Edubiotik : Jurnal Pendidikan, Biologi dan Terapan
ISSN 2528-679X (print), ISSN 2597-9833 (online)
Vol. 10, No. 01, May 2025, pp. 110 – 116

Available online at:
https://ejurnal.uibu.ac.id/index.php/edubiotik

Research Article

OPEN ACCESS

Potential candidate probiotic *Bacillus amyloliquefaciens* strain NO10 SA8 in cholesterol assimilation and amino acid production

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Article Information

Article History:
Submitted: 2025-01-30
Revision: 2025-05-23
Accepted: 2025-06-30
Published: 2025-07-07

Keywords:
Amino acids production; cholesterol assimilation; probiotic.

ABSTRACT

Probiotics are known as beneficial microorganisms that, when administered in appropriate amounts, can provide health benefits to their host. The ability of probiotics to produce amino acids is crucial to consider, as amino acids are essential components for the growth and development of organisms. The objective of this study is to analyze the ability of *Bacillus amyloliquefaciens* strain NO10 SA8 to assimilate cholesterol and its capability to produce amino acids. This study employed a descriptive method, including cholesterol assimilation and analysis of amino acid types produced by the potential probiotic bacterium *Bacillus amyloliquefaciens* strain NO10 SA8. The results of this study showed that *Bacillus amyloliquefaciens* strain NO10 SA8 ability demonstrated a relatively high cholesterol assimilation capacity—approximately 69.75% compared to the positive control. This indicates that the strain has potential to be used as a cholesterol-lowering agent in functional applications, such as probiotics or dietary supplements.it was capable of assimilating cholesterol. Furthermore, it was able to produce various amino acids, except for *L-Histidine, L-Tyrosine, and L-Tryptophan*. The conclusion of this study is that *Bacillus amyloliquefaciens* strain NO10 SA8 has potential as a probiotic strain because it demonstrated the ability to assimilate cholesterol 69.75% compared to the positive control, and producing amino acids.

Publisher
Biology Education Department
Universitas Insan Budi Utomo, Malang, Indonesia

How to Cite
Sukmawati, Hardianti , F., Amalia, F., & Kaway, S. (2025). Potential candidate probiotic Bacillus amyloliquefaciens strain NO10 SA8 in cholesterol assimilation and amino acid production. Edubiotik : Jurnal Pendidikan, Biologi Dan Terapan, 10(01), 110–116. https://doi.org/10.33503/ebio.v10i01.1038

INTRODUCTION

Aquaculture has rapidly emerged as one of the fastest-growing sectors in the global food industry, playing a vital role in fulfilling the increasing demand for animal protein worldwide. This industry is crucial in providing a sustainable source of high-quality protein, especially as wild fish stocks face overexploitation. However, alongside its rapid expansion, aquaculture faces significant challenges, particularly in maintaining

the health of cultured fish and optimizing production efficiency. A major concern is the heavy reliance on antibiotics and premium feed inputs to ensure fish growth and disease control (Telaumbanua et al., 2024). The overuse of antibiotics in aquaculture raises serious issues beyond fish health. Excessive antibiotic application can promote the development of antibiotic-resistant bacteria, posing threats not only to aquatic organisms but also to human health. Additionally, antibiotic residues discharged into the environment may disrupt aquatic ecosystems and biodiversity (Larsson et al., 2022). These concerns highlight the urgent need for safer, environmentally friendly alternatives to support fish health and sustainable aquaculture practices.In this context, probiotics have gained considerable attention as a promising approach. Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits to the host—in this case, the fish (Fijan et al., 2023). The use of probiotics can enhance fish digestive efficiency, boost immune responses, and help maintain a balanced microbial environment within aquaculture systems. Consequently, probiotics represent not only a health-promoting agent but also a strategic tool to improve productivity and sustainability in the aquaculture industry.

Probiotics in aquaculture have been shown to improve fish health, enhance digestion, and increase nutrient absorption, including cholesterol and amino acids (Sionek et al., 2023). Certain probiotic strains have demonstrated the ability to assimilate cholesterol in the host's digestive system (Puri et al., 2022), which can help regulate blood cholesterol levels and reduce excessive fat accumulation in fish (Wongrattanapipat et al., 2023; Agolino et al., 2024). This is particularly important for ensuring long-term fish health and improving meat quality. Some examples of probiotic bacteria include Streptococcus thermophilus, Lactobacillus bulgaricus (Ahmed et al., 2025), *Bacillus* subtilis, Pediococcus pentosaceus BBS1, and Lactiplantibacillus plantarum BBS13 (Botthoulath et al., 2024). Furthermore, the ability of probiotics to produce amino acids is another key aspect to consider. Amino acids are essential for fish growth and development (Liu et al., 2024), especially essential amino acids that cannot be synthesized by fish and must be obtained from their diet. Adequate amino acid intake is crucial for protein synthesis, muscle tissue development, and cellular repair (Xing et al., 2024). Certain probiotic strains have been reported to produce essential amino acids, thereby enhancing the nutritional value of fish and potentially reducing dependence on expensive commercial feeds.

Probiotic bacteria are microorganisms that provide significant health benefits to their host. A screening study of potential probiotic bacteria in the Klawalu mangrove area, Sorong City (Sukmawati et al., 2019), identified three promising bacterial strains: *Bacillus safensis* strain C251 SA3, *Bacillus amyloliquefaciens* strain NO10 SA8, and *Bacillus* australimaris strain IIHR GAPB01 SL1 (Sukmawati et al., 2022). *Bacillus* safensis strain C251 SA3 was catalase-negative, while *Bacillus amyloliquefaciens* strain NO10 SA8 and *Bacillus* australimaris strain IIHR GAPB01 SL1 were catalase-positive. These bacteria also exhibited negative oxidative-fermentative properties and demonstrated nitrate reduction capabilities. Additionally, pathogenicity tests confirmed that none of these strains were pathogenic (Sukmawati & Fahrizal, 2024). Further physiological studies identified *Bacillus amyloliquefaciens* strain NO10 SA8 as the most promising probiotic candidate (Sukmawati et al., 2024). Strain NO10 SA8 is preferred due to its stronger antimicrobial activity, better adaptability and growth, and higher production of beneficial metabolites compared to the other two strains. Based on preliminary findings, further research is needed to explore the ability of *Bacillus amyloliquefaciens* strain NO10 SA8 to assimilate cholesterol and produce amino acids. This capability is important because probiotic bacteria can help regulate blood cholesterol levels, reduce fat accumulation in fish, and produce essential amino acids for growth. The objectives of this study are: (1) to evaluate the cholesterol assimilation ability of *Bacillus amyloliquefaciens* strain NO10 SA8, and (2) to identify the types of amino acids it produces.

RESEARCH METHODS

Research design; the research was designed using a descriptive method, encompassing cholesterol assimilation and the analysis of amino acid types produced by the potential probiotic bacterium *Bacillus amyloliquefaciens* strain NO10 SA8. Cholesterol assimilation assay by *Bacillus amyloliquefaciens* strain NO10 SA8. Preparation of cholesterol-containing medium, pure cholesterol was added to bacto agar medium at a concentration of 200 µg/mL. To ensure cholesterol solubility in the medium, it was first dissolved in ethanol as an organic solvent. The cholesterol-containing medium was sterilized prior to inoculation to prevent contamination. The medium was inoculated with *Bacillus amyloliquefaciens* strain NO10 SA8 and incubated at 30°C for 24 hours under aerobic conditions. The experiment was conducted with three independent replications to ensure the reliability and reproducibility of the results. The number of colonies capable of growing on the cholesterol-containing medium was observed and compared to both the negative and positive controls.

Amino acid analysis test of *Bacillus amyloliquefaciens* strain NO10 SA8; It was conducted by growing the bacterium on MRSB medium, followed by incubation at 29°C for 24 hours. The bacterial culture was then filtered using a Millipore filter with a 0.22 µm diameter. The filtered sample was subsequently analyzed for amino acids (test of 18 amino acids; L-Alanine, L-Arginine, L-Aspartic Acid, Glycine, L-Glutamic Acid, L-Histidine, L-Isoleucine, L-Cystine, L-Leucine, L-Lysine, L-Methionine, L-Tryptophan, L-Valine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine) using UPLC-PDA methods (AOAC, 2005; Weber et al., 2022), for amino acids (test of L-Cystine and L-Methionine) using LC-MS/MS methods (Lassen, 2018).

FINDING AND DISCUSSION

The following figure illustrates the results of cholesterol assimilation tests conducted on *Bacillus amyloliquefaciens* strain NO10 SA8. The figure compares the performance of the test strain (*Bacillus amyloliquefaciens* strain NO10 SA8) with the negative control (*E. coli*) and the positive control (*Bacillus* sp.), highlighting the ability of *Bacillus amyloliquefaciens* strain NO10 SA8 to assimilate cholesterol (Figure 1).

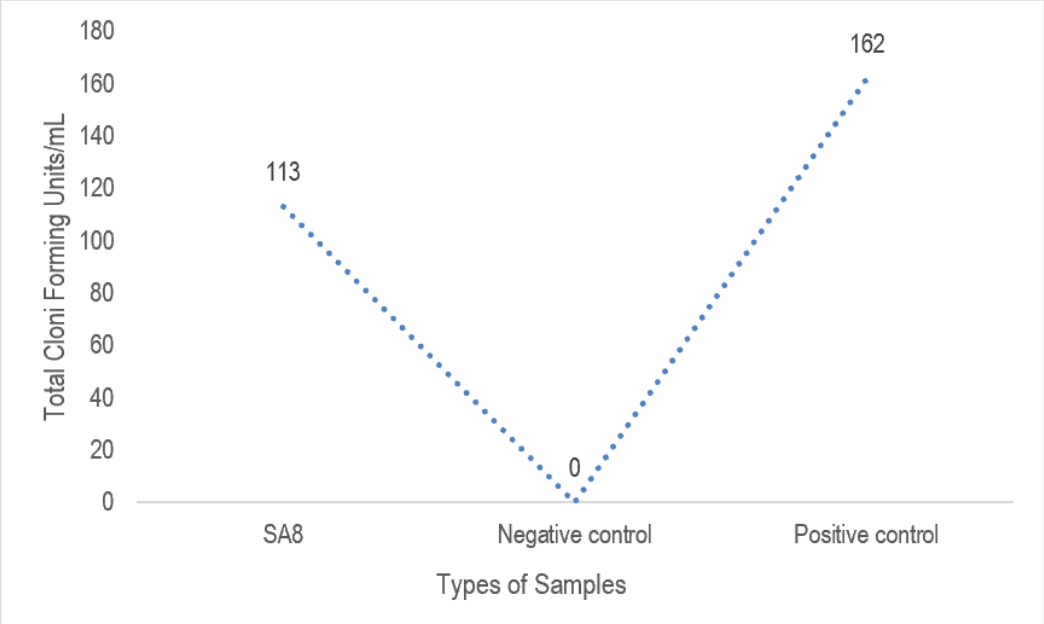

Figure 1. Results Of Cholesterol Assimilation Test By SA8: *Bacillus Amyloliquefaciens* Strain NO10 SA8; *E. Coli*: Negative Control And *Bacillus* Sp: Positive Control.

The Table 1 presents the results of the amino acid analysis conducted on *Bacillus amyloliquefaciens* strain NO10 SA8. It lists the types and concentrations of amino acids produced by the strain, providing insight into its metabolic capabilities (Table 1).

Table 1. Results Of Amino Acid Analysis Produced By *Bacillus Amyloliquefaciens* Strain NO10 SA8.

Amino acid type	Units (mg/L)
L-Alanin	599.45
L-Arginin	842.85
L-Asam Aspartat	352.18
Glisin	646.50
L-Asam Glutamat	1785.43
L-Histidin	Not detected
L-Isoleusin	345.61
L-Sistin	1308.05
L-Leusin	591.15
L-Lisin	402.96
L-Metionin	<386.33
L-Triptofan	Not detected
L-Valin	522.88
L-Fenilalanin	<476.07
L-Prolin	1877.77
L-Serin	392.21
L-Treonin	<163.11
L-Tirosin	Not detected
L-Sistin	1308.05
L-Metionin	<386.33
L-Triptofan	Not detected

Cholesterol assimilation test was conducted to evaluate the ability of *Bacillus amyloliquefaciens* strain NO10 SA8 to utilize cholesterol as a source of carbon or energy. This ability can be measured by assessing the reduction of cholesterol concentration in the medium after the incubation period. The negative control is a medium containing cholesterol inoculated with bacteria incapable of assimilating cholesterol. Bacteria in this negative control will not grow on the medium as they lack the ability to assimilate cholesterol as a carbon source. The positive control is a medium inoculated with bacteria known to effectively assimilate cholesterol. It is used as a comparison to confirm the effectiveness of the *Bacillus amyloliquefaciens* strain NO10 SA8 (Figure 1). *Bacillus amyloliquefaciens* strain NO10 SA8 was shown to grow on Bacto agar medium containing only cholesterol, compared to the negative control, indicating that this strain is capable of assimilating cholesterol.

Bacillus amyloliquefaciens strain NO10 SA8 is capable of producing amino acids, except for L-Histidine, L-Tryptophan, L-Tyrosine, and L-Tryptophan (Table 1). The absence of these amino acids is most likely due to limitations in the biosynthetic pathways possessed by the strain, such as the lack of genes encoding key enzymes or the inactivity of gene expression. To confirm this, further analysis in the form of genome sequencing and transcriptomic expression is highly recommended to identify the metabolic pathways involved. The concentrations of amino acids produced by *Bacillus amyloliquefaciens* strain NO10 SA8, from the highest to the lowest, are as follows: L-Proline 1877.77 mg/L, L-Glutamic Acid 1785.43 mg/L, L-Cysteine 648.50 mg/L, L-Arginine 842.85 mg/L, Glycine 646.50 mg/L, L-Alanine 599.45 mg/L, L-Leucine 591.15 mg/L, L-Valine 522.88 mg/L, L-Phenylalanine <476.07 mg/L, L-Lysine 402.96 mg/L, L-Serine 392.21 mg/L, L-Methionine <386.33 mg/L, L-Aspartic Acid 352.18 mg/L, L-Isoleucine 345.61 mg/L, and L-Threonine <163.11 mg/L (Table 1).

Several studies have reported that *Bacillus amyloliquefaciens* ACCC11060 and *Bacillus amyloliquefaciens* BA01 are capable of producing amino acids such as L-Proline (Wu et al., 2018; Eswaran et al., 2024). Additionally, *Bacillus amyloliquefaciens* HM618 can produce lipopeptides (Shang et al., 2024). *Bacillus amyloliquefaciens* HM-4KSMSO also produces proline (Zhang et al., 2024). *Bacillus amyloliquefaciens* LL3 is capable of producing L-Glutamic Acid or γ-Polyglutamic acid (γ-PGA) (Zhu et al., 2024), and *Bacillus amyloliquefaciens* W25 also produces γ-PGA amino acids (Liu et al., 2024). However, studies regarding its ability to produce amino acids other than proline and glutamic acid have not been reported.

Comparison with previous literature shows that several *B. amyloliquefaciens* strains such as ACCC11060, BA01, and HM618 have been reported to produce L-proline and L-glutamate, which are also produced by strain NO10 SA8 (Wu et al., 2018; Eswaran et al., 2024; Shang et al., 2024). However, scientific documentation regarding the production of other amino acids by this strain remains limited. Therefore, this study provides an important preliminary contribution in expanding the understanding of the biosynthetic profile of strain NO10 SA8. In an applied context, the ability of this strain to produce L-proline and L-glutamate is highly promising for use as a probiotic in fish feed. These two amino acids are known to play a key role in enhancing fish growth and immunity by optimizing protein metabolism. Thus, *Bacillus amyloliquefaciens* NO10 SA8 has the potential to be an effective probiotic candidate for improving feed utilization efficiency and reducing reliance on expensive protein sources such as fishmeal.

However, since this strain does not produce all essential amino acids, its use should be considered as part of a more holistic feed formulation—either in combination with other complementary microbes or through external amino acid supplementation. Further research into the synergistic interactions between probiotic strains and application trials in aquaculture systems is essential to ensure their functional effectiveness. Overall, these findings open new opportunities for the development of probiotics based on local microbial strains with specific metabolic profiles that not only support feed efficiency but also enhance fish health and reduce aquaculture production costs.

CONCLUSION

Bacillus amyloliquefaciens strain NO10 SA8 exhibits strong potential in assimilating cholesterol, as shown by its high 113 CFU value compared to the negative control. The amino acid analysis of *Bacillus amyloliquefaciens* strain NO10 SA8 revealed the production of various amino acids in different concentrations. The highest concentration was observed for L-Proline (1877.77 mg/L) and L-Glutamic acid (1785.43 mg/L), followed by L-Cystine (1308.05 mg/L) and L-Isoleucine (1308.05 mg/L). Moderate levels of Glycine (646.50 mg/L), L-Arginine (842.85 mg/L), L-Leucine (591.15 mg/L), and L-Alanine (599.45 mg/L) were also detected. Some amino acids, such as L-Histidine, L-Tryptophan, and L-Tyrosine, were not detected. These findings suggest that *Bacillus amyloliquefaciens* NO10 SA8 has potential as a probiotic candidate in fish feed, both to improve feed efficiency and to replace expensive protein sources such as fishmeal. In the future, this strain may also be further developed as an alternative to antibiotics through its probiotic properties..

ACKNOWLEDGMENT

We are grateful that this research was funded by the Higher Education Research and Development Council of Muhammadiyah Central Leadership through a development research scheme (Funding and Implementation of Muhammadiyah Research Grants Batch VIII Year 2024; number 0258.809/I.3/D/2025. We also thank the Institute for Research, Publication, and Community Service (LP3M) of Universitas Muhammadiyah Sorong.

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