JoSSEd
Journal of Science and Science Education
ISSN: 2774-1869 eISSN: 2774-2105
Research Paper

Growth and Antibiotic Sensitivity Status of *Bacillus* spp. associated with Abalone (*Haliotis asinina*) as Probiotic Candidate Selection

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DOI: 10.29303/jossed.v4i1.3089

Article Info
Received: February 5, 2023
Revised: April 25, 2023
Accepted: April 28, 2023
Published: April 30, 2023

Abstract: The use of probiotics is an innovation in an effort to increase production and fight pathogens in aquaculture cultivation environments. Probiotic candidate microorganisms are selected selectively to ensure that the probiotics used are safe and provide benefits for the host and the environment. This study aims to select probiotic candidates based on the aspects of growth and sensitivity to antibiotics from 4 isolates of Bacillus from abalone (*Haliotis asinina*). Growth observations were carried out by turbidimetric method using a spectrophotometer at a wavelength of 600 nm in SWC media, and antibiotic sensitivity was tested using Rifampicin, Gentamicin, Kanamycin, and Erythromycin antibiotics using the well-diffusion agar method. The growth analysis of isolates was described by a growth curve based on the OD value, and antibiotic susceptibility based on the diameter of the inhibition zone. The results showed that the growth of the four isolates of Bacillus spp. had a short lag phase, ranging from 1-2 hours, an exponential phase ranging from 15 hours to *B. pumilus* SLK1 and *B. licheniformis* SLK2 isolates, and 12 hours to *B. coagulans* CaK1 and *B. coagulans* CaK6 isolates. Antibiotic sensitivity showed that *B. coagulans* CaK1 isolates were sensitive to all the tested antibiotics, while *B. pumilus* SLK1 isolates, *B. licheniformis* SLK2, and *B. coagulans* CaK6 were only sensitive to Rifampicin and Gentamicin antibiotics. The ideal probiotic candidate is an isolate with good growth and does not have resistance properties, based on the results of this study CaK1 isolate has the potential as a probiotic candidate.

Keywords: Antibiotic resistance; Abalone; Aquaculture probiotics; Bacillus

Citation: Faturrahman, F., Fitriana, N.Z., Sarkono, S., & Hidayati, E. (2023). Growth and Antibiotic Sensitivity Status of Bacillus spp. associated with Abalone (Haliotis asinina) as Probiotic Candidate Selection. *Journal of Science and Science Education*, *4*(1), 55–64. https://doi.org/10.29303/jossed.v4i1.3089

INTRODUCTION

Aquaculture is a food-producing sector with the most rapid development and is a vital activity in efforts to improve food security (Chauhan and Singh, 2018; El-Saadony, *et al*. 2021). In The State of World Fisheries and Aquaculture 2020 it was reported that the contribution of world aquaculture to global fish production reached 46.0 percent in 2018, up from 25.7 percent in 2000, and 29.7 percent in other countries in the world, excluding China, compared with 12.7 percent in 2000. At the regional level, aquaculture accounts for 17.9 percent of total fish production in Africa, 17.0 percent in Europe, 15.7 percent in the Americas and 12.7 percent in Oceania. The share of aquaculture in Asian fish production (excluding China) was 42.0 percent in 2018, up from 19.3 percent in 2000 (FAO, 2020). However, production in the aquaculture sector cannot be separated from various problems, including a low rate of survival of organisms, slow growth rates, infection with pathogenic bacteria and viruses, to the presence of toxic compounds in the environment (Permadi, *et al*. 2018).

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Nowadays, the use of probiotics as an innovation in overcoming the problems of aquaculture cultivation is considered more effective (Alonso, *et al*. 2018; Amin *et al*. 2019). Because control using antibiotics or disinfectants can cause resistance, especially in ineffective use so that it is detrimental to cultivated organisms, and can have a negative impact on environmental health (Lara-Flores, 2011; Mingmongkolchai, *et al*., 2017).

Aquaculture probiotics are living and dead microorganisms or components of non-pathogenic microorganisms cells that provide benefits by balancing microorganisms in the body and in the host growth environment (Merrifield, *et al*., 2010; Hai, 2015). Probiotics can be in the form of single cultures or mixed cultures formulated in the form of foods and drugs (Permadi, *et al*. 2018; Praja, 2011). The probiotic market was valued at 35.9 billion dollars in 2016 and is expected to reach 52 billion by 2020 (Market Research Report, 2018).

Probiotic candidate bacteria must meet several criteria so that they are suitable for use as probiotic microorganisms, including having good growth, to be able to balance the microbial ecosystem in the host's digestive tract, and meet the safety requirements of probiotic products, probiotic candidate bacteria must be sensitive or not resistant to common antibiotics. used on animals and humans (Faturrahman, 2013; Ayichew, *et al*., 2017). *Bacillus* spp. as aquaculture probiotic bacteria, it is known to be able to increase the resistance of aquatic organisms against pathogenic microorganisms in the form of bacteria and viruses, increase the survival of organisms, and improve water quality with the ability to reduce toxic compounds (Soltani, *et al*., 2019; Kuebutornye, *et al*., 2020. The ability of Bacillus to form spores and survive for a long time makes it superior to other bacteria as probiotic microorganisms (Mingmongkolchai *et al*., 2017).

Our previous research has obtained four *Bacillus* spp. isolates as probiotic candidates associated with abalone. The four isolates were tested for their antagonism against *Vibrio* spp., the enzymatic activity of the isolates in degrading organic compounds and the resistance of the isolates under various pH conditions. In this study, further tests were carried out by measuring the growth and sensitivity of probiotic candidate bacteria to antibiotics. In the future, bacterial isolates that have the potential as probiotics will be applied to abalone cultivation and are expected to increase production of abalone cultivation, especially in the West Nusa Tenggara area, considering that abalone is an ethnofauna of West Nusa Tenggara and is a very promising export commodity (Faturrahman, 2012; Hayati, *et al*., 2018

METHOD

Preparation of Research Tools and Materials

The tools and materials used were first prepared, then sterilized for glassware and media using an autoclave for 15 minutes at a temperature of 121 °C and a pressure of 2 atm.

Culture Preparation of Bacillus spp.

The bacterial isolate used was an isolate of Bacillus sp. obtained from our previous research activities, namely four isolates of *Bacillus coagulans* CaK1, *Bacillus coagulans* CaK6, *Bacillus pumilus* SLK1, and *Bacillus licheniformis* SLK2. Four isolates of Bacillus spp. this is the result of isolation from the shell and digestion of abalone Haliotis asinina. The four isolates were rejuvenated and inoculated on SWCA media. The isolate preparation was divided into two parts, namely preparation for growth test and preparation for antibiotic sensitivity test. Each of the test bacteria isolates that had been rejuvenated were taken 2 oses, put into a 0.9% physiological NaCl solution, and adjusted to the standard Mc. Farland 0.5. Furthermore, as much as 100 l of the solution was inoculated into the Sea Water Completed Broth medium (HiMedia: peptone 5 gram; yeast extract 1 gram; glycerol 3mL; sea water 750 mL; water destilates 250 mL), then incubated at 30°C for 24 hours.

Preparation of Test Media

The culture media used to test the growth of Bacillus spp. isolates. is SWCB, while for antibiotic susceptibility test using Mueller Hinton Agar media (HiMedia: Beef Extract, 2 gram; Acid Hydrolysate of Casein, 17,5 gram; Starch, 1,5 gram; Agar, 17 gram; Aquadest, 1 liter). All components are mixed, then heated to boiling, then sterilized using an autoclave for 15 minutes, then the SWCB media is poured into a test tube and the MHA media is poured into a petri dish, allowed to solidify.

Growth Test of Bacillus spp. Isolates Using a Spectrophotometer

A total of 100µL of fresh culture of Bacillus isolate was injected aseptically into test tubes containing 5 ml of SWCB media each. Then this culture was incubated at room temperature for 1×24 hours, and every 3 hours the absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm, referring to the modified Seniati, *et al.,* (2019) and Rosmania and Yanti (2020) method.

Sensitivity test for Bacillus spp. isolates. Against Various Antibiotics

Antibiotic susceptibility test was carried out using MHA media, using the agar diffusion method with the technique of agar wells with a diameter of 7 millimeters. Prepared petri dishes containing MHA that had been poured and cooled previously, then cultured Bacillus spp. isolates. which has been prepared in SWCB media is taken using a sterile cotton swab and then streaked evenly over the entire surface of the MHA media. After evenly distributed and the surface to dry, 4 wells were made which were then injected with Gentamicin antibiotics, Erythromycin antibiotics, Kanamycin antibiotics, and Rifampicin antibiotics, then incubated for 1×24 hours.

Measurement and Interpretation of Antibiotic Inhibitory

The measurement of antibiotic inhibition was carried out by measuring the diameter of the inhibition zone, namely the area that was not overgrown with the test strain, using a millimeter ruler. The results are interpreted according to the Clinical Laboratory Standards Institute, (2012) as follows:

Table 1. Standard levels of resistance to antibiotics

Antibiotics	Diameter of inhibition zone (mm)		
	Sensitive	Intermediates	Resistant
Rifampicin	>20	17-19	<16
Gentamycin	>15	13-14	<12
Kanamycin	>18	14-17	<13
Erythromycin	>22	14-22	<13

RESULT AND DISCUSSION

Utilization of microorganisms as aquaculture probiotics is carried out with strict selection, starting from the necessity to provide benefits to the host, and for the aquatic environment, the safety of the probiotic products produced is also a major concern in the selection of bacterial strains as probiotic candidates (Chauhan and Singh, 2018). One of the requirements for probiotic candidate bacterial strains is to have good growth and be free from virulence and resistance properties that can be transferred to other microorganisms and to the host (Ayichew, *et al*., 2017). In this study, the growth and sensitivity to antibiotics of Bacillus *coagulans* CaK1, *Bacillus coagulans* CaK6, *Bacillus pumilus* SLK1, dan *Bacillus licheniformis* SLK2 isolates was studied.

Growth of Bacillus spp. Isolates

Growth measurements of isolates of *Bacillus coagulans* CaK1, *Bacillus coagulans* CaK6, *Bacillus pumilus* SLK1, and *Bacillus licheniformis* SLK2 were performed using the turbidimetric method, using a spectrophotometer at a wavelength of 600 nm. Measurements were carried out every three hours starting from the 0th hour to the 24th hour during incubation at room temperature, so that nine points of optical density (OD) value were obtained for each test isolate. Then, based on the results of the OD values obtained, the conversion was carried out into a graphic form, which describes the growth of each isolate. The results of the OD measurement are presented in the form of a growth curve (Figure 1) and the interval data between growth phases in Table 2.

The growth curves of the four isolates of *Bacillus* spp. show different growth patterns. Growth during the lag phase or different adaptation periods and gives an overview of the lag phase of all test isolates is short. The growth in the logarithmic phase, stationary phase and death phase also showed various growth patterns. Interval of time required by each isolate to grow in the four growth phases is presented in Table 2.

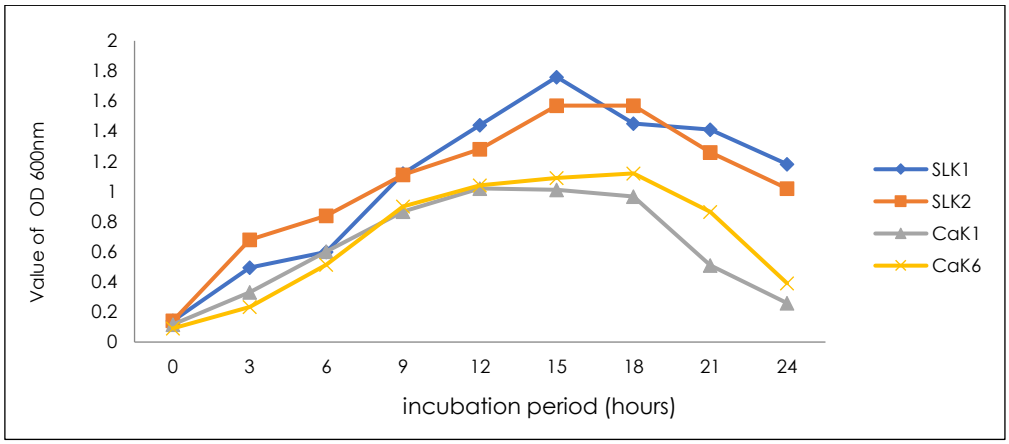

Figure 1. Growth curves of isolates *B. pumilus* SLK1, *B. licheniformis* SLK2, *B. coagulans* CaK1, and *B. coagulans* CaK6 grown on sea water completed media for 24 hours at 30°C

Table 2. he time interval required for the four *Bacillus* spp isolates in each growth phase

Isolates	Time Interval in Growth Phase (hours)			
	Lag	Logarithmic	Stationer	Death
B. pumilus SLK1	0	15	0	IP* 15
B. licheniformis SLK2	0	15	3	IP* 18
B. coagulans CAK1	1-3	12	6	IP* 18
B. coagulans CAK6	1-3	12	6	IP* 18

Notes: IP = incubation period

Growth can be interpreted by increasing the size, total weight, mass, of an individual. In unicellular microorganisms, division means increasing the number of cells (multiplication), bacteria multiply asexually by transverse binary fission into 2 cells and multiples thereof. Each offspring individually can continue the reproductive process indefinitely in the same way as the parent, or the previous individual with the requirements of the availability of sufficient food and energy, as well as supportive environmental conditions (Pelczar and Chan, 2013).

The four isolates of *Bacillus* spp. the origin of abalone growth has been measured by turbidimetric method using a spectrophotometer. This method is a rapid method to determine bacterial growth through the level of turbidity (turbidity) in the test bacterial culture. The basic principle of the turbidimetric method is to pass light in a culture that was previously filled in a special tube called a cuvette, then there is a process of light absorption and transmission. The amount of light absorbed (absorption) will be directly proportional to the number of bacterial cells in the test culture, while the light transmitted (transmission) will be inversely proportional to the number of bacterial cells, so that the less light that is transmitted, the more the number of bacterial cells measured (Karki, 2020).

SLK1 isolate had the highest exponential point with a time range of 15 hours, followed by SLK2 isolate with the same time range, but with an OD value at a lower exponential point. CaK1 and CaK6 isolates also showed OD values with a lower exponential point, with a time range of 12 hours. The stationary phase based on the curve showed that SLK1 and CaK1 isolates occurred for 6 hours, SLK2 with a time range of 3 hours, while CaK6 was not clearly depicted and was seen directly entering the death phase at 21 hours. SLK2 and CaK1 were also seen to enter the death phase at 21 hours, and SLK1 occurred at 24 hours.

Graph 1 shows that the exponential phase of each test isolate looks varied. The four isolates showed different ranges of adding OD values, as well as the time range for the exponential phase. *B. pumilus* SLK1 isolate experienced a sharp increase in turbidity (OD) at 6 to 9 hours, from 0.599 to 1,120, in *B. licheniformis* SLK2 isolate a sharp increase occurred in the 0 hour range until at the 3rd hour, from 0.141 to 0.680, while in *B. coagulans* CaK1 and *B. coagulans* CaK6 isolates the measured OD value was fairly constant, there was no significant increase between one measurement time and the next measurement, this is in accordance with Pelczar and Chan (2013) stated that the characteristics shown in the exponential phase are that bacterial cells divide and carry out metabolic activity constantly, their mass doubles at the same rate, and a balanced growth state. Maier and Pepper (2015) also added that in the exponential phase there was a rapid multiplication of bacteria which was in line with the exponential equation, so that the growth graph showed an increasing line with increasing levels of turbidity or cell density.

Variations in the time range for the exponential phase to occur were also seen in the four isolates, where isolates *B. pumilus* SLK1 and *B. licheniformis* SLK2 had exponential phases ranging from 15 hours, while in *B. coagulans* CaK1 and *B. coagulans* CaK6 the exponential phase ranges from 12 hours. Dewi (2014) found that the exponential phase of *Bacillus* sp. probiotic candidates was able to survive up to 16 hours on control media in the form of fish meal, molasses, and yeast, and were the best growth compared to other treatments. Sari *et al.* (2017) also added that *Bacillus* sp. D2.2 isolated from shrimp on technical media was around 18 hours, while on SWC media it lasted for 23 hours. This is thought to occur due to differences in the utilization of nutrients in the media, and physiological differences in the test bacteria (Pelczar and Chan, 2013). The exponential phase is strongly influenced by the presence of nutrients in the test medium and the physiological ability of bacteria to utilize available nutrients as an energy source for cleavage, the volume of inoculum added also has a major influence on the sustainability of this phase (Rolfe, *et al*., 2012; Nurhajati, *et al*., 2016).

Bacterial culture in the exponential phase is an ideal culture for the application of aquaculture probiotics, in this phase the bacteria experience the highest growth so it is very effective as a bicontrol agent and as feed because it contains a complete nutritional composition (Dewi, 2014).

The Sensitivity of Bacillus spp. Isolates to Antibiotics

Sensitivity to antibiotics is one of the criteria for ideal probiotics based on the safety of the probiotic products produced. Sensitivity test for Bacillus spp. isolates. against several antibiotics commonly used in animals and humans such as the antibiotics rifampicin, gentamicin, kanamycin, and erythromycin. Sensitivity testing was carried out using standard media for antibiotic testing, namely MHA (Mueller Hinton Agar), and with the well method. The culture was incubated in an incubator for 1×24 hours at 30 C. The measurement of the test results in the form of the diameter of the inhibition zone formed is presented in Figure 2 and Table 3 along with the interpretation of the sensitivity of each test antibiotic in Table 4.

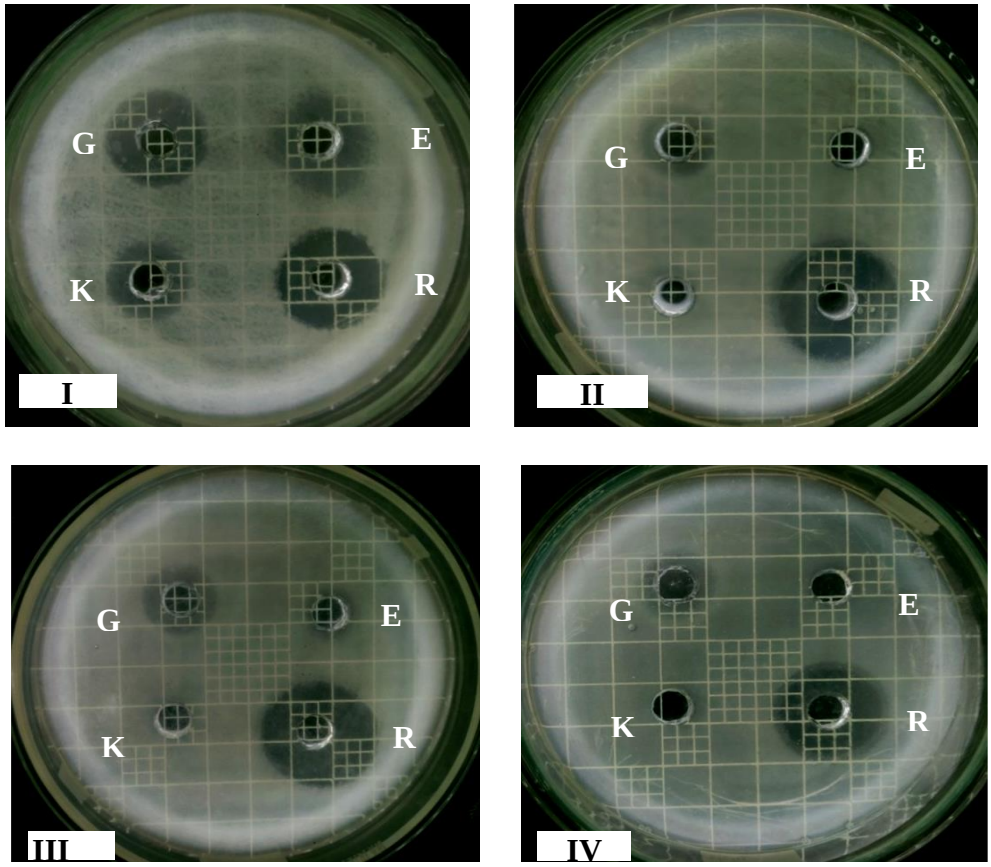

Figure 2. Inhibition zones produced by isolates (I) *B. coagulans* CaK1, (II) *B. coagulans* CaK6, (III) *B. pulminans* SLK1, (IV) *B. licheniformis* SLK2, which were tested using the antibiotic (G) Gentamicin, (E) Erythromycin, (K) Kanamycin, and (R) Rifampicin, after incubation at 30 ◦C for 24 hours.

Table 3. Data from the measurement of the diameter of the inhibition zone

Code of Isolates	Diameter zona hambat (mm)			
	R (5 µg)	G (10 µg)	K (30 µg)	E (15 µg)
B. coagulans CAK1	23	20	18	24
B. coagulans CAK6	27	15	-	-
B. pumilus SLK1	28	17	-	-
B. licheniformis SLK2	24	16	-	-

Notes: R (Rifampicin), G (Gentamycin), K (Kanamycin), E (Erythromycin), is the type of test antibiotic used.

Table 4. Interpretation of sensitivity of the four *Bacillus* spp. isolates to antibiotics

Code of Isolates	Kategori kepekaan			
	R(5 µg)	G (10 µg)	K (30 µg)	E (15 µg)
B. coagulans CAK1	S	S	S	S
B. coagulans CAK6	S	S	R	R
B. pumilus SLK1	S	S	R	R
B. licheniformis SLK2	S	S	R	R

Notes: R (Rifampicin), G (Gentamycin), K (Kanamycin), E (Erythromycin), is the type of test antibiotic used. Interpretation of Sensitivity: S (sensitive), I (intermediate), R (resistance).

Antibiotic susceptibility test is one of the requirements for probiotic candidate microorganisms to be suitable for use as probiotic products, especially for several types of antibiotics used in animals and humans. Ayichew *et al*., (2017) further explained that probiotic microorganisms must have a clear regulatory status to be used as components in food, this concerns the safety of probiotic products, the strains used must not contain virulence and resistance genes or antibiotic resistance genes. In this study, the sensitivity of *Bacillus* spp. isolates was tested. to gentamicin, kanamycin, rifampicin and erythromycin antibiotics. Antibiotic susceptibility testing was carried out using a well-drilled technique on MHA (Mueller Hinton Agar) media which is the standard medium for susceptibility testing to antibiotics recommended by CLSI (Clinical and Laboratory Standard Institute), besides that this media provided satisfactory growth (Mc.Pherson and Pincus, 2011), contains starch (starch) which absorbs toxic compounds produced by bacteria and contains low sulphonamide, trimethoprin, and tetracycline inhibitors so that they do not interfere with antibiotic activity (Atmojo, 2016).

The use of antibiotics gentamicin, kanamycin, erythromycin and riampicin as test antibiotics in this study is because these antibiotics are several types of antibiotics that are often used for the treatment of several types of fish and shrimp (Monica, *et al*., 2013; Kusmarwarti *et al*., 2017), and is a type of antibiotic used as a test material in antibiotic resistance testing in probiotic candidates (Jeon *et al*., 2018; Lee *et al*., 2019; Hu *et al*., 2021). Based on the results of sensitivity testing of *Bacillus* spp. to several antibiotics, it was found that the four isolates tested had different sensitivity responses.

B. coagulans CaK1 isolate had a sensitive sensitivity response to all types of tested antibiotics with an inhibitory zone of 23 mm for rifampicin, 20 mm for gentamicin, 18 mm for kanamycin and 24 mm for erythromycin antibiotics. *B. coagulans* CaK6 isolate had an inhibition zone value of 27 mm for rifampicin and 15 mm for gentamicin. Furthermore, in the tests carried out for kanamycin and erythromycin antibiotics on *B. coagulans* CaK6 isolates there was no inhibition zone formed, indicating that *B. coagulans* CaK6 isolates were resistant to kanamycin and erythromycin antibiotics.

The isolate *B. pumilus* SLK had an inhibition zone value of 28 mm against rifampin and 17 mm for the antibiotic gentamicin, which indicated that *B. pumilus* SLK1 was sensitive to rifampin and gentamicin, whereas in the test there were no kanamycin and erythromycin antibiotics. zone of inhibition, which indicates that *B. pumilus* SLK1 is resistant to erythromycin and kanamycin. *B. licheniformis* SLK2 isolate based on the test results had an inhibition zone value of 24 mm for rifampicin antibiotics, and 16 mm for gentamicin antibiotics, which showed sensitive sensitivity, while the isolates tested for kanamycin and erythromycin antibiotics did not have an inhibition zone, indicating presence of resistance. Up to four isolates of Bacillus spp. which have been tested are sensitive to rifampin and gentamicin antibiotics, while for kanamycin and erythromycin antibiotics 3 isolates (*B. coagulans* CaK6, *B. pumilus* SLK1, and *B. licheniformis* SLK2) showed resistance, and *B. coagulans* CaK1 shows a sensitive to the antibiotics.

Rifampicin is a semisynthetic antibiotic from the ansamycin group produced from *Amycolatopsis mediterranei*, rifampicin has a broad antibacterial spectrum, acts on gram-negative, gram-positive and mycobacterium, is bactericidal, and is active in low concentrations (Mosaei and Harbottle, 2019). Some microorganisms also show resistance to rifampicin which is largely due to chromosomal mutations in the

*rpo*B gene that encodes changes in the subunit of RNA-polymerase, as has been known in several *Mycobacterium tuberculosis*, *Escherichia coli* and several other microorganisms (Goldstein, 2014; Mosaei and Zenkin, 2020).

Gentamicin and kanamycin are aminoglycoside antibiotics that are bactericidal and it is suspected that these antibiotics have the same mechanism of action. Chromosomal resistance of microbes to aminoglycosides (16S rRNA Methylation) in the form of modification of target sites and blocking the attachment of aminoglycosides with the help of 16S-rRNA methyltransferases enzymes, resistance through plasmids depends on the formation of adenylate, phosphorylate and acetylated enzymes that can damage drugs such as Aminoglycoside cetyltransferases, Aminoglycosides Phosphotransferases , and Aminoglycoside nucleotidyltransferases and other resistances can occur due to the effect of permeability, namely changes in the outer membrane that can reduce the active transport of aminoglycosides into cells so that drugs cannot reach the ribosomes (Wachino and Arakawa, 2012; Krause et al., 2016).

Erythromycin is a macrolide antibiotic that is effective against most Gram-positive bacteria, some Gram-negative bacteria, and some atypical pathogens (Myer and Clark, 2021). Some macrolide-resistant bacteria do not have appropriate receptors on the ribosome through tRNA methylation catalyzed by the enzyme erythromycin-resistance methyltransferases, this mechanism can be through plasmid or chromosomal control (Vazquez-Laslop and Mankin, 2018).

We have also tested the ability of *Bacillus* spp. isolates. this is to grow in blood heart infusion broth media containing the antibiotics colistin and polymyxin B. These two antibiotics are drugs commonly used in humans to treat infectious diseases caused by Gram negative bacteria. The results are presented in Table 5.

Table 5. Test the ability of *Bacillus* spp isolates to grow on BHI broth media containing antibiotics

Antibiotic	*Bacillus* spp. Isolates			
	B. coagulans CaK1	*B. coagulans* CaK6	SLK1 *B. pumilus*	SLK2 *B. licheniformis*
Colistin	-	-	-	-
Polymyxin B	-	-	-	-

The data in table 5 shows that the four isolates of Bacillus spp. did not have the ability to grow on media containing the antibiotics colistin and polymyxin B, this means that the four isolates were sensitive to antibiotics that could inhibit the growth of Gram-negative bacteria. However, because *B. coagulans* CaK6, *B. pumilus* SLK1, and *B. licheniformis* SLK2 showed resistance to the antibiotics kanamycin and erythromycin 3, these three isolates could not be considered as probiotic candidates.

Resistance is a form of bacterial defense to adapt to antibiotic exposure in order to survive, bacteria can have antibiotic resistance properties caused when antibiotics are not used properly or when antibiotics are used not at the right dose and or the duration of use is not correct (Romich, 2003). 2010; Sánchez and Demain, 2015; Aprilliani *et al*., 2016). Resistance genes in bacteria can come from antibiotic-producing strains that are used to protect themselves from harmful compounds or as natural protective mechanisms. Resistance is encoded by several genes so that these genes have the potential to be transferred to other bacteria (Blair *et al*., 2015; Sánchez and Demain, 2015). The nature of resistance to antibiotics involves genetic changes that are stable and passed from one generation to another, and the existence of mutations, transduction (transfer of DNA through bacteriophages), transformation (DNA comes from the environment) and conjugation (DNA comes from direct bacterial contact with one another). Other bacteria through pili can cause resistance.

Bacteria that are not resistant can become resistant when they acquire genes that code for resistance. Some genes are transferred from members of the same bacterial species or from different bacterial species by plasmid transfer (Romich, 2010). Resistant bacteria consumed by humans can cause the spread of resistant genes to other bacteria in the human digestive tract (Forslund *et al*., 2014). Carol *et al*. (2016) stated that there are several mechanisms that cause bacteria to be resistant to drugs, including bacteria producing enzymes that destroy the active substances contained in drugs, bacteria changing their permeability to drugs, developing other target structures for drugs, developing other metabolic pathways that are bypassed when they occur. inhibitory reactions by drugs, and develop certain enzymes and metabolites so that they can still carry out their metabolic functions but are less affected by drugs.

The potential to transfer resistance properties between bacteria makes probiotic candidate microorganisms must be ensured to be safe and required to be free from antibiotic resistance, especially to antibiotics that are often used in humans and animals (Faturrahman, 2013; Ayichew *et al*., 2017) research conducted by Lee *et al.* (2019) also mentioned that Bacillus strains that are safe as probiotic products for

humans are strains that are free from resistant properties. So based on the antibiotic resistance test of the four Bacillus spp. isolates, the isolate with the most potential as a probiotic candidate was *B. coagulans* CaK1 isolate, which was able to show sensitive or sensitive properties to all the tested antibiotics in the form of Rifampicin, Gentamycin, Kanamycin, and Erythromycin antibiotics. Based on the growth test and antibiotic sensitivity test, the isolates recommended to be probiotic candidates based on this study were *B. coagulans* CaK1 isolates.

CONCLUSION

Based on growth measurements and testing for sensitivity to antibiotics on four isolates of *Bacillus* spp. probiotic candidates, it was concluded that the sensitivity to antibiotics of *Bacillus* spp. varied, isolates of *B. coagulans* CaK1 were sensitive to rifampicin, gentamicin, kanamycin, and erythromycin, while isolates of *B. coagulans* CaK6, *B. pumilus* SLK1, and *B. licheniformis* SLK2 were only sensitive to rifampin and gentamicin, and were resistant to kanamycin and erythromycin. Based on the status of sensitivity to antibiotics, *B. coagulans* CaK1 isolate was the isolate that had the most potential to be used as a probiotic candidate compared to other isolates.

ACKNOWLEDGEMENTS

This research is part of the Capacity Building research scheme funded from the University of Mataram PNBP funds in 2022. We would like to thank the Chancellor, Head of LPPM and Dean of FMIPA Mataram University for their support for this research.

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