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EXPLORING LACTIC ACID BACTERIA FROM ACEH'S KEUMAMAH FISH AS POTENTIAL NATURAL ANTIBIOTICS TO INHIBIT ESCHERICHIA COLI

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ABSTRACT
Aceh is known for its abundant fishery resources, particularly tuna, which has an annual production of 7,903 tons. However, tuna is highly perishable, necessitating effective preservation methods to maintain its quality. Keumamah fish, a traditional Acehnese dish, is renowned for its dried texture, allowing it to be stored for extended periods and making it a common choice for various events. Objective: This study aims to identify lactic acid bacteria (LAB) present in keumamah fish that contribute to the fermentation process and serve as potential sources of natural antibiotics to inhibit the pathogenic bacteria Escherichia coli. Method: Lactic Acid Bacteria (LAB) isolation was conducted using dilution and inoculation techniques on MRS agar media supplemented with $CaCO_3$. The results indicated that the inhibition zones of the isolated LAB were as follows: isolate IK4 showed an inhibition zone of 9.81 mm, isolate IK5 exhibited 11.3 mm, isolate IK6 had 9.4 mm, and isolate IK9 displayed 10.56 mm. Based on Gram staining and biochemical tests (temperature, pH, and salt tolerance), seven isolates were classified into three genera: Lactobacillus (IK3, IK5, and IK7), Enterococcus (IK4, IK6, and IK9), and Leuconostoc (IK8).

Keywords: escherichia coli; fermentation; keumamah fish; lab; natural antibiotics

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INTRODUCTION

Indonesia is well-known for its abundance of ancestry from various local cultures, such as Acehnese food. Aceh's status as an international port makes it a significant cultural destination, including in terms of cuisine. Food intake, a fundamental and universal human necessity, is linked to culinary arts (Fitrisia et al., 2020). The Province of Aceh is located in the westernmost part of Indonesia with a coastline of up to 1,660 km and sea area of 295,370 km2. With this condition, the fishery processing industries have the brightest potential to grow in Aceh Province (Anwar et al., 2020). Because of its great nutritional content and status as an animal source of protein, fish is one of the most popular foods consumed by humanity. Fish also includes minerals and lipids that are necessary for human development. On the other hand, once fish is caught, it quickly deteriorates and becomes perishable. Coastal regions had abundant fish production, but maintaining fish quality required intensive post-handling(Mustaqimah et al., 2019). Among the various types of seafood produced in this region, tuna stands out as one of the most significant. With an impressive annual catch of approximately 7,903 tons, this fish is not only a staple in the local diet but is also marketed extensively for consumption in Banda Aceh and its surrounding areas (Garuda608036, n.d.).

Fresh tuna, while highly regarded, presents a challenge due to its perishable nature, which leads to rapid spoilage. As a result, there is a pressing need to explore and implement alternative preservation methods to extend the shelf life of this valuable resource and maintain its quality for consumers. Effective preservation techniques would ensure that tuna remains safe and nutritious for longer periods, benefiting both local communities and the economy (Siddiqui et al., 2024). In the majority of developing nations, fisheries play a significant part in lowering food and nutrition insecurity among the very poor. It is well known that fish contributes to healthy eating and well-being due to its remarkable nutritious makeup. The high bioavailability of key amino acids, essential fatty acids, and minerals including iron, zinc, calcium, vitamin A, and vitamin B12 makes fish a great source of high-quality protein (Gutema & Hailemichael, 2021).

One of the most cherished traditional dishes made from tuna is known as keumamah. As of right now, the community still uses natural heat transfer to dry salted fish and keumamah, which directly harnesses sun energy (Fitri et al., 2022). The product is typically dried using this process by laying it out in the sun on mats, fishing nets, hanging trays, a section of cement flooring, or woven bamboo (Sulaiman & Khairi, 2024). Because birds, insects, cats, and other animals eat the fish that is being dried in the sun, this process is not very hygienic and can result in significant yield loss rates. Additionally, the product will be susceptible to contamination and dust exposure, both of which could harm the health of the user (Anwar et al., 2020). These issues result in lower-than-expected output levels and quality (Mustaqimah et al., 2019). This specialty is unique to Aceh, featuring a distinctive flavor profile and a texture reminiscent of wood, achieved through a meticulous sun-drying process. Keumamah is not just a delicacy; it holds cultural significance and is commonly served at various social gatherings and events, symbolizing hospitality and local heritage. Given its importance in Acehnese culture, there is a compelling need to study the fermentation process involved in creating keumamah, particularly the role of lactic acid bacteria (LAB) in enhancing its taste and shelf stability (Warisman et al., 2017).

The fermentation process is vital as it transforms the flavor of the tuna and can enhance its nutritional content (Saryono et al., 2023). There are two main techniques to make fish silages, which are the acid silage made by the addition of a mineral and/or organic acid, or, alternatively, the fermented silage, prepared by anaerobic microbial fermentation (Kuley et al., 2020).Fish proteins are primarily composed of three categories: myofibrillar proteins (which make up about 65-75%), sarcoplasmic proteins (20-30%), and stromal proteins (5-8%). Understanding the composition of these proteins is essential for improving the nutritional attributes of fermented fish products like keumamah (Mahmud et al., 2021).

Lactic acid bacteria are the main bacteria in the fermentation process of food and beverages (Hutajulu et al., 2021). The main role of these bacteria is to acidify raw materials by producing the majority of lactic acid (homofermentative bacteria), acetic acid, ethanol and CO_2 (heterofermentative bacteria) (Kulla & Retnaningrum, 2019). These bacteria are widely used in food fermentation products such as yogurt, sour cream, cheese, butter, and acid production, and pickles. Food fermentation is typically dominated by LAB, which not only enhances flavor but also increases the food's nutritional value (Sionek et al., 2023). These bacteria produce beneficial compounds, including antimicrobial agents, antifungal substances, and gamma-aminobutyric acid (GABA) (Ula, 2022). LAB are recognized as effective probiotics, known to inhibit the growth of pathogenic bacteria in the digestive system. Furthermore, they can act as natural preservatives in food, producing compounds such as lactic acid, acetate, diacetyl, and bacteriocin that help prolong shelf life and ensure safety (Duche et al., 2023).

Escherichia coli is a harmful gram-negative bacterium. The cell walls of gram-positive and gram-negative bacteria differ from one another. Acute diarrhea, particularly in toddlers, is caused by some strains of the E. coli bacteria. Low birth weight newborns are the term used to describe some of these strains. These bacteria can be found in toilets (WCs), excrement, or contaminated water. If bacteria are the cause of the diarrhea, the small intestine has a large number of E. coli germs. These bacteria produce a particular kind of toxin that harms the small intestine's mucous membrane (Antibakteri et al., n.d.). To properly evaluate the potential of LAB as probiotics, comprehensive testing protocols must be implemented, starting with antibacterial assays. These tests aim to identify the ability of LAB isolates to combat pathogenic bacteria by inhibiting their growth and producing antibiotic compounds (Quinto et al., 2014). The research focuses on isolating LAB from keumamah and assessing their effectiveness in preventing or eliminating pathogenic bacterial growth. By understanding the antibiotic properties of these beneficial bacteria, we can contribute to improved food safety, longer shelf life for traditional products, and enhanced public health in the region. The objective of this study is to identify lactic acid bacteria (LAB) present in Acehnese keumamah fish, which contribute to the fermentation process and have the potential as a natural antibiotic source to inhibit the growth of the pathogenic bacterium Escherichia coli.

METHOD

Isolation of Lactic Acid Bacteria (BAL)

a. Preparation of Media
   Prepare 100 mL of MRSA media by dissolving 6.82 g in distilled water and sterilizing it in an autoclave. After sterilization, add 1 g of $CaCO_3$.
b. Isolation.
   Crush a 1 g sample in 9 mL of distilled water and perform dilutions up to 10^-3. Spread 0.1 mL of each dilution on the MRSA media using the spread plate method and incubate at 37 °C for 72 hours.

c. Purification
   Purify isolates showing clear zones using the quadrant streaking technique on fresh MRSA media, incubating again at 37 °C for 72 hours.
d. Characterization
   Macroscopic observations of colony morphology were recorded. For microscopic examination, perform Gram staining:
   1. Clean a glass slide and flame it.
   2. Transfer bacteria using a sterile needle.
   3. Stain with crystal violet, rinse, and air dry.
   4. Add iodine, rinse, and dry.
   5. Apply 95% alcohol, rinse, and dry.
   6. Stain with safranin, rinse, and dry.
   7. Observe under a microscope (purple indicates gram-positive BAL).

Biochemical Tests

a. Temperature Test
   Inoculate MRSB media and incubate at 15°C, 30°C, and 45°C for 48 hours. Measure growth with UV-Vis spectrophotometry at 560 nm: an increase in absorbance indicates growth.
b. pH Test
   Adjust sterilized MRSB media to pH 8 using NaOH, and inoculate bacteria. Repeat for pH 2 and 4 using HCl. Incubate at 37 °C for 48 hours and measure absorbance.
c. Salt Test

Inoculate MRSB media with salt concentrations of 5% and 10%, incubate at 37 °C for 48 hours, and measure growth via absorbance.

Antibacterial Test

Inoculate *Escherichia coli* on NA media and incubate at 37 °C for 24 hours. Prepare a suspension matching McFarland standard 0.5 and evenly spread on Mueller Hinton Agar. Place BAL isolates in the agar and incubate at 37 °C for 24 hours. Measure inhibition zones using a digital slide.

RESULT

Colony and Bacterial Cell Morphology of LAB

Isolation of lactic acid bacteria from the fish keumamah was based on the morphology of the colonies and the bacterial cells. The colony from the seventh isolate exhibited a round shape with color variations ranging from white to yellowish-white and yellow. All colonies had a convex elevation with smooth edges. The variation in colony color indicates different characteristics among isolates: isolates IK3 and IK9 showed a yellowish-white color, while isolates IK4, IK6, and IK8 were yellow, and isolates IK5 and IK7 were white.The cell morphology of the seven isolates revealed differences in shape and Gram staining results. Three isolates (IK3, IK5, and IK7) had rod-shaped (bacilli) cells and were Gram-positive. In contrast, the other four isolates (IK4, IK6, IK8, and IK9) had round-shaped (cocci) cells, which also exhibited Gram-positive results. These findings indicate that the lactic acid bacteria isolated from the community consist of both round and rod shapes, with common Gram-positive characteristics typically found in lactic acid bacteria (Table 1).

Table 1.
Morphological Characteristics of Lactic Acid Bacteria in Keumamah Fish

Isolate	Bacterial Colony Morphology				Bacterial cell morphology	
	Form	Color	Elevation	Edge	Form	Gram Staining
IK3	Round	Yellowish white	Convex	Smooth	Basil	+
IK4	Round	Yellow	Convex	Smooth	Cocus	+
IK5	Round	White	Convex	Smooth	Basil	+
IK6	Round	Yellow	Convex	Smooth	Cocus	+
IK7	Round	White	Convex	Smooth	Basil	+
IK8	Round	Yellow	Convex	Smooth	Cocus	+
IK9	Round	Yellowish white	Convex	Smooth	Cocus	+

Biochemical Characteristics of LAB

Temperature Test

Table 2.
Temperature Test of Lactic Acid Bacteria in Keumamah Fish

Isolate	Temperature 15℃								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,126	0,089	0,120	0,111	0,325	0,237	0,102	0,221	Up	Grow
IK4	0,165	0,117	0,173	0,151	0,155	0,118	0,120	0,131	Down	Not Grow
IK5	0,159	0,151	0,192	0,187	0,149	0,140	0,205	0,164	Down	Not Grow
IK6	0,136	0,127	0,225	0,162	0,236	0,282	0,239	0,252	Up	Grow
IK7	0,11	0,093	0,121	0,108	0,254	0,338	0,376	0,322	Up	Grow
IK8	0,112	0,132	0,124	0,122	0,140	0,187	0,204	0,177	Up	Grow
IK9	0,108	0,120	0,155	0,127	0,206	0,164	0,153	0,174	Up	Grow
Isolate	Temperature 30℃								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		

Isolate	Temperature 15°C								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
	P1	P2	P3		P1	P2	P3			
IK3	0,070	0,080	0,112	0,087	0,119	0,124	0,168	0,137	Up	Grow
IK4	0,102	0,172	0,173	0,149	0,177	0,128	0,266	0,190	Up	Grow
IK5	0,081	0,115	0,145	0,113	0,196	0,160	0,126	0,160	Up	Grow
IK6	0,103	0,156	0,166	0,141	0,163	0,212	0,158	0,177	Up	Grow
IK7	0,082	0,074	0,146	0,100	0,082	0,088	0,174	0,114	Up	Grow
IK8	0,083	0,157	0,126	0,122	0,091	0,109	0,116	0,105	Down	Not Grow
IK9	0,070	0,103	0,115	0,096	0,173	0,166	0,149	0,162	Up	Grow

Isolate	Temperature 45°C								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,114	0,140	0,159	0,137	0,150	0,158	0,133	0,147	Up	Grow
IK4	0,163	0,163	0,205	0,177	0,132	0,155	0,126	0,137	Down	Not Grow
IK5	0,108	0,142	0,130	0,126	0,122	0,142	0,149	0,137	Up	Grow
IK6	0,114	0,173	0,151	0,146	0,166	0,197	0,174	0,179	Up	Grow
IK7	0,105	0,186	0,127	0,139	0,110	0,162	0,181	0,151	Up	Grow
IK8	0,272	0,217	0,177	0,222	0,145	0,158	0,140	0,147	Down	Not Grow
IK9	0,092	0,109	0,117	0,106	0,178	0,185	0,156	0,173	Up	Grow

pH Test

Table 3.
pH Test of Lactic Acid Bacteria in Keumamah Fish

Isolate	pH 2 (acid)								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,420	0,43	0,435	0,429	0,138	0,139	0,148	0,141	Down	Not Grow
IK4	0,425	0,69	0,500	0,536	0,156	0,148	0,134	0,146	Down	Not Grow
IK5	0,444	0,5	0,47	0,468	0,129	0,140	0,136	0,135	Down	Not Grow
IK6	0,450	0,53	0,47	0,484	0,143	0,141	0,139	0,141	Down	Not Grow
IK7	0,420	0,450	0,43	0,431	0,143	0,131	0,158	0,144	Down	Not Grow
IK8	0,402	0,41	0,43	0,427	0,154	0,137	0,165	0,152	Down	Not Grow
IK9	0,509	0,475	0,45	0,478	0,140	0,158	0,123	0,140	Down	Not Grow

Isolate	pH 4 (acid)								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,298	0,302	0,300	0,300	1,168	1,052	0,675	0,965	Up	Grow
IK4	0,358	0,35	0,390	0,364	0,961	0,927	1,042	0,976	Up	Grow
IK5	0,314	0,37	0,32	0,332	0,998	0,928	0,991	0,972	Up	Grow
IK6	0,321	0,42	0,33	0,357	1,141	1,177	0,916	1,078	Up	Grow
IK7	0,308	0,3	0,32	0,307	1,369	1,266	0,859	1,164	Up	Grow
IK8	0,319	0,31	0,28	0,301	1,247	1,176	0,887	1,103	Up	Grow
IK9	0,322	0,346	0,390	0,364	0,713	0,821	0,953	0,829	Up	Grow

Isolate	pH 8 (base)								Absorbance Value	Description
	Before Incubation			mean	After Incubation			mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,098	0,109	0,111	0,106	0,118	0,128	0,115	0,120	Up	Grow
IK4	0,130	0,16	0,18	0,157	0,131	0,124	0,118	0,124	Down	Not Grow
IK5	0,124	0,09	0,136	0,118	0,114	0,121	0,159	0,141	Up	Grow
IK6	0,181	0,14	0,15	0,157	0,132	0,108	0,119	0,119	Down	Not Grow

IK7	0,119	0,16	0,14	0,138	0,119	0,137	0,20	0,152	Up	Grow
IK8	0,118	0,15	0,11	0,106	0,199	0,118	0,130	0,149	Up	Grow
IK9	0,131	0,131	0,16	0,141	0,116	0,133	0,132	0,127	Down	Not Grow

Salt Test

Table 4.
Salt Test of Lactic Acid Bacteria in Keumamah Fish

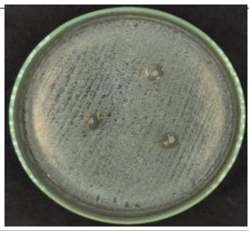
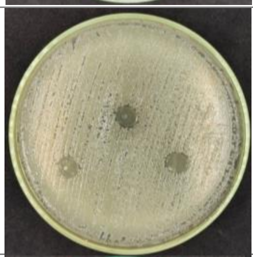
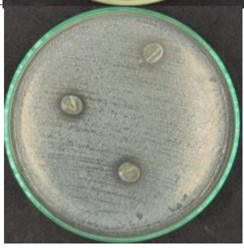
Isolate	Salt 5%								Absorbance Value	Description
	Before Incubation			Mean	After Incubation			Mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,096	0,098	0,139	0,11	0,101	0,136	0,110	0,115	Up	Grow
IK4	0,135	0,123	0,205	0,154	0,221	0,102	0,135	0,152	Up	Grow
IK5	0,146	0,145	0,147	0,15	0,374	0,530	0,369	0,424	Up	Grow
IK6	0,121	0,191	0,177	0,16	0,140	0,184	0,178	0,167	Up	Grow
IK7	0,110	0,118	0,087	0,105	0,100	0,125	0,109	0,111	Up	Grow
IK8	0,097	0,125	0,182	0,13	0,307	0,128	0,170	0,201	Up	Grow
IK9	0,132	0,167	0,172	0,13	0,308	0,381	0,361	0,350	Up	Grow

Isolate	Salt 10%								Absorbance Value	Description
	Before Incubation			Mean	After Incubation			Mean		
	P1	P2	P3		P1	P2	P3			
IK3	0,087	0,085	0,099	0,090	0,103	0,092	0,094	0,096	Up	Grow
IK4	0,178	0,190	0,190	0,19	0,170	0,142	0,152	0,154	Down	Not Grow
IK5	0,100	0,130	0,179	0,14	0,083	0,126	0,122	0,110	Down	Not Grow
IK6	0,082	0,175	0,151	0,14	0,090	0,243	0,171	0,168	Up	Grow
IK7	0,080	0,102	0,110	0,1	0,096	0,089	0,127	0,104	Up	Grow
IK8	0,104	0,162	0,161	0,14	0,091	0,115	0,114	0,106	Down	Not Grow
IK9	0,106	0,106	0,150	0,120	0,101	0,127	0,140	0,122	Up	Grow

Antibacterial Activity

The investigation into the antibacterial properties of potential lactic acid bacteria (LAB) derived from keumamah fish against the common bacterium Escherichia coli has yielded informative results. Four specific isolates—designated as IK4, IK5, IK6, and IK9—were tested for their ability to inhibit the growth of E. coli.The results revealed that all four isolates exhibited noticeable inhibition zones, which are indicative of antibacterial activity. Among them, isolate IK5 demonstrated the most robust effect, with an average inhibition zone measuring 11.3 mm. Following closely was isolate IK9, which produced an average inhibition zone of 10.56 mm. Isolate IK4 showed an inhibition zone of 9.81 mm, while isolate IK6 had a slightly lower measurement at 9.4 mm. These findings underscore the significant antibacterial activity of these isolates, suggesting their potential role in inhibiting the growth of E. coli in relevant contexts.In contrast, isolates IK3, IK7, and IK8 exhibited no antibacterial activity at all; no inhibition zones were detectable in their respective tests. This lack of activity suggests that not all lactic acid bacteria sourced from keumamah fish have the capability to function as effective antibacterial agents. The observed variations in antibacterial potency among the different isolates may stem from inherent differences in the genus and species of the bacteria.

Table 5.
Antibacterial Activity Test against Escherichia coli Bacteria

Isolate	Antibacterial Test Results	Inhibition Zone Diameter (mm)			
		P1	P2	P3	Average
IK3		0	0	0	0
IK4		9.7	9.9	9.85	9.81
IK5		10.95	11.3	11.65	11.3
IK6		9.1	10	9.1	9.4
IK7		0	0	0	0
IK8		0	0	0	0
IK9		9.4	11.25	11.05	10.56

DISCUSSION

Bacteria isolated from Keumamah produced 1 white oval-shaped bacterial colony and provided a clear zone around the bacteria. Because when MRSA + CaCO1% media reacting with lactic acid which was metabolized from lactic acid bacteria, it will form calcium lactate which dissolves in the media and produces a clear zone around the bacterial colony (Nasri et al., 2021). Table 1 presents the morphological characteristics of lactic acid bacteria isolated from keumamah fish, categorized into two main aspects: colony morphology and cell morphology. All bacterial isolates have round-shaped colonies with varying colors, including yellowish-white, yellow, and white. The colonies of each isolate have a convex elevation and smooth edges, indicating uniformity in colony shape. Regarding cell morphology, there are two types of cell shapes: bacillus (rod-shaped) and coccus (round-shaped). The isolates with bacillus shape include IK3, IK5, and IK7, while the isolates with coccus shape include IK4, IK6, IK8, and IK9. All isolates show a positive Gram stain (+), indicating that these bacteria are Gram-positive, a characteristic commonly found in lactic acid bacteria. Moreover, this study has similar findings with (Taye et al., 2021) who identified isolation and identification of lactic acid bacteria from cow milk and milk products got gram stain characteristics of the bacterium revealed that all of the bacteria were Gram-positive. Gram-positive bacteria, such as lactic acid bacteria, differ in their Gram staining reactions according to the makeup of their cell walls. Gram-positive bacteria have 90% peptidoglycan in their cell walls, with the remaining 10% being tekoic acid. Because they can create a complicated association with the primary dye, crystal violet, which is also purple, gram-positive bacterial cells appear purple. Gram-negative bacteria have a 5–20% peptidoglycan cell wall, with polysaccharides making up the remaining portion. By dissolving the lipids in the outer membrane, a 95% alcohol solution can enhance the porosity of the cell wall, causing the purple complex to be released and the cells to turn colorless (9072-21451-1-SM, n.d.).

In order to identify the prevalence of Lactic Acid Bacteria (LAB), the researchers conducted a series of biochemical assays on 7 isolates, as described by (Khushboo et al., 2023). The temperature test results of lactic acid bacteria isolated from keumamah fish, assessing their growth at two different temperatures, 15°C and 30°C. The absorbance values before and after incubation at each temperature were measured to determine the bacteria's growth. At 15°C, the isolates showed varying growth responses. Isolates IK3, IK6, IK7, IK8, and IK9 exhibited an increase in absorbance after incubation, indicating growth ("Up Grow"). In contrast, isolates IK4 and IK5 showed a decrease in absorbance, indicating no growth ("Down Not Grow"). At 30°C, all isolates demonstrated an increase in absorbance, indicating growth ("Up Grow"). This suggests that the bacteria in the tested isolates are capable of growing at both temperatures, with some isolates (IK3, IK6, IK7, IK8, IK9) growing better at 15°C, while others like IK4 and IK5 did not grow at 15°C but grew at 30°C.

The pH test results of lactic acid bacteria isolated from keumamah fish, measuring their growth under acidic conditions (pH 2 and pH 4). The absorbance values before and after incubation indicate bacterial growth at each pH level. At pH 2 (acidic), all isolates showed a decrease in absorbance after incubation, indicating no growth ("Down Not Grow"). The values remained low across all isolates, suggesting that the bacteria are not able to thrive in highly acidic conditions. In contrast, at pH 4 (slightly acidic), all isolates demonstrated an increase in absorbance after incubation, indicating growth ("Up Grow"). This suggests that the bacteria are capable of growing in less acidic conditions, with absorbance values rising significantly for each isolate. Overall, the test shows that the lactic acid bacteria from keumamah fish are better adapted to grow in mildly acidic environments (pH 4) rather than highly acidic ones (pH 2). Lactic acid produced by LAB during fermentation lowers the product's pH, which in turn lowers the quantity of other microorganisms. Once fermentation has begun, the normal acid-forming bacteria quickly proliferate, take over as the predominant germs, and reach their maximum density at the end of the fermentation process (Ngasotter et al., 2020).

The salt test results of lactic acid bacteria isolated from keumamah fish, assessing their growth in different concentrations of salt (5% and 10%). The absorbance values before and after incubation were used to evaluate bacterial growth under these conditions. At the 5% salt concentration, all isolates showed an increase in absorbance after incubation, indicating growth ("Up Grow"). For example, isolates like IK3, IK4, and IK5 experienced noticeable growth with absorbance values increasing significantly. This suggests that the lactic acid bacteria can tolerate and thrive in moderately saline environments. However, when exposed to a 10% salt concentration, only some isolates demonstrated growth, with a significant decrease in absorbance values for others, indicating no growth ("Down Not Grow"). For instance, isolates IK4 and IK5 showed no significant growth at 10% salt concentration, while isolates like IK3, IK6, IK8, and IK9 still showed growth, albeit at a reduced level. This indicates that the bacteria are more adaptable to lower salt concentrations than higher ones. The results of the research on the antibacterial activity of lactic acid bacteria (LAB) isolated from keumamah fish revealed that not all isolates possess the ability to inhibit the growth of Escherichia coli. Among the tested isolates, four demonstrated notable antibacterial properties: IK4, IK5, IK6, and IK9. The inhibition zones varied across these isolates, ranging from 9.4 mm to 11.3 mm. Notably, isolate IK5 exhibited the largest inhibition zone of 11.3 mm, indicating it has the most significant potential to act as an antibacterial agent against E. coli. In contrast, isolates IK3, IK7, and IK8 showed no antibacterial activity, demonstrated by the absence of inhibition zones.

The observed differences in antibacterial capabilities among the isolates may be attributed to several factors, including differences in genus and species among the isolated LAB strains. Based on gram staining and biochemical tests, it was determined that the isolates with antibacterial activity—specifically IK4, IK5, IK6, and IK9 belong to the genera Enterococcus and Lactobacillus. These genera are well-known for harboring several species capable of producing antimicrobial compounds, such as lactic acid, bacteriocins, and hydrogen peroxide, all of which can inhibit the growth of pathogenic bacteria like E. coli.The antibacterial activity of these LAB strains is crucial in the context of food fermentation processes (Zapaśnik et al., 2022). Such activity not only extends the shelf life of fermented products but also enhances food safety by reducing the levels of pathogenic bacteria that could pose health risks to consumers (Wang et al., 2021).Moreover, the variation in inhibition zones among the different BAL isolates suggests that there are differences in their production of antimicrobial compounds. For instance, IK5, with its larger zone of inhibition, may be producing antimicrobial substances either in higher concentrations or with greater effectiveness against E. coli when compared to the other isolates. Conversely, the lack of inhibition zones in IK3, IK7, and IK8 implies that these isolates either do not produce sufficient amounts of effective antimicrobial compounds or lack adequate defense mechanisms against E. coli. Environmental factors, such as pH, temperature, and the types of substrates used during antibacterial testing, may also impact the production of antimicrobial compounds by these isolates.

The lactic acid fermentation with the bacteria has long been known and practical by the humans for making different food stuffs. Lactic acid bacteria (LAB) produce different compounds such as organic acids, diacetyl, hydrogen peroxide & bacteriocin or bacteriocidal proteins during lactic fermentations, in this research to isolate lactic acid bacteria that have antibacterial activity of pathogenic bacteria that are in future use as probiotic for the food industry (Islam et al., 2020). This research highlights the importance of selecting appropriate BAL isolates for food fermentation processes. Isolates with potent antibacterial capabilities not only contribute to enhancing the flavor and nutritional value of fermented products but also significantly improve food safety by minimizing the risk of contamination from pathogenic bacteria. Given the findings, isolates such as IK5 and IK9 present substantial potential for application within the food fermentation industry, particularly in fish-based products like keumamah.I n summary, this investigation demonstrated that lactic acid bacteria (LAB) isolated from keumamah fish exhibit notable antibacterial activities against Escherichia coli. Several isolates showed significant potential for application in fermentation products, suggesting their suitability as natural preservatives or functional ingredients in food systems. Furthermore, LAB strains identified in this study have the potential to enhance food safety by inhibiting pathogenic bacteria through the production of organic acids, bacteriocins, or other antimicrobial metabolites. However, further research is required to fully characterize the specific antimicrobial compounds produced by these LAB strains, assess their safety for human consumption, and evaluate their effectiveness against a broader spectrum of pathogenic microorganisms. Such research will be crucial for optimizing their application in food safety, fermentation technology, and even as natural therapeutic agents in clinical settings (Garcia-Gutierrez et al., 2024).

CONCLUSION

This study shows that lactic acid bacteria (LAB) isolated from Acehnese keumamah fish have varying antibacterial activity against pathogenic bacteria Escherichia coli. Of the seven isolates tested, four isolates (IK4, IK5, IK6, and IK9) showed significant inhibition zones, with isolate IK5 having the strongest effect with an inhibition zone of 11.3 mm. These isolates, which belong to the genera Lactobacillus and Enterococcus, are known to produce antimicrobial compounds such as lactic acid and bacteriocins that can inhibit the growth of pathogenic bacteria.

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