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RESEARCH ARTICLE

Molecular identification of the bacterium acute conjunctivitis by the method of sequensing gen 16S rRNA

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Abstract

Conjunctivitis is the most common eye disease, characterized by contextual inflammation, which can be caused by bacteria. The diagnosis of conjunctivitis is established based various factors, including the patients's medical history, onset of eye symptoms, non-ocular symptoms, previous disease history, family medical history, allergies, and physical examination of the eyes, which may include assessments of visual acuity and vision field. Some cases of conjunctivitis require laboratory examinations to confirm the diagnosis. These may include cytological examination with Giemsa staining, Chlamydia Diagnostic Test, Nucleic acid amplification tests (NAATs) or Polymerase chain reaction (PCR), and microbiological tests. Microbiological examination helps identify the bacteria responsible for conjunctivitis and aids in treatment by prescribing antibiotics to suppress the growth of the infecting bacteria. While identification tests are not routinely performed, researchers often seek to determine the specific type of bacteria causing conjunctivitis infections, which may require several laboratory tests. Bacterial virulence plays a significant role, with genetic mutations potentially leading to severe infections of varying severity. Virulence genes encode proteins that express pathogenic properties. The species responsible for conjunctivitis can be definitively identified definitively through microbiological examination, utilizing methods such as the 16S Ribosomal RNA Sequencing (rRNA) technique, known for its accuracy and speed. This study aims to analyze the results of rRNA sequencing in cases of acute bacterial conjunctivitis caused by 16S rRNA genotyping. The research employed an exploratory metholodolot, with the results analyzed using the The Basic Local Alignment Search Tool (BLAST) tracking program database on the National Center for Biotechnology Information (NCBI) website. The findings revealed that *Sphingomonas paucimobilis* encoded the 16S rRNA using Universal Primary 27 F and 1492 R, obtained in a sequence size of 1351bp. The isolate demonstrated similarities to *Sphingomonas paucimobilis*.

1. INTRODUCTION

Conjunctivitis is an inflammation of the conjunctiva caused by microorganisms invasion, hypersensitivity reactions, and degenerative changes (1). It is the most common eye disease that causes complaints of red eyes (2). Symptoms associated with conjunctivitis includes redness of the eyes, excessive tearing, sticky eyelids, oedema eyelids, and increased eye secretions (3).

In Indonesia, it ranked in the top 10 most outpatient diseases in 2009, with 135,749 patients who visited the eye clinic, 73% were cases of conjunctivitis (4). The incidence of conjunctivitis at the Bandung City Health Centre in 2017 was 13,690 cases (5). The Department of Ophthalmology in Brisbane states that

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keratitis and conjunctivitis are the top problems among the five common problems in primary systemic health care (6).

Conjunctivitis can result from bacteria infections, chlamydia, allergies, and exposure to viral toxins, which associated with systemic diseases. Bacterial conjunctivitis, specifically, is characterized by inflammation of the conjunctiva caused by microorganisms such as *Streptococcus pneumoniae, Moraxella catarhalis, Neisseria gonorrhoeae, Neisseria meningitidis,* and Gram-negative bacteria originating from the gastrointestinal tract (7).

Diagnosis conjunctivitis extends beyond anamnesis and physical eye examination. In many cases, laboratory test is essential to confirm diagnosis. These may include cytological examinations using Giemsa staining, fungal diagnostics, microbiological cultures, and Nucleic acid amplification tests (NAATs), such as Polymerase Chain Reaction (PCR) (8). Bacterial identification involves a methodical process of observing, testing, recording, and identifying specific types of organisms based on test results (9).

Microbiological examination is carried out through conjunctival swab culture examination of patients with conjunctivitis. The bacterial culture examination results are intended for the patient who received antibiotics suitable to the patient's condition by considering the causative bacteria and antibiotic resistance. Bacterial culture technique helps determine the morphological and biochemical properties of bacteria (10).

Molecular identification methods, like 16S ribosomal RNA (rRNA) gene sequencing, offer a precise approach to identify bacterial species by analyzing their genetic makeup. By targeting specific regions of the bacterial genome, such as the 16S rRNA gene, these methods provide insight into the evolutionary relationships among different bacterial taxa. This approach is valuable in fields like microbiology and epidemiology for accurate species identification and tracking microbial evolution. The 16S rRNA gene is about 1550 base pairs in size and about 500 bases at the end of the sequence in a region called hypervariable regions. The hypervariable region is the part that distinguishes between organisms. This technique analyzes the DNA bases structure or arrangement within the 16S DNA region. The most commonly used DNA sequencing method is the Sanger method, which has the advantage of a complete base arrangement than the DNA template. With the development of the world of biotechnology, efforts to determine the specific type of bacteria that cause a disease accurately and efficiently are needed, to make it easier to overcome the spread of diseases caused by pathogenic bacteria (11).

2. MATERIALS AND METHODS

The research methodology employed a combination of exploratory and descriptive approaches to analyze conjunctival swab samples collected from patients diagnosed with Acute Conjunctivitis. The study was conducted between April and December 2022 at the Bacteriology and Parasitology Laboratory of IKES Rajawali, Indonesia.

2.1. Sampling and DNA Extraction

The study population comprised patients diagnosed with conjunctivitis at the PMN Laboratory Installation of Cicendo Eye Hospital. A purposive sampling technique was employed to collect conjunctival swab samples from these patients. Inclusion criteria for the study encompassed symptoms such as redness of the eye, eyelid swelling, and excessive discharge, while patients with healthy eyes and no eyelid swelling were excluded.

The research utilized conjunctival swab samples obtained from conjunctival secretions. Inclusion criteria involved symptoms such as red eyes, eyelid swelling, and excessive secretions. Subsequently, a single bacterial isolate was chosen for sequencing. The etiological agent of Acute Conjunctivitis was identified from swab samples isolated from patients' conjunctival secretions at Cicendo Eye Hospital, Bandung. Genomic DNA extraction was conducted according to the Quick-DNA Fungal/Bacterial Miniprep Kit protocol (Zymo Research, D6005).

2.2. Amplification of 16S rDNA

The extracted DNA was utilized as a template for amplifying a segment of approximately 500 or 1,500 base pairs from the 16S rRNA gene sequence through Polymerase Chain Reaction (PCR). The PCR was performed under optimal conditions using the Rotor-Gene Q PCR system. The amplification process employed the MyTaq™ HS Red Mix method (Bioline, BIO-25048). Universal primers 27 F (5'-AGAGTTTGATCMTGGTCCAG-3') and 1492 R (5'-GGTTACCTTGTTACGACTT-3') were utilized in the PCR technique. Subsequently, the DNA isolation results were quantitatively tested to determine the level of purity. The concentration of the DNA isolation results was measured, and its purity was assessed by calculating the absorption ratio at wavelengths Aλ260/Aλ280 nm using a spectrophotometer.

2.3. 16S rDNA Sequencing

DNA sequencing or DNA base sequencing aims to determine the sequence of nitrogen bases (adenine, guanine, cytosine and thymine) in a sample. The method used is the Sanger method. The Sanger method is a determination of the nucleotide sequence based on specific base termination by ddNTP (Dye Terminator labeling) when DNA synthesis is carried out in vitro by the polymerase enzyme using one primer. The sequencing results obtained the DNA sequence of the bacteria that causes Acute Conjunctivitis. The Sanger method is basically the same, but does not use chemicals to determine the base composition. The Sanger method refers to the premature termination of DNA synthesis resulting in chain-terminating dideoxynucleotides i.e. there is no 3' hydroxyl deoxyribose in the DNA polymerase reaction. DNA synthesis is initiated by primers resulting in four separate reactions that occur simultaneously. Each reaction contains one dideoxynucleotide (A, C, G, T) which in the next stage will stop DNA synthesis. This phenomenon occurs due to the absence of a 3' hydroxyl group necessary for the addition of the next nucleotide in the DNA strand. As a result, a series of labeled DNA molecules is generated, with each molecule experiencing termination at the specific base introduced by the dideoxynucleotide.

2.4. Phylogenetic Analyses

A phylogenetic tree was designed using comparison of 16S rRNA sequences from other bacteria in the Basic Local Alignment Search Tool (BLAST) database tracking program with the website address https://blast.ncbi.nlm.nih.gov/Blast.cgi. Alignment was visualized using the ClustalW program. Phylogenic tree formation was carried out using the MEGA11 program.

2.5. Nucleotida Sequence Accesion Numbers

The results of sequencing in the form of nucleotide sequences were analyzed using the BLAST (Basic Local Alignment Search Tools) program on the NCBI website (http://www.ncbi.nlm.nih.gov). Results from BLAST (Basic Local Alignment Search Tool). The results of DNA sequencing analysis of bacterial isolates that cause acute conjunctivitis using BLAST showed that this isolate had a homology or level of similarity of 99.85% with the species *Sphingomonas paucimobilis* strain GSG_2 which had a max score of 2484, total score of 2484, query cover of 100%, and e-value. 0.0. Nucleotida sequence accesion numbers is MK802906.1.

3. RESULTS AND DISCUSSION

The five samples used in this study were collected from patients diagnosed with Acute Conjunctivitis who presentwd themselves for examination at e PMN Laboratory Installation within Cicendo Eye Hospital. Conjunctival swab samples were then transported to the Bacteriology and Parasitology Laboratory of the Rajawali Health Institute using Amies transport media (merck) for microbiological testing.

The swab sample were inoculated on Nutrient Agar media (Merck, Cat. No. 70148) for bacterial isolation and subsequent identification. The isolated colonies were identified through macroscopic, microscopic, biochemical, and DNA sequencing methods. The results of the isolation of bacteria on the Nutrient Agar were observed macroscopically (Table 1). Microscopic observations were conducted using Gram staining, and the findings are outlined in Table 2 and depicted in Figure 1. Additionally, the results of biochemical tests are summarized in Table 3.

Table 1. Characteristics of colonies on nutrient agar plates inoculated from conjunctival swab samples

Sample Code	Colony Code	Colony Character				
		Shape	Color	Elevation	Edge	Size (Diameter) mm
AC01	YAC1	Circular	White	Raised	Entire	1-4
AC02	YAC2a	Circular	White	Convex	Entire	3
	YAC2b	Circular	Yellow	Convex	Entire	3-5
AC03	XAC3	Circular	White	Raised	Entire	Full
AC04	XAC4	Circular	White	Raised	Entire	1-2
AC05	YAC5	Circular	White	Raised	Entire	1-3

Table 2. Microscopic observation of the bacteria isolated from conjunctival swab samples

Colony Code	Microscopic observation			
	Cell Shape	Arrangement	Color	Gram Stain
YAC1	Coccus	Swarm	Purple	+
YAC2a	Coccus	Two by two	Red	-
YAC2b	Basil	Chain	Red	-
XAC3	Coccus	Swarm	Purple	+
XAC4	Basil	Swarm	Purple	+
YAC5	Coccus	Swarm	Purple	+

Table 3 indicates that only five bacterial colonies were available for biochemical testing, as there was only one colony, namely YAC2b. The failure of the prufication culture to grow could be attributed to the inappropriate selection of media and treatment of bacterial isolates. Due to these limitations, the selection of isolates for the next step of PCR is based on representative results. The YAC2b bacterial isolates were selected to illustrate the PCR results.

Table 4 provides the molecular identification results for these isolates, accompanied by the corresponding DNA concentration and purity values. DNA concentration and purity were assessed by spectrophotometry, specifically measuring absorbance at a wavelength ratio of A260/280, providing insights into both the quantity and quality of the DNA sample (12). The results of DNA amplification were confirmed through electrophoresis. Electrophoresis of PCR amplification of DNA samples of YAC2b isolates of the causative bacteria Acute Conjunctivitis presented in Figure 2.

In Figure 2, the PCR amplification results are visualized through electrophoresis. Electrophoresis was carried out for 45 minutes at a voltage of 220 volts. The resulting amplicons, generated using primers 27 F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492 R (5'-GGTTACCTTGTTACGACTT-3'), represent the 16S rRNA genes with an approximate size of ± 1500 bp. The 27 F and 1492 R are indeed universal primers commonly used for PCR amplification of bacterial 16S rRNA genes. The forward primer 27 F targets a conserved region near the 5' end, while the reverse primer 1492 R targets a conserved region near the 3' end of the 16S rRNA gene. These primers are designed to amplify a broad range of bacterial taxa, making them valuable tools for microbial diversity studies and bacterial identification.

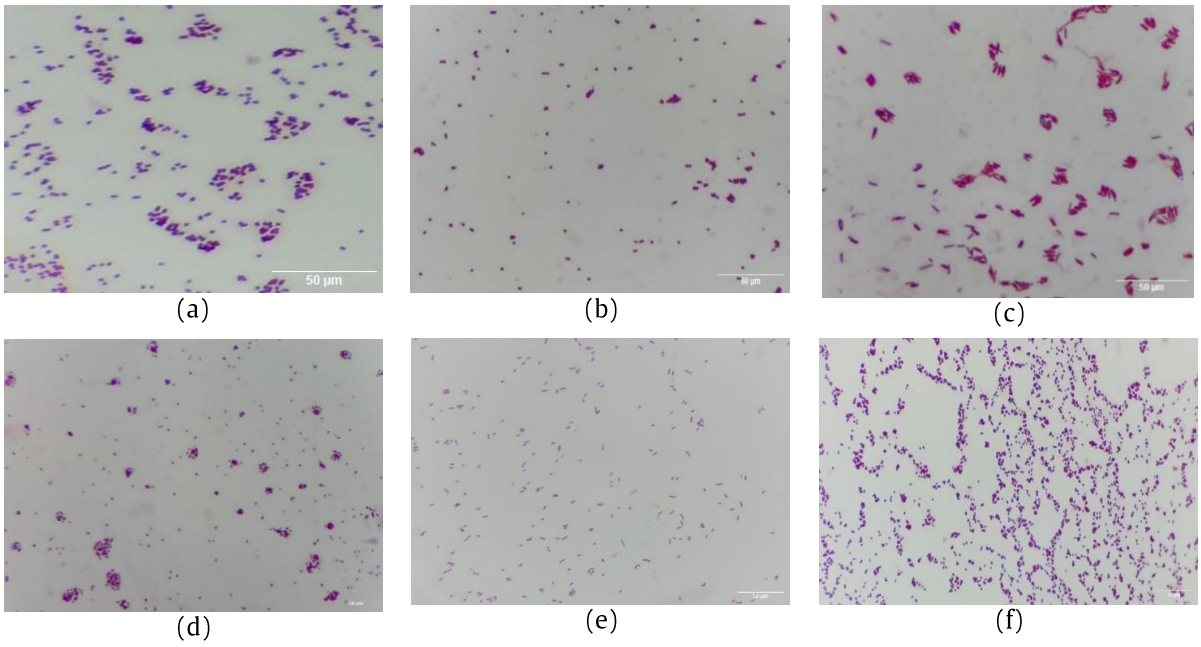

Figure 1. Bacterial isolate Gram staining results from conjunctival swab samples: (a) YAC1 isolate; (b) YAC2a isolate; (c) YAC2b isolate; (d) XAC3 isolate; (e) XAC4 isolate; (f) YAC5 isolate

Table 3. Biochemical test results for the isolated colonies

	Colony Code				
	YAC1	YAC2b	XAC3	XAC4	YAC5
Sugar Fermentation					
Glucose	+	-	+	+	+
Sucrose	+	-	+	+	+
Lactose	+	-	+	-	+
Fructose	+	-	+	+	+
Mannitol	+	-	-	+	+
Biochemical Test					
Citrat	-	+	-	+	-
MR	+	-	+	+	+
VP	-	-	-	+	-
Indol	-	-	-	-	-
Urease	-	+	-	+	-
Motility	-	+	-	+	-
Catalase	+	-	+	-	+
Coagulase	+	-	-	-	+

Table 4. Concentration and purity of DNA isolate YAC2b cause acute conjunctivitis

Isolates ID	DNA Concentration (ng/µl)	Purity λ260/280
YAC2b	70.9	2.00

The PCR product sequencing stage was carried out to determine the nucleotide base sequence of the bacterial isolate YAC2b, which is considered the causative agent of Acute Conjunctivitis. This process involves amplifying specific regions of the bacterial genome using PCR and then sequencing the amplified DNA to identify the order of nucleotide bases. The resulting sequence data provides valuable information for identifying the bacterial isolate and understanding its genetic characteristics. The nucleotide base sequence of the YAC2b isolate associated with Acute Conjunctivitis is detailed in Table 5.

The sequencing results revealed nucleotide sequences attributed to YAC2b bacteria, which were subsequently analyzed using BLAST (Basic Local Alignment Search Tool) on the NCBI website (http://www.ncbi.nlm.nih.gov). The outcomes of this analysis are summarized in Table 6, providing valuable information about the genetic identity and potential relationships of the YAC2b isolate with known sequences in the GenBank database.

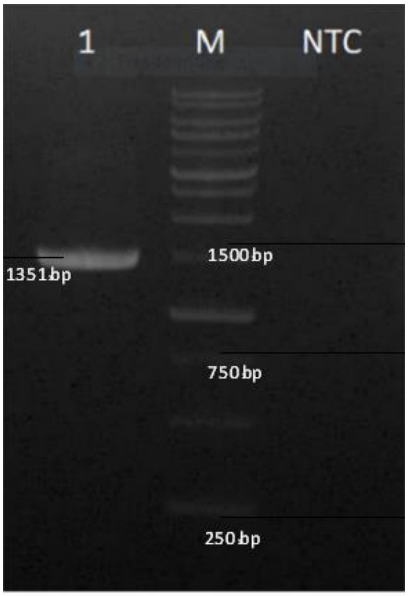

Figure 2. Electrophoresis results of 16S rRNA gene amplification on YAC2b isolate; Non-template control (NTC) is a control that does not contain DNA samples but contain nuclease free water (NFW)

Table 6 presents the results of the BLAST analysis of the 16S rRNA gene partial sequence of YAC2b bacteria *S. paucimobilis* strain GSG_2. This strain was selected as the closest match to the obtained DNA strain based on its maximum score and total score, both of which were 2484. The alignment also showed 100% query coverage, an e-value of 0, and a maximum identity of 99.85%. A comparison of nucleotide base sequences between isolates of YAC2b bacteria causing Acute Conjunctivitis and *S. paucimobilis* is illustrated in Figure 3.

Table 5. Base sequence of the causative YAC2b isolate acute conjunctivitis

Isolates ID	Language Order
YAC2b	AAGTCGAACGAAGGCTTCGGCCTTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTAG GTTCGGAATAACAGCTGGAAACGGCTGCTAATACCGGATGATATCGCGAGATCAAAGATTTATCG CCTGAGGATGAGCCCGCGTTGGATTAGGTAGTTGGTGGGGTAAAGGCCTACCAAGCCGACGATCC ATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGA AGGCCCTAGGGTTGTAAAGCTCTTTTACCCGGGAAGATAATGACTGTACCGGGAGAATAAGCCCC GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGGGCTAGCGTTGTTCGGAATTACTGGGC GTAAAGCGCACGTAGGCGGCTTTGTAAGTCAGAGGTGAAAGCCTGGAGCTCAACTCCAGAACTG CCTTTGAGACTGCATCGCTTGAATCCAGGAGAGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATT CGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTGACTGGACTGGTATTGACGCTGAGGT GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAAC TAGCTGTCC
	GGGCACTTGGTGCTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGCCGCA AGGTTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAA GCAACGCGCAGAACCTTACCAGCGTTTGACATGGTAGGACGACTTCCAGAGATGGATTTCTTCCCT TCGGGGACCTACACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTCGCCTTTAGTTACCATCA

Figure 3 elucidates that the YAC2b bacterial strains obtained from the order 1 to 1351 are similar to *S. paucimobilis* strain GSG_2 sequences 42 to 1393. BLAST alignment was performed on the two nucleotide chains used, the query is the nucleotide sequence of the YAC2b isolate obtained after sequencing, and the subject is the nucleotide sequence in the database. The symbol "|" indicates a match or alignment between the two sequences. The gap is indicated by the sign if there is no symbol showing a mismatch or non-match between the two sequences. The nucleotide base is replaced by the letter "N" indicating that it can be replaced by any of the four-existing base.

The phylogenetic tree was used to determine the relationship or similarity between YAC2b isolates and species in GenBank based on the sequence of nucleotide bases it is evident in Figure 4. Figure 4 illustrates the causative bacteria Acute Conjunctivitis YAC2b isolate, has a branch separate from the other ten isolated bacteria in the phylogenetic tree.

However, according to the BLAST homology table (Table 6), it is evident that the YAC2b isolate shares the closest 16S rRNA homology with the YAC2b isolate *S. paucimobilis*, exhibiting a similarity percentage of 99.85%. Although the YAC2b isolate shares a common ancestor, it underwent different changes as it evolved. Therefore, the YAC2b isolate bacteria cannot be considered a new bacterial species, as the homology value of YAC2b isolates is 99.85%.

Bacteria are designated as new species if their nucleotide homology is less than 70% similarity (13). The analysis of the 16S rRNA gene sequences, which have the same nucleotide base sequence, indicates that a similarity of at least 97% suggests the species is the same. Conversely, if the similarity is less than 97%, the isolated bacteria are considered a new species (14).

Based on the observations presented in Table 2 and Figure 1, it was observed that the five isolates consisted of both Gram-positive and Gram-negative bacteria with varying cell shapes. To determine the species, biochemical tests were conducted. Bacterial biochemical testing involves employing methods or procedures to identify and characterize a pure culture of isolated bacteria based on their physiological properties.

The YAC2a colony, based on the Gram stain results, raised suspicion that the bacteria belonged to the Neisseria genus. For specific treatment of Neisseria species, media such as brown blood agar or Thayer Martin media were employed due to their higher sensitivity and specificity. These media were then incubated at 37°C under 5% CO_2 pressure. Maintaining a 5% CO_2 pressure can be achieved using the wax mask method or an anaerobic jar. It's worth noting that Neisseria species have limited survival in atmospheric air, quickly perish in dry conditions, and cannot withstand temperatures exceeding 39°C (15).

Based on the results of macroscopic, microscopic and biochemical tests, it was determined that the selected isolates, YAC2B were Gram-negative bacteria. Severe eye infections result from virulent conjunctivitis caused by Gram-negative bacteria (16). To confirm the species of YAC2b isolate, molecular identification was perfomed.

Table 6. BLAST analysis results of YAC2b bacterial nucleotide sequence on GeneBank

Query Sequence	Matched Organisms	% Identity	E-Value	Accesion Number	Reference
100%	*Sphingomonas paucimobilis* strain GSG 2 16S ribosomal RNA gene partial sequence	99.85	0.0	MK802906.1	NCBI
99%	*Sphingomonas paucimobilis* strain S_WE.S7.040 16S ribosomal RNA gene_ partial sequence	99.93	0.0	OL636271.1	NCBI
100%	*Sphingomonas paucimobilis* strain P8-44 16S ribosomal RNA gene _partial sequence	99.70	0.0	MN181176.1	NCBI
100%	*Sphingomonas paucimobilis* strain P8-31 16S ribosomal RNA gene _partial sequence	99.70	0.0	MN181165.1	NCBI
100%	*Sphingomonas paucimobilis* strain P8-1 16S ribosomal RNA gene _partial sequence	99.70	0.0	MN181148.1	NCBI
100%	*Sphingomonas paucimobilis* JCM 7521 gene for 16S ribosomal RNA _partial sequence	99.70	0.0	LC504035.1	NCBI
100%	*Sphingomonas paucimobilis* JCM 7519 gene for 16S ribosomal RNA _partial sequence	99.70	0.0	LC504033.1	NCBI
100%	*Sphingomonas paucimobilis* JCM 7518 gene for 16S ribosomal RNA _partial sequence	99.70	0.0	LC504032.1	NCBI
100%	*Sphingomonas paucimobilis* JCM 7511 gene for 16S ribosomal RNA _partial sequence	99.70	0.0	LC504025.1	NCBI
100%	*Sphingomonas paucimobilis* JCM 7509 gene for 16S ribosomal RNA _partial sequence	99.70	0.0	LC504025.1	NCBI

The three fundamental processes for molecular identification of microbes are DNA extraction, DNA amplification by PCR, and electrophoresis. The basic principle of DNA extraction is a series of processes that separate DNA from other components, such as proteins (17). The Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) used in the DNA extraction. The first step in DNA separation is breaking or destroying the membrane and cell wall process. Cell division (lysis) is the first step in DNA separation, which aims to remove the cell contents (18). Following DNA extraction, PCR amplification of ITS1 and ITS4 fragments from isolates was conducred using the MyTaq™ HS Red Mix (Bioline, BIO-25047).

MyTaq Red Mix. The PCR process is carried out by making a mastermix, namely mixing universal primer material 27 F (5'-AGAGTTGATCMTGGTCAG-3') 0.5 µl; universal primer 1492 R (5'-GGTTACCTTGTTACGACTT-3') 0.5 µl; 4 µl DNA template; 12.5 µl TAQ I Hs DNA Red; and 7.5 µl ddH2O. Primers 27 F and 1492 R are two primers commonly used to identify bacterial 16S rRNA genes (19). PCR involves three stages of the temperature cycles, namely DNA denaturation, annealing and extension (20). redenaturation was carried out at 95°C for a minute with one cycle, then denaturation at 95°C for 15 seconds with 35 cycles. Annealing or primary attachment was carried out at 56°C for 15 seconds with 35 cycles. Extension at 72°C for 15 seconds with 35 cycles. The PCR amplification process uses Using specific bacterial primers, namely 27F and 1492R, can obtain 16S rRNA gene fragment amplicons with a size of ± 1500 bp. Primers 27F and 1492R are two primers commonly used to identify bacterial 16S rRNA genes (19).

The success of a PCR reaction is determined by several factors, including 1) Deoxyribonucleotide triphosphate (dNTP); 2) Primary oligonucleotides; 3) DNA template (mould); 4) Composition of buffer solution; 5) Number of reaction cycles; 6) Enzymes used and 7) Other technical and non-technical factors, for example, contamination (21). Suboptimal PCR conditions may lead to non-specific banding (22).

According to Table 4, the results of the purity of the isolated DNA of YAC2b show that it has a concentration of 70.9 ng/µl, the absorbance value of DNA at a wavelength of 260/280 nm is 2.00. A good purity of DNA is characterized by a ratio of A260/A280 falling within the range of of 1.8-2.0. A purity value below 1.8 suggests the

presence of protein contamination, while a value exceeding 2.0 indicates the presence of small molecular weight compounds such as RNA contamination (23).

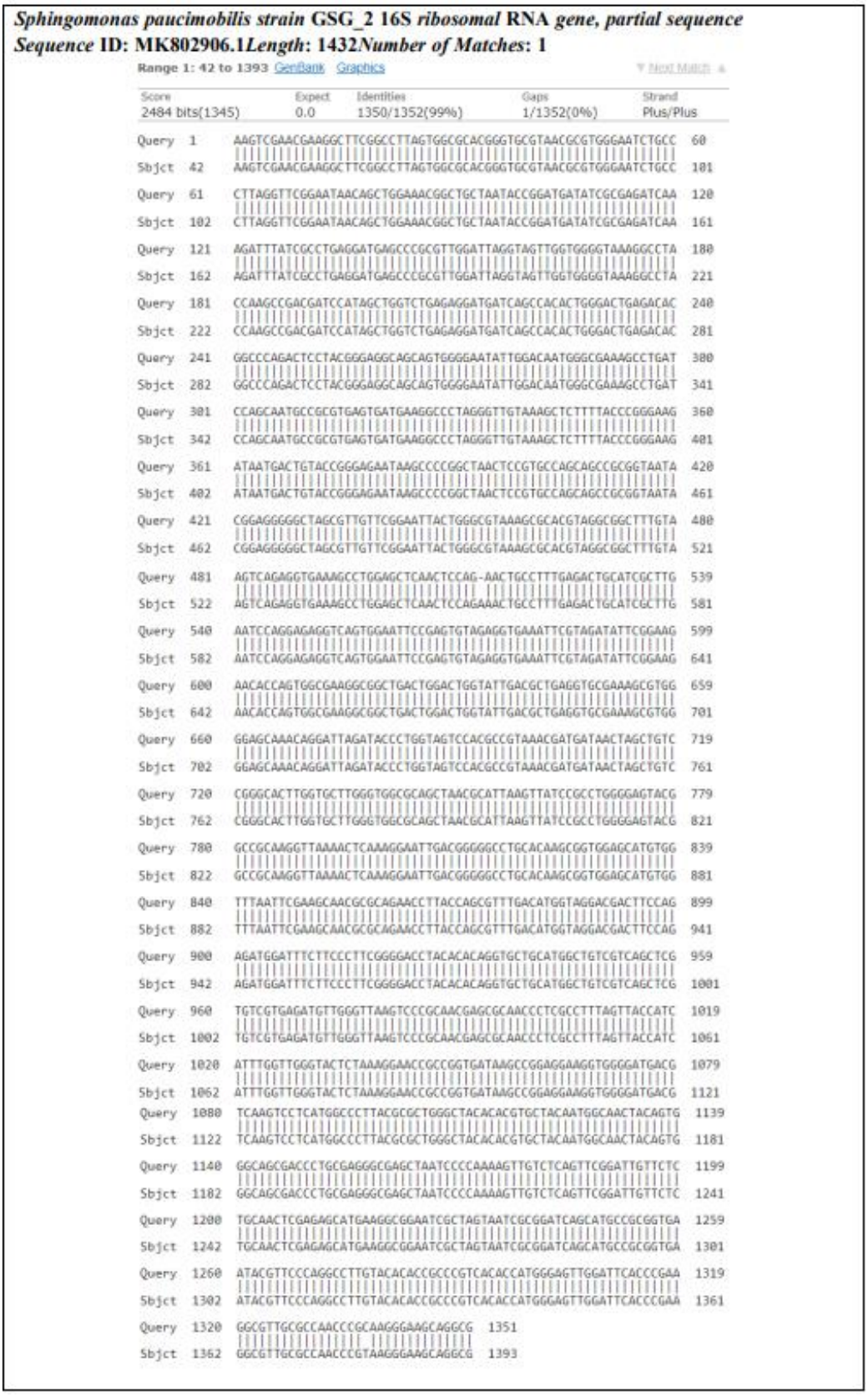

Figure 3. Comparison of base sequences of YAC2b isolate and bacterial dan *Sphingomonas paucimobilis*

Figure 2 shows that number 1 is the PCR product of the YAC2b isolate, M is the marker of DNA Ladder, and NTC is the negative control. Electrophoresis results on YAC2b isolates causing Acute Conjunctivitis have a DNA length of about 1351 bp. The amplicon band of the YAC2b isolate produced in PCR looks thick and does not smear, meaning that the PCR amplification process has been carried out well. Then, the nucleotide sequence is translated into the amino acid arrangement of the protein polypeptide chain to obtain a 16S rRNA product (22).

The success of electrophoresis is influenced by the selection of the separating medium. In this study, agarose gel was utilized, offering the advantage of of simplicity, faster fragments separation, and non-toxicity. Agarose gel is particularly suitable for separating medium to large DNA fragment. Additionally, DNA fragment amplification is influenced by various factors, including primer selection, temperature, and cycle length. PCR optimization was carried out to enhance the specificicty of PCR products (23).

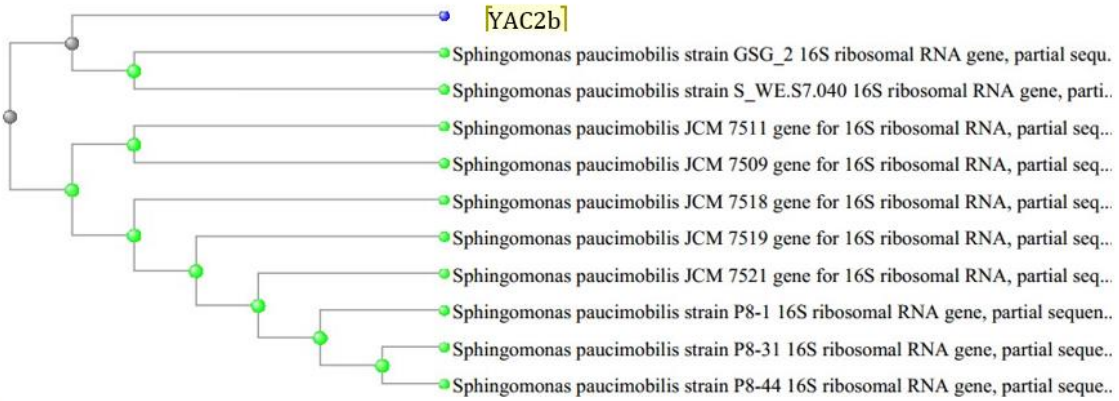

Figure 4. Pylogenitic tree Base on Comparison of 16S rRNA Sequences

The molecular identification results on YAC2b isolates causing Acute Conjunctivitis indicate the presence of *S. paucimobilis*. Although *S. paucimobilis* is rarely associated with intraocular infection, it is important to note that in the context of eye disease, its pathogenic potential is limited (24). *S. paucimobilis* belongs to the genus *Sphingomonas*, which encompassess 13 species. Among these, *S. paucimobilis* is the only species known to infects humans, often associated with nosocomial infections. It is characterized by Gram-negative, aerobic, motile flagellar properties, non-fermentative and yellow-pigmented. The prevalence of *S.paucimobilis* infections has increased in recent times (25).

S. paucimobilis, a type of bacterial bacilli characterized by low virulence, has been implicated in various eye infections, including endophthalmitis, panophthalmitis, and keratitis. These infections are often associated with underlying risk factors such as the peripartum or postpartum phase, cataract surgery, the use of contact lenses, neurotic keratopathy, or ocular trauma. Dryness of the eyes can lead to irritation, while marked inflammation results in the dilation of blood vessels, causing redness in the conjunctiva and sclera, along with edema, pain, and mucopurulent discharge (30).

Therefore, the ability to measure or infer genetic sequences is of paramount importance in biological research. This review comprehensively examines how researchers have addressed the challenge of DNA sequencing over the years. It explores the methodologies employed by researchers across different generations, highlighting the unique characteristics that define each era of sequencing technology (26).

There have been no previous reports linking *S. paucimobilis* to conjunctivitis causing pathogens. This may be attributed to the natural habitat of *S. paucimobilis* in environment, primarily found in water and soil. Water contaminated with bacteria is one of the causes of conjunctivitis. Based on cases found that *S. paucimobilis* causes keratitis, endophthalmitis, and canaliculitis (27). The previous research found that *S. paucimobilis* is the cause of conjunctivitis through molecular identification by analysis of 16S rRNA gene sequencing on the causative bacteria isolates Acute Conjunctivitis. Observation without treatment was strongly recommended in non-herpetic viral conjunctivitis and bacterial conjunctivitis (except chlamydial and gonorrhoeal conjunctivitis) due to the usually self-limiting nature of the conditions. Cold compress, artificial tears and lubricating ointments were suggested for symptomatic relief of infectious conjunctivitis (28). *S. paucimobilis* was initially thought to cause infections in immunocompromised individuals with long-term catheters. Compared to other Gram-negative bacteria, the cell wall of this bacterium lacks lipopolysaccharide, and this deficiency may be the reason for the low virulence of this bacterium (29).

4. CONCLUSIONS

The most commonly identified bacteria causing Acute Conjunctivitis predominantly belong to Gram-positive species, including two strains of *Staphylococcus aureus*, one strain of *Bacillus subtilis*, and one strain of *Staphylococcus epidermidis*. Additionally, there was one sample identified as containing Gram-negative bacteria. Molecular identification of the 16S rRNA gene sequence revealed a sequence encoding 1351 bp, exhibiting a remarkable similarity percentage of 99.85% to *S. paucimobilis*, as determined by the BLAST (Basic Local Alignment Search Tool) database tracking program. This finding suggests a potential association between *S. paucimobilis* and Acute Conjunctivitis, although further research with larger sample sizes is warranted to comprehensively represent the microbial causes of this condition.

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