ISOLATION AND IN-SILICO ANALYSIS OF THE POLYKETIDE SYNTHASE (PKS) GENE FRAGMENT FROM ACTINOBACTERIA *Kocuria palustris* 19C38A1

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Abstract. Marine sponges also known with able to produce antibiotic compounds which are widely used medically, but unfortunatelly they are difficult to cultivate en masse, so symbiont microbes that can be obtained from sponge tissue such as actinobacteria are utilized. One of marine sponge actinobacteria symbiont that has been successfully cultured is *Kocuria palustris* 19C38A1 which reported able to inhibit the growth of pathogenic fungi, characterized by the polyketide synthase (PKS) gene. This research was carried out to isolate and carry out in silico expression analysis to the PKS gene fragment from the marine actinobacteria *Kocuria palustris* 19C38A1, began with culturing and rejuvenating the culture of *Kocuria palustris* 19C38A1 isolates using ISP-2 media. Continued by observing microscopically, DNA extraction, gene amplification by PCR using specially designed specific primers, visualization by electrophoresis, and sequencing of the PKS 2 gene. The sequencing results were analyzed using BIOEDIT software and continued by characterizing the expression/conformation in silico via the NCBI website. The similarities were analyzed to look for matches with the deposited PKS genes in the UNIPROT database. Furthermore, the ligand binding domain is predicted using the conformation that has been deposited in the PUBCHEM database using SWISSMODEL. Through ORFFINDER, 3 potential regions coding for a protein were found, namely ORF1 (498 nt), ORF2 (447 nt), and ORF3 (84 nt). It was discovered that only the ORF 1 region was confirmed have 53.74% similarity to the Multidrugs Efflux gene RND Transporter Permease Subunit of Shewanella oneidensis bacteria (ID: WP_011073987.1).

Keywords: polyketide synthase (PKS), gene, fragment, *Kocuria palustris* 19C38A1, in silico analysis.

1 Introduction

Antibiotics are unique drugs among all drug formulations used in the world of health because they are the only class of drug agents whose primary target is not human tissue

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or its products, but rather pathogenic bacteria with a mechanism of exerting evolutionary pressure on the bacteria (Gould et al, 2015). The challenge currently faced is the large number of bacteria that are resistant to antibiotics. Steps such as antibiotic management and infection control have been implemented, but these are only able to targeting one patient and are temporary and do not address the main problem, namely antibiotic resistance. So, one way to overcome this is that new bioactive compounds are needed that come from organisms that have antibiotic potential (Gould et al, 2015).

One natural source of antibiotics comes from sea sponges. Sponges have been known to produce several bioactive compounds. such as terpenoids, alkaloids and polyketides. These compounds have antiviral, antitumor, antimicrobial and other cytotoxic activities which play a major role in the health sector. However, sponges are difficult to cultivate en masse, so symbiont microbes that can be obtained from sponge tissue are utilized. Microorganisms in symbiosis with sponges are estimated to be around 40% of the volume and 50-60% of the dry weight of the sponge tissue itself. Therefore, genes characterizing these bioactive compounds can be isolated from microorganisms, such as actinobacteria that are in symbiosis with marine sponges.

Actinobacteria from the sea show the potential performance of new bioactive compounds, but information regarding their diversity, distribution and secondary metabolites is still limited. One type of marine sponge actinobacteria symbiont that has been successfully cultured is *Kocuria palustris* 19C38A1, which we have deposited in GenBank with access code LC659429. Through our previous research which is Setiawan et al (2022), antifungal activity was recorded where this isolate showed inhibition of the growth of the pathogenic fungus Fusarium oxysporum and had the potential to inhibit the growth of pathogenic bacteria such as methicillin-resistant S. aureus from *Kocuria palustris* strain RB-107 (Santosa et al ., 2023) and Kocuria sp. (Zhang et al, 2021). One type of gene that produces bioactive compounds that can inhibit S. aureus includes the polyketide synthase (PKS) gene (Braña et al., 2017) which interacts with the compound thiophene (Wilson et al., 2013), benzofur (Aggarwal et al., 2017), β-lactones (Lehmann et al., 2017). Therefore, it is necessary to carry out research to isolate the PKS gene from the actinobacteria symbiont of the marine sponge *Kocuria palustris* 19C38A1, so that further antibiotic-producing bacteria from *Kocuria palustris* 19C38A1 can be assembled. To increase the efficiency and accuracy of isolation results, the research was carried out using an in silico approach. In silico molecular approach methods have the ability to carry out analysis with a higher level of specificity and sensitivity, shorter time, and earlier detection (Liu et al, 2023). This research is very possible to carry out because similar research has been carried out by Vatansever et. al. (2016) who have succeeded in isolating the DREB1A gene fragment and characterizing its expression using docking and molecular dynamics approaches. This research aims to isolate the polyketide synthase (PKS) gene fragment from the marine actinobacteria *Kocuria palustris* 19C38A1 which is associated with sponges and in silico expression analysis to the PKS gene fragment.

2 Materials and Methods

This research began with pre-research which was carried out from December 2021 to February 2024. Meanwhile, the research was carried out from May 2024 to October

2024 at the Biomolecular Laboratory, Biology Department, Faculty of Mathemathics and Natural Science, Lampung University. Isolation and analysis were carried out at several institutions, including DNA Extraction, DNA Nanophotometer Analysis, PCR, and Electrophoresis carried out at the Biomolecular Laboratory, Biology Department, Faculty of Mathemathics and Natural Science, Lampung University, sequencing using the Sanger method was carried out at 1st BASE DNA Sequencing Division, Apical Scientific, Malaysia.

2.1 Culture of the Actinobacterial Isolate *Kocuria palustris* 19C38A1

The actinobacterial isolate *Kocuria palustris* 19C38A1 was rejuvenated for 7 days on International Streptomyces Project 2 (ISP 2) standard media with the composition (1% malt, 0.4% glucose, 0.4% yeast extract) (Nord et al, 2019) with solvent artificial sea water (Setiawan et al, 2022).

2.2 Isolation of the Polyketide Synthase Gene *Kocuria palustris* 19C38A1

1 mL of culture from each actinobacterial isolate was taken aseptically for DNA extraction following the procedure of the Wizard® Genomic DNA Purification Kit (Promega, USA). The extracted DNA is then stored at -20℃ and ready to proceed to the next procedure.

2.3 Concentration and Purity Analysis of Extracted DNA

The concentration and purity of the extracted DNA were analyzed using a Nanophotometer (IMPLEN, Germany) following the equipment manual.

2.4 Analysis of Concentration and Purity of Extracted DNA

The extracted DNA was amplified using the Polymerase Chain Reaction (PCR) method using a Sensoquest Sensodirect thermocycle (Germany). The primers actinobacterial Polyketide Synthase gene primer was designed using the NCBI Primer BLAST website which was then validated using MEGA 11 software.

Next, each PCR reagent consists of 10.5 µL of MyTaqTM HS Red Mix mastermix (Bioline, England), 0.25 µL of forward primer PKS5 (GTCCTCTACTCGTCGGTGT), 0.25 µL of reverse primer PKS5 (GACGAAGATCAGCCGCG), 8 µL of ddH2O, and 2 µL of DNA sample which is placed in a 0.2 mL microtube. The thermocycling program used has 6 stages, namely the initial denaturation stage (94℃ for 5 minutes), denaturation stage (94℃ for 1 minute), primary attachment stage (56℃ for 1 minute), elongation (72℃ for 1 minute), final extension (72℃ for 5 minutes), and cooling (20℃ for 10 minutes). The denaturation, primer attachment and elongation stages were repeated for 35 cycles. The microtube containing the PCR sample is stored in a refrigerator at 0℃.

2.5 Visualization of PCR Results by Electrophoresis

Visualization was carried out using the capillary electrophoresis method using a QiaxCel Advanced digital electrophoresis device (Qiagen, Germany) using the DNA HIGH RESOLUTION KIT. The procedure used follows the QiaxCel Advanced manual from Qiagen, Germany.

2.6 Sequencing

DNA base sequence sequencing of the chitinase gene from the actinobacterial isolate *Kocuria palustris* 19C38A1 was carried out using the Sanger Sequencing method using the services of 1st BASE DNA Sequencing Division, Apical Scientific, Malaysia via PT. Genetic Science Indonesia. The results of the analysis in the form of DNA base sequences are then analyzed further.

2.7 Analysis of Sequencing Results

The resulting sequence in the form of an electropherogram was validated by combining forward and reverse sequences using BIOEDIT software. The six sequencing results (sequences with 6 pairs of primers) were sequenced and aligned to then estimate the open reading frame (ORF) from NCBI website (https://www.ncbi.nlm.nih.gov/orffinder/). Each ORF obtained was analyzed for similarity to look for matches with the chitinase gene that has been deposited in the UNIPROT database. Estimated sequence of the constituent amino acids from start codon to stop codon using the GenBank database at NCBI. Next, these amino acids were predicted by polypeptide modeling via the SWISSMODEL website (https://swissmodel.expasy.org/interactive). After obtaining the model, we predict the ligand binding domain with the shrimp chitin monomer conformation that has been deposited in the PUBCHEM database using modeling from SWISSMODEL (https://swissmodel.expasy.org/interactive) by in silico. If there are similarities, then the orf that has been obtained can be assumed to encode the chitinase gene from the actinobacterial isolate being worked on.

3 Result

3.1 Rejuvenation of Actinobacterial Isolates on Colloidal Chitin Agar Media

Actinobacterial isolates were rejuvenated using colloidal chitin media (Fig 1) with a composition of 1 gram of colloidal chitin and 2 grams of agar dissolved in 100 ml of sterile seawater. Referring to several previous studies, colloidal chitin media was chosen because it is a selective medium for isolating marine actinomycetes, because not all microorganisms are able to convert chitin into a nutritional source (Setiawan et al, 2022).

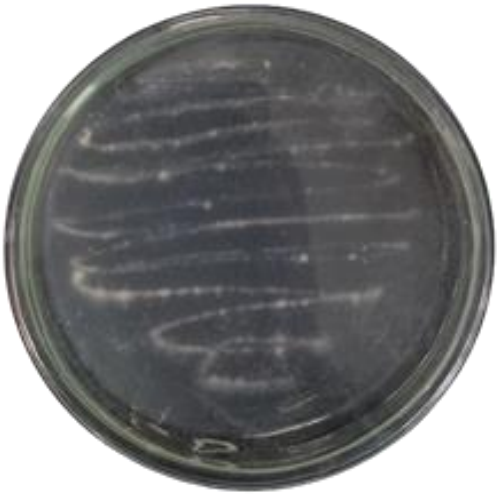

Fig 1. Macroscopic visualization of *Kocuria palustris* colonies.

The *Kocuria palustris* species as shown in Fig 1 has a white, powdery surface on colloidal chitin agar media. This is in line with research (Laila et al, 2023) that *Kocuria palustris* has white colonies with a powdery surface, which is a collection of hyphae, composed of many spores.

3.2 **Microscopic Visualization**

After the isolate has grown and there is no contamination based on macroscopic observations, purity is then verified using microscopic visualization under a microscope with the aim of making it easier to observe the cellular morphology of actinobacteria (for example, rod-shaped, cocci or spiral) and also to verify the Gram type of actinobacteria, a Gram stain is carried out. The results of the Gram staining of *Kocuria palustris* can be seen in Fig 2.

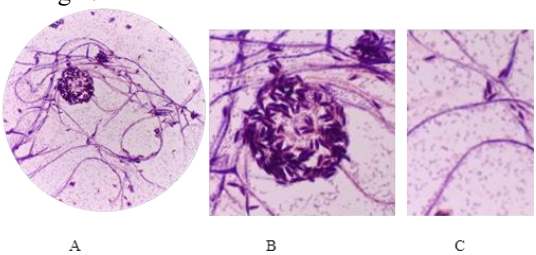

Fig 2. (a) Gram-staining to *Kocuria palustris* staining (100x magnification). (b) Observed spores (c) Observed mycelia.

After macroscopic visualization of the colony, purity verification is then carried out by microscopic visualization with a microscope to observe the cellular morphology of the actinobacteria and also verify the Gram type.

The results of the Gram staining of the *Kocuria palustris* isolate showed that the round-purple isolate also contained oval-shaped spores that were collected or separated around the mycelium. Based on verified microscopic observations, the *Kocuria palustris* isolate is a Gram-positive bacterium.

3.3 Recultured of K. palustris isolates on Liquid ISP-2 media

The isolate of *Kocuria palustris* on colloidal chitin agar media which had been verified by Gram staining treatment was then taken 1 cycle and then inoculated into liquid ISP-2 media and then incubated for 7 days in an incubator shaker. The results of the re culture shown in Fig 3.

Fig 3. Re cultured of *Kocuria palustris* isolates on liquid ISP-2 media

We get results in the form of a change in the color of the media which becomes cloudier as well as the formation of colonies that are round like white powder, in line with the results of research by Araujo-Melo et al (2019), the growth of actinobacteria on ISP-2 media liquid is characterized by the formation of sediment and turbidity in the media, as well as powder or granules depending on the type of actinobacteria being grown.

3.4 DNA Extraction

After optimizing the DNA extraction using the Wizard Genomic DNA Purification Kit and then analyzing the DNA quality using a nanophotometer (IMPLEN, Germany), the results show in Table 1 below :

Table 1. Purity and DNA concentration of the Actinobacter *Kocuria palustris*

Number	Sample	A_{260}/A_{280}	Concentration (ng/µl)
1	F2	1.914	838
2	A2	1.937	409
3	F1	1.969	287

4	A1	1.998	60.9

Based on analysis of the results of DNA extraction using a nanophotometer, the results obtained were that all four extracted DNA results (F2, A2, F1, A1) are pure DNA. Pure DNA samples were indicated by absorbance values of A260/A280 ranging between 1.8–2.0 (Lucena-Aguilar et al, 2016). DNA with a purity of 1.8–2.0 and a concentration of ≥50 ng/µL can proceed to the amplification process using Polymerase Chain Reaction (PCR). The highest bacterial DNA concentration results were found in sample F2 and the lowest bacterial DNA concentration was found in sample A1.

3.5 Gene Amplification using The PCR Method and Visualization using Electrophoresis

Based on the results of the DNA purity and concentration analysis, the extraction results can be continued to the DNA amplification process using the Polymerase Chain Reaction (PCR) process. Bacterial DNA was amplified using the polyketide synthase (PKS) gene using the PKS primer with a combination of forward primer PKS5F and reverse primer PKS5R. In this study, PCR underwent process optimization at the annealing stage where the initial annealing temperature of the PKS5 gene primer was at 57°C, experiencing a temperature decrease of 1°C to 56°C. The annealing stage usually ranges between 37-72°C, depending on the number of base pairs and the type of template being amplified (Lorenz, 2012).

Electrophoresis on an agarose gel held to visualization PCR result with a concentration of 1.2% was carried out with a voltage of 100V for 25 minutes using 5µl of PCR results and 7µl of DNA marker. The visualization of the electrophoresis process shows positive results in the PKS 5 sample with sample code RA5 shown by Fig 4.

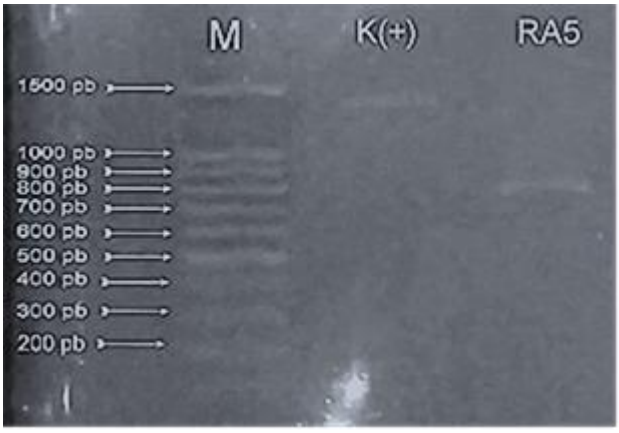

Fig 4. Sample RA5 visualized at ±800 bp by gel electrophoresis.

Based on electrophoresis visualized using gel doc in Fig 4, the result was a single band in the PKS5 RA5 sample with an amplicon size of around 800 bp.

3.6 Sequencing and Analysis of Sequencing Results Data

The sequencing anlaysis was carried out by sending samples to PT. Genetic Science. Samples were sent together with a set of PKS5 Gen primers which are packaged in one box and include the names of the sender and recipient. The sequencing test method used by PT. Genetic Science is the Sanger method. The Sanger method uses template DNA and requires specific primers for the sequencing reaction.

The sequencing results are in the form of a nucleotide sequence electropherogram in the form of an ABI file. Based on the electropherogram results, the K. palustris. sample had good results, indicated by one clear peak in the nitrogen base sequence and no build-up on the electropherogram graph (Crossley et al, 2020). Electropherogram from sequencing results shown in Fig 5 and 6.

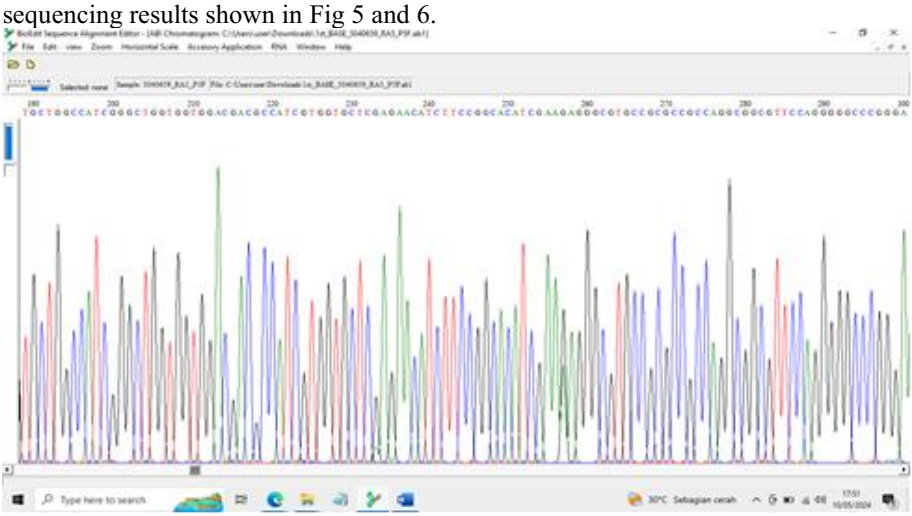

Fig 5. PKS gene electropherogram in RA5 forward.

Fig 6. PKS gene electropherogram in RA5 reverse.

Based on the good results of the good appearance of the electropherogram, proceed to the next analysis, which are the alignment and consensus of the nucleotide base sequence using the help of the BIOEDIT application. The consensus sequence are nucleotide base sequences which will then be used as a query in NCBI BLAST analysis. The consensus results obtained were then subjected to the Basic Alignment Search Tool (BLAST) test for further sequence analysis. This analysis is carried out via the NCBI website by accessing this link : https://www.ncbi.nlm.nih.gov/Blast.cgi.

Sequence alignment is a way to match DNA, RNA or protein sequences to determine the level of similarity where the arrangement process is carried out on two or more sequences so that the similarity of these sequences appears real. The result of this matching process can be called sequence alignment or alignment. The results of the consensus sequence are nucleotide base sequences which will then be used as a query in NCBI BLAST analysis. The consensus results were then subjected to the Basic Alignment Search Tool (BLAST) test for further sequence analysis. This analysis is carried out via the NCBI website by accessing the link https://www.ncbi.nlm.nih.gov/Blast.cgi.

The BLAST results shown in Fig 7, the PKS gene fragment fragment that emerged from the RA5 sample has the highest similarity to *Bordetella petri* which has a percent identity of 90.71%, the error value has reached 0.0 and the query cover is at 99%. After carrying out the BLAST test, screening is then carried out on the ORFFINDER (Open Reading Frame Finder) page https://www.ncbi.nlm.nih.gov/orffinder/.

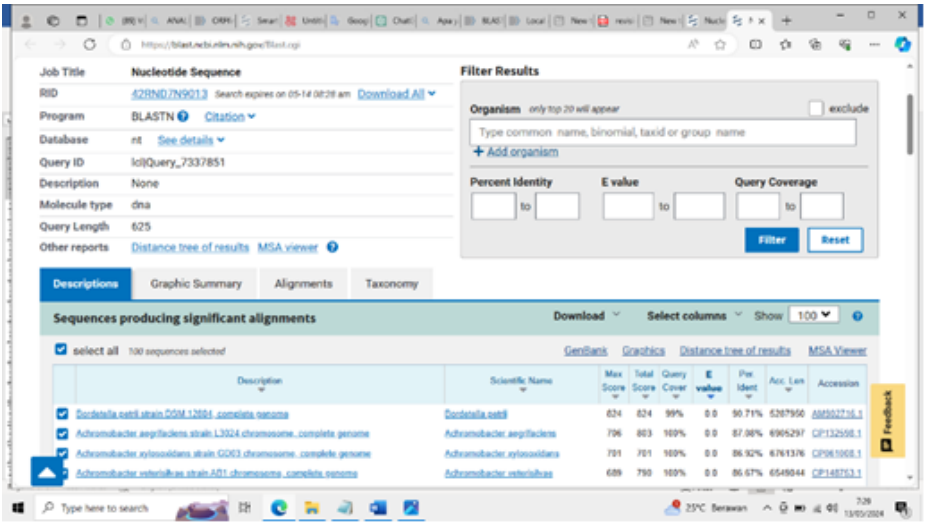

Fig 7. BLAST analysis results for RA5.

Through ORFFINDER, 3 potential regions coding for a protein belonging to the RA5 sample were found, which is ORF1 with a size of 498 nt, ORF2 447 nt, and ORF3 84 nt, then a SMART BLAST was carried out to find potential proteins based on the data bank at NCBI and compared with the nucleotide sequence which is owned by the RA5. After smart blasting, it was discovered that only the ORF 1 region was confirmed to have 53.74% similarity to the Multidrugs Efflux RND Transporter Permease Subunit gene of the Shewanella oneidensis bacteria ID WP_011073987.1, which is shown by Figure 8.

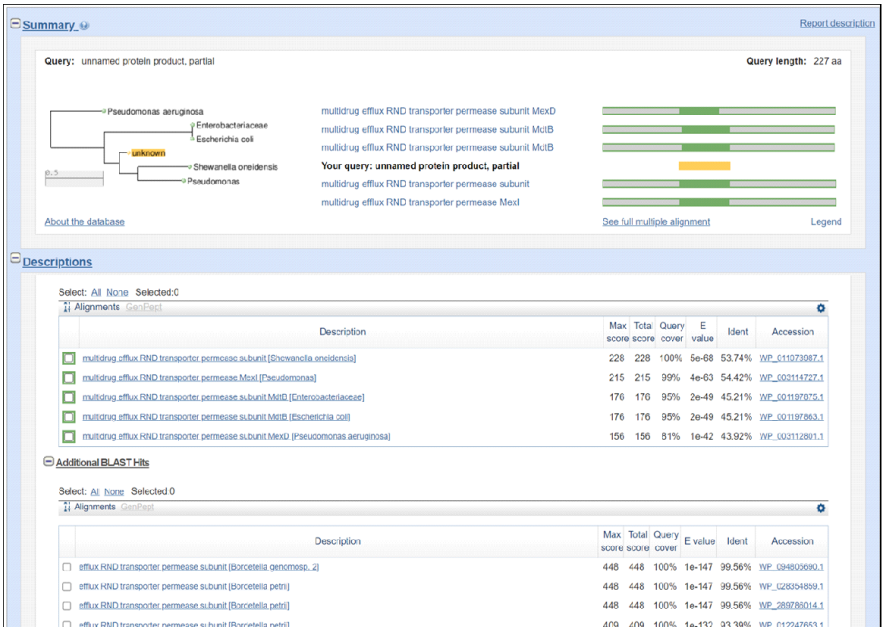

Fig 8. Smart BLAST ORF1 results for RA5.

After carrying out the BLAST analysis, potential antimicrobial activity is then carried out on the ORF (Open Reading Frame Finder) page. ORF Finder (Open Reading Frame Finder) is a program used to find and identify potential regions in a DNA sequence that have possible code for proteins (Yunus et al 2019). The main principle by analyzing DNA sequences and looking for sequences of bases that meet certain criteria, such as the minimum length expected to code for a protein or contain common start and end signs of a gene (for example, start and stop codons). SMART BLAST analysis was carried out on the ORF Finder, which then found that the ORF 1 region was confirmed positive for encoding an antibiotic gene protein, which is the Multidrugs Efflux RND Transporter Permease Subunit (RND transporter permease subunit for multi-drug efflux).

The amino acid sequence of ORF1 was predicted by model in SWISSMODEL (https://swissmodel.expasy.org/interactive). This structural model is shown in Fig 9.

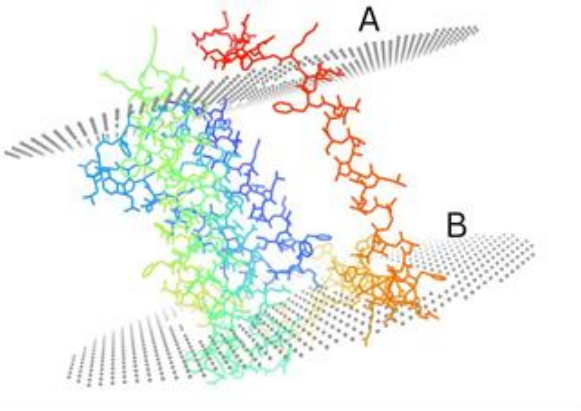

Fig 9. Protein structure model from SWISSMODEL. A. Outer lipid membrane and B. Inner lipid membrane of the *Kocuria palustris* 19C38A1.

4 Discussion

Actinobacterial colonies generally have a convex, round and smooth surface, even wrinkled, irregular edges and are attached to the growth medium. The collection of hyphae consisting of many spores makes the actinobacterial colony on its surface look powdery like flour. Actinobacterial colonies are covered with hyphae which are surrounded by a hydrophobic sheath and point towards the surface of the colony into the air and are covered by free air mycelium (Laila et al, 2023). The observation continued with microspically observation to observe the cellular morphology of the actinobacteria and also verify the Gram type. Gram staining aims to classify bacteria based on their Gram type. Gram staining is also useful for determining extraction methods based on the grouping of Gram types of bacteria. Gram positive bacteria will be purple and Gram-negative bacteria will be red. Gram staining functions to give color to bacterial cells so that they appear more clearly when observed in a microscope (NauE et al, 2022). Gram staining is based on differences between the structure of bacterial cell walls which ultimately causes color differences (Mahmudah et al, 2016). Gram-negative bacteria are composed of thick lipids so that when they are given alcohol, the violet crystals trapped in the lipid layer will fade and the peptidoglycan will absorb the second color, namely safranin. The results of the staining appear to be in line with the results of research by Kandi et al (2016), *Kocuria palustris* bacteria on blue Gram stains tend to be purple which is Gram positive bacteria, also based on research by Setiawan et al (2022) which shows that isolates of *Kocuria palustris* are oval-shaped spores that collect or separate around the mycelium (Setiawan et al, 2022).

Re cultured of *Kocuria palustris* isolates was carried out on liquid ISP-2 media as preparation for the DNA extraction process, where to facilitate and increase the success of DNA extraction it is necessary to pay attention to several factors, one of which is the choice of media, because the media will affect the density of growing colonies and It also influences the cell breakdown process of actinobacterial cells. ISP-2 media has ingredients and nutrients that are very suitable for supporting the growth of actinobacterial, such as yeast extract, which is a product of the breakdown of yeast cells

which consists of soluble components including protein, amino acids, peptides, which is a source of nutrition that is widely used as a medium. microbial growth (Zarei et al, 2016). According to Widiastoety et al (2003) yeast extract is used as a supplement in microbial growth media because it contains peptides, amino acids and is known as a source of nitrogen which has a role in physiological processes because nitrogen is a component of nucleic acids, proteins and other important substances that will have an effect. in the formation of actinobacterial cell structures.

DNA extraction is the starting point in any molecular biology study. DNA stores all biological information in every organism in the form of a linear sequence of polynucleotides which are then translated into mRNA and then undergo a transcription process into a sequence of amino acids which determine the structure of the proteins that make up the cells of every living creature, which will have a big influence on the optimal biological function of cells. Therefore, DNA provides instructions for maintaining biological functions and is considered the blueprint of life (Minchin and Lodge, 2019). Previously, the DNA extraction method for isolating the polyketide synthase (PKS) gene from the *Kocuria palustris* 19C38A1 which is in symbiosis with Sponges was carried out using a method derived from the Wizard® Genomic DNA Purification Kit, however the quality and quantity of the DNA, both concentration and purity of the extraction results was not detected, which should have been under 50 ng/ µL. Therefore, optimization was carried out on DNA extraction of the polyketide synthase (PKS) gene from the *Kocuria palustris* 19C38A1.

Modifications to the *Kocuria palustris* DNA extraction were carried out at the initial stage, namely the lysis process by adding 200 µl chloroform and 2 bullets into the tube for the tissue lyser process. This aims to maximize the cell wall breakdown process so that the materials in the cells such as DNA, RNA, protein and other cell organelles can be released. The lysis process in DNA extraction is a crucial process in determining the level of DNA concentration and the success of the extraction process, because cell lysis is the initial process which will influence the purification, precipitation and rehydration processes of DNA extraction. The cell lysis or cellular disruption process is a method in which the outer boundary or cell membrane is broken or destroyed to release intercellular materials such as DNA, RNA, proteins or organelles from the cell (Islam et al, 2019). Cell lysis is an important unit operation for molecular diagnostics. Optimization of DNA extraction was also carried out at the precipitation stage, namely by adding the volume of precipitated protein with a volume increase of 50 µl, the initial volume before optimization was from 200 µl to 250 µl. This is done with the aim of maximizing the precipitation process so that DNA can be free from protein contamination by adding a protein precipitation solution.

The DNA extraction results are then continued to the DNA sequence amplification process using Polymerase Chain Reaction (PCR). Polymerase Chain Reaction (PCR) is a nucleic acid amplification technique used to denature and renature short segments of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) using the DNA polymerase enzyme. PCR is a monumental tool used in biomolecular science because of its profound capabilities in examining and detecting amplified DNA components. PCR consists of three main phases: denaturation, hybridization/annealing, elongation/amplification. During the denaturation phase, DNA is heated to 95°C to break apart hydrogen bonds between complementary base pairs of double-stranded DNA. After completion of the denaturation process, the annealing process occurs;

Annealing is the process of cooling DNA that has been separated by a previous denaturation process. The annealing process usually ranges at a temperature between 37-72°C, where this temperature allows hydrogen bonds to re-form. Although PCR is an advanced innovation in the molecular world because it can produce a large supply of a particular DNA segment (amplicon) from only a small amount of starting material, namely, a DNA template or target sequence. However, sometimes there are obstacles that complicate the reaction, resulting in false results.

PCR failure can cause multiple non-specific DNA products of varying sizes to appear as ladders or band spots on agarose gels. Sometimes no product is even formed at all. Another potential problem occurs when mutations are accidentally introduced into the amplicon, resulting in a heterogeneous population of PCR products (Lorenz, 2012). This of course requires optimization of the PCR process, both in terms of stages, temperature, stages and time of each stage so that it can produce good and accurate results.

The amplified sequence was then visualized using electrophoresis, which in this study used gel electrophoresis. Gel electrophoresis is useful for separating macromolecules, such as DNA, RNA fragments, or proteins, based on molecular size or charge so that the range of sizes of DNA bands that have been amplified can be known. The results of electrophoresis will be visible in the form of glowing bands when the agarose gel is visualized on a gel doc with the help of Blue Light rays, the glowing bands visible on the agarose gel are formed due to the interaction between positive ions in the SYBR safe DNA gel stain and negative ions in the DNA (Anam et al, 2021). The amplicons visualized in this study correspond to the size limitations of the primary amplicons used. Despite from functioning as an attachment to the target DNA, the primer also functions to estimate the length of the DNA visible on the electrophoresis gel. According to that fact, the DNA size that appears in the visualization of electrophoresis results needs to be compared with the estimated amplicon length based on primer design (Ye et al, 2012).

The well-visualized DNA then continue to sequencing. DNA sequencing aims to determine the sequence of nitrogen bases (adenine, guanine, cytosine and thymine) in a DNA sample. The sequencing method in this research refers to the Sanger method, Sanger sequencing technology and its modifications have dominated sequencing methods for 30 years (Crossley et al, 2020). The sequencing results are in the form of a nucleotide sequence electropherogram in the form of an ABI file. An electropherogram is a graph formed from the detection of ddNTP molecules marked with a fluorescent marker on the DNA amplicon. Each ddNTP molecule has a fluorescent marker with a different color, green is indicated by adenine bases, red is indicated by thymine bases, blue is indicated by cytosine bases, and black is indicated by guanine bases. A good electropherogram is an electropherogram that has one clear peak in the nitrogen base sequence. Based on the electropherogram results, the Kocuria palustris. sample had good results, indicated by one clear peak in the nitrogen base sequence and no buildup on the electropherogram graph (Crossley et al, 2020).

The sequencing results showed the highest similarity to the Multidrug Efflux RND Transporter Permease Subunit. Multidrug Efflux RND Transporter Permease Subunit is a component of a complex system found in many Gram-negative bacteria but also some in Gram-positive bacteria.The mechanism for pumping these drugs from inside the bacterial cell to the environment that threatens or introduces toxic substances

originating from outside the environment into the periplasm of the bacterial cell, then transporters and periplasmic proteins work together to direct and pump it out of the bacterial cell to be returned to the environment (Delmar et al ., 2014). According to research by Matsuo et al (2013), Multidrug RND Transporter is a group of proteins that cause antibiotic resistance in most Gram-negative bacteria, especially bacteria originating from the digestive organs, especially E. coli. The multi drug efflux group was identified in five main groups, namely the RND (Resistance Nodulation cell Division) group, the MF (major facilitator) group, the MATE (Multidrug and Toxic compound Extrusion) group, the SMR (Small Multidrug Resistance) group, and the ABC (ATP Binding) group. cassette). The RND group consists of three constituent components, those are the inner membrane protein (IMP) known as AcrB, the periplasmic membrane fusion protein (MFP) known as AcrA, and the outer membrane protein (OMP) known as ToIC, all three of which work together to eliminate the effects of toxic compounds that enter bacterial cells which have the potential to threaten bacterial life (Matsuo et al, 2013). However, Matsuo et al (2013) reported the existence of Multidrugs RND Transporter also has the potential to produce antibiotic compounds, exposed to an unfavorable environment or conditions that threaten the survival of bacteria. Based on that similarity, it can be said that the ORF1 sequencing results that we found is an efflux for certain compounds, including bioactive compounds. In the future, it will be very interesting to compare further with the bioactive compounds produced by *Kocuria palustris* 19C38A1.

5 Conclusion

Based on the research results and discussion above, it can be concluded that the polyketide synthase (PKS) gene fragment from the actinobacteria *Kocuria palustris* 19C38A1 has been successfully isolated and have 53.74% similarity to the Multidrugs Efflux gene RND Transporter Permease Subunit of *Shewanella oneidensis* bacteria (ID: WP_011073987.1).

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