiracin JM79 F	5'-TGAATTCAAACTACTTTTTATGACG-3'	(Sánchez et al., 2008)
Hiracin JM79 R	5'-TGGGACTGATGAATCAGAATTG-3'	
Enterocin CRL35 F	5'-GCAAACCGATAAGAATGTGGGAT-3'	(Saavedra et al., 2004)
Enterocin CRL35 R	5'-TATACATTGTCCCCACAACC-3'	

RESULTS AND DISCUSSION

PCR Detection of Enterocin Genes in *Enterococcus faecium* ATCC 19434

Gel electrophoresis revealed clear bands for enterocin A (400 bp) and B (121 bp) (Figure 1). No band was obtained for other

enterocins. Homology searches showed that the PCR product for enterocin A shares 100 % homology with the gene encoding enterocin A immunity protein in a strain of *E. faecium* while that of enterocin B shares 97 % homology with the gene encoding enterocin B.

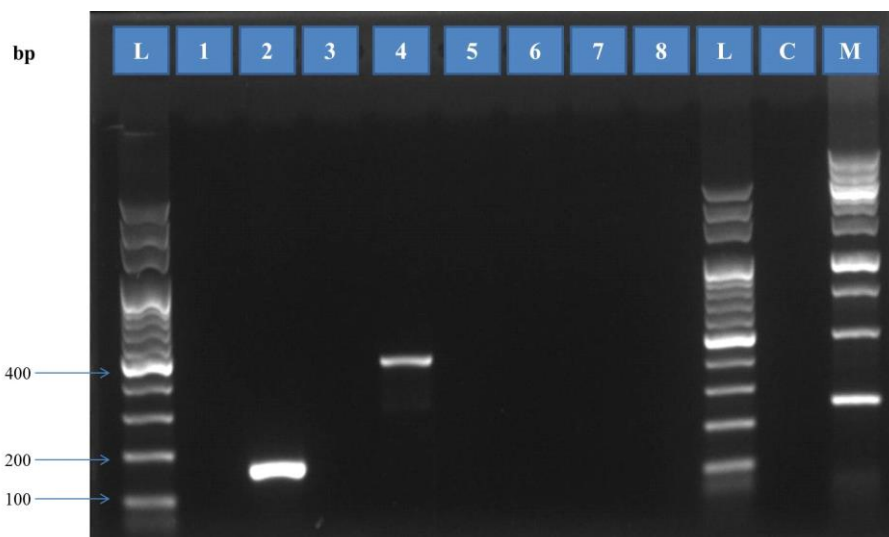


Figure 1: Gel electrophoresis of PCR products from *E. faecium* ATCC 19434. L, 100 bp ladder; 1, enterocin GM-1; 2, enterocin B; 3, enterocin P; 4, enterocin A; 5, enterocin Q; 6, enterocin L50A and B; 7, hiracin JM 79; 8, enterocin CRL35; C, negative control

Nucleotide Sequences of Enterocin A Immunity Protein and Enterocin B

The nucleotide sequences of genes encoding enterocin A immunity protein and enterocin B are shown in Figure 2.

Gene encoding enterocin A immunity protein is made up of 359 nucleotides while gene encoding enterocin B comprises of 122 nucleotides (Figure 2).

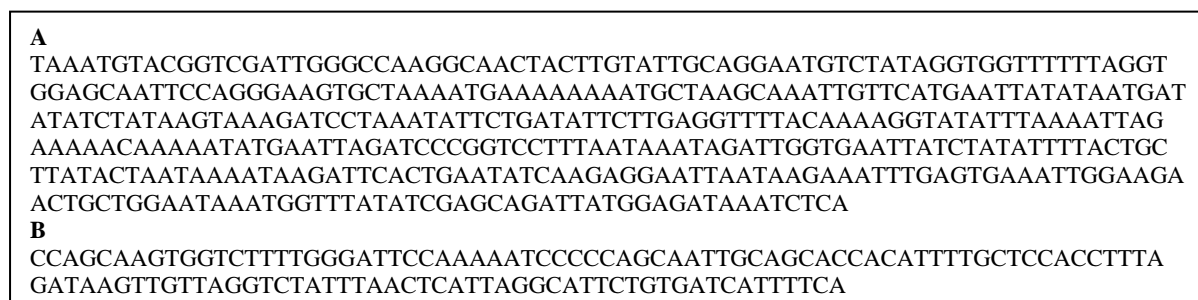


Figure 2: Nucleotide sequences of (A) Enterocin A Immunity Protein and (B) Enterocin B

Amino Acid Sequences of Enterocin A Immunity Protein and Enterocin B

Homology searches (using blastx) of PCR product for enterocin A revealed 100 % homology with enterocin A

immunity protein while PCR product for enterocin B showed 100 % homology with enterocin B (Figure 3).

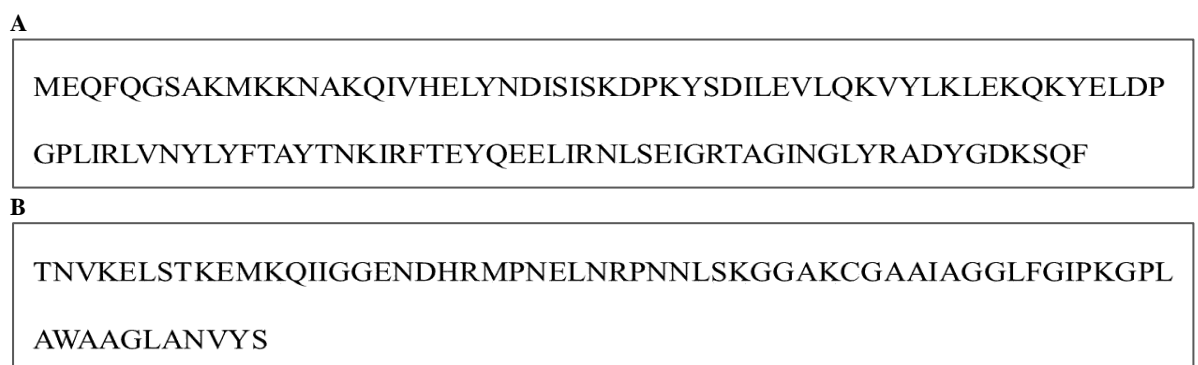


Figure 3: Amino acid sequence of (A) enterocin A immunity protein and (B) enterocin B

Discussion

We reported for the first time that *Enterococcus faecium* ATCC 19434 harbors genes encoding Enterocin B and Enterocin A immunity protein. It has been reported that all enterococci that harbor Enterocin B gene also possess gene

encoding Enterocin A (Vuyst et al., 2003). *Enterococcus faecium* ATCC 19434 is an exception in that it produces Enterocin B without possessing gene encoding Enterocin A. This could be due to mutational changes leading to the loss or inactivation of the gene as revealed by the presence of gene

encoding Enterocin A immunity protein in *Enterococcus faecium* ATCC 19434 (Figure 2 and 3). The presence of gene encoding Enterocin A immunity protein suggests its lack of susceptibility to Enterocin A. *Enterococcus faecium* is an important component of the gastrointestinal tract of humans. It has also been isolated from various foods (Goh & Philip, 2015; Kang & Lee, 2005; Vuyst et al., 2003). They are mainly known for their ability to inhibit the growth of pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (Abdel-Hamid et al., 2018; Franz et al., 2024). This finding suggests the potential future application of *Enterococcus faecium* ATCC 19434 in the treatment of diseases associated with *Listeria monocytogenes* and *Staphylococcus aureus*.

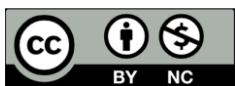
CONCLUSION

The escalating prevalence of antimicrobial resistance presents a dire global health crisis, with current trends suggesting a dramatic rise in annual deaths and substantial economic repercussions by 2050. Our study underscores the critical necessity for novel antimicrobial agents, spotlighting bacteriocins as a promising solution. Through comprehensive genetic analysis, we identified the gene encoding enterocin B in *Enterococcus faecium* ATCC 19434, a bacteriocin with potent activity against significant pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. Additionally, the presence of the enterocin A immunity protein gene was confirmed, which provides further insight into the bacteriocin production and immunity mechanisms of this strain. These findings offer a foundational step toward the genetic enhancement of *Enterococcus faecium* ATCC 19434, aiming to boost enterocin B production. Such advancements could significantly contribute to the development of alternative antimicrobial therapies, addressing the urgent need to combat antibiotic-resistant infections. Future research should focus on the genetic modification techniques to optimize enterocin B synthesis, exploring its potential for large-scale commercial applications and its efficacy in clinical settings. This approach not only promises to mitigate the impending health crisis but also aligns with the global endeavor to find sustainable and effective antimicrobial strategies.

REFERENCES

- Abdel-Hamid, M., Anis, A., Elbawab, R., Mohammed, A., Orabi, S., & Fathalla, S. (2018). Distinctive antagonistic role of new *Enterococcus faecium* ER-3M strain and its bacteriocin effect against *Staphylococcus aureus* Pneumonia. *Rendiconti Lincei. Scienze Fisiche e Naturali*, *1*(3), 3. <https://doi.org/10.1007/s12210-018-0722-7>
- Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., & Kuipers, O. P. (2016). Bacteriocins of lactic acid bacteria: extending the family. *Applied Microbiology and Biotechnology*, *100*(7), 2939–2951. <https://doi.org/10.1007/S00253-016-7343-9>
- Cotter, P., Hill, C., & Ross, R. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, *3*, 777–788. <https://www.nature.com/articles/nrmicro1273>
- Cryslar, A., & Streu, C. (2022). Directed Evolution of Antibacterial Nanobodies Using Novel Antigen Production Strategies. *The FASEB Journal*, *36*(S1). <https://doi.org/10.1096/FASEBJ.2022.36.S1.0R324>
- Denku, C., Ambelu, A., & Mitike, G. (2022). Enteric bacterial pathogens and their antibiotic-resistant patterns from the environmental sources in different regions of Ethiopia: A laboratory-based cross-sectional. *Wiley Online Library* *CY Denku, A Ambelu, G Mitike Health Science Reports, 2022*•Wiley Online Library, 5(2). <https://doi.org/10.1002/hsr2.521>
- Franz, C., Pot, B., Vizoso-Pinto, M., Arini, A., Coppolecchia, R., & Holzapfel, W. (2024). An update on the taxonomy and functional properties of the probiotic *Enterococcus faecium* SF68. *Beneficial Microbes*, *15*, 211–225. https://brill.com/view/journals/bm/15/2/article-p211_7.xml
- Goh, H., & Philip, K. (2015). Isolation and mode of action of bacteriocin BacC1 produced by nonpathogenic *Enterococcus faecium* C1. *Journal of Dairy Science*, *98*(8), 5080–5090. <https://www.sciencedirect.com/science/article/pii/S0022030215003483>
- Hasan, M. R., Sundaram, M. S., Sundararaju, S., Tsui, K. M., Karim, M. Y., Roscoe, D., Imam, O., Janahi, M. A., Thomas, E., Dobson, S., Tan, R., Tang, P., & Lopez, A. P. (2020). Unusual accumulation of a wide array of antimicrobial resistance mechanisms in a patient with cytomegalovirus-associated hemophagocytic lymphohistiocytosis: A case report. *BMC Infectious Diseases*, *20*(1). <https://doi.org/10.1186/S12879-020-04966-Z>
- Hocking, L., Ali, G., d'Angelo, C., Deshpande, A., Stevenson, C., Virdee, M., & Guthrie, S. (2021). A rapid evidence assessment exploring whether antimicrobial resistance complicates non-infectious health conditions and healthcare services, 2010–20. *JAC-Antimicrobial Resistance*, *3*(4). <https://academic.oup.com/jacamr/article-abstract/3/4/dlab171/6428492>
- Javed, A., Masud, T., Ul Ain, Q., Imran, M., & Maqsood, S. (2011). Enterocins of *Enterococcus faecium*, emerging natural food preservatives. *Annals of Microbiology*, *61*(4), 699–708. <https://doi.org/10.1007/S13213-011-0223-8>
- Jernigan, J. A., Hatfield, K. M., Wolford, H., Nelson, R. E., Olubajo, B., Reddy, S. C., McCarthy, N., Paul, P., McDonald, L. C., Kallen, A., Fiore, A., Craig, M., & Baggs, J. (2020). Multidrug-Resistant Bacterial Infections in U.S. Hospitalized Patients, 2012–2017. *New England Journal of Medicine*, *382*(14), 1309–1319. <https://doi.org/10.1056/NEJMOA1914433>
- Kaczor, A., Witek, K., Podlewska, S., Sinou, V., Czekajewska, J., Źesławska, E., Doroz-Płonka, A., Lubelska, A., Latacz, G., Nitek, W., Bischoff, M., Alibert, S., Pagès, J., Jacob, C., Karczewska, E., Bolla, J., & Handzlik, J. (2021). Molecular insights into an antibiotic enhancer action of new morpholine-containing 5-arylideneimidazolones in the fight against MDR bacteria. *International Journal of Molecular Sciences*, *22*(4). <https://www.mdpi.com/1422-0067/22/4/2062>
- Kang, J., & Lee, M. (2005). Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. *Journal of Applied Microbiology*, *98*(5), 1169–1176. <https://doi.org/10.1111/j.1365-2672.2005.02556.x>
- Kaškonienė, V., Stankevičius, M., Bimbraitė-Survilienė, K., Naujokaitytė, G., Šernienė, L., Mulkytė, K., Malakauskas, M., & Maruška, A. (2017). Current state of purification, isolation and analysis of bacteriocins produced by lactic acid

- bacteria. *Applied Microbiology and Biotechnology*, 101(4), 1323–1335. <https://doi.org/10.1007/S00253-017-8088-9>
- Klaenhammer, T. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews*, 12, 168–6445. <https://doi.org/10.1111/j.1574-6976.1993.tb00012.x>
- Saavedra, L., Minahk, C., De Ruiz Holgado, A. P., & Sesma, F. (2004). Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. *Antimicrobial Agents and Chemotherapy*, 48(7), 2778–2781. <https://doi.org/10.1128/AAC.48.7.2778-2781.2004>
- Sánchez, J., Borrero, J., Gómez-Sala, B., Basanta, A., Herranz, C., Cintas, L. M., & Hernández, P. E. (2008). Cloning and heterologous production of hiraicin JM79, a Sec-dependent bacteriocin produced by *Enterococcus hirae* DCH5, in lactic acid bacteria and *Pichia pastoris*. *Applied and Environmental Microbiology*, 74(8), 2471–2479. <https://doi.org/10.1128/AEM.02559-07>
- Simons, A., Alhanout, K., & Duval, R. (2020). Bacteriocins, antimicrobial peptides from bacterial origin: Overview of their biology and their impact against multidrug-resistant bacteria. *Microorganisms*, 8(5). <https://www.mdpi.com/2076-2607/8/5/639>
- Vuyst, L. De, Moreno, M., & Revets, H. (2003). Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. *International Journal of Food Microbiology*, 84(3), 299–318. <https://www.sciencedirect.com/science/article/pii/S0168160502004257>
- Wayah, S. B., & Philip, K. (2018). Characterization, yield optimization, scale up and biopreservative potential of fermencin SA715, a novel bacteriocin from *Lactobacillus fermentum* GA715 of goat milk origin. *Microbial Cell Factories*, 17(1). <https://doi.org/10.1186/S12934-018-0972-1>
- Wayah, S. B., Philip, K., Auta, R., Waziri, P. M., & Yahaya, G. (2022). Salivaricin mmaye1 Production is Enhanced in a New Medium and Acts Synergistically with Pentocin MQ1. *FUDMA Journal of Sciences*, 6(3), 214–221. <https://doi.org/10.33003/fjs-2022-0603-983>



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