

Genus-specific Primer Design for Molecular Identification of *Rhodobacter* sp. Origin of the *16S rRNA* Gene

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Introduction

Rhodobacter is a Gram-negative bacterium, oval or rod-shaped, non-motile to motile, because it has flagella, produces capsules and mucus, and can form chain formations. *Rhodobacter capsulatus* is a kind of species with a characteristic zigzag chain formation. *Rhodobacter* sp. grows well in areas with high organic substrates and low oxygen concentrations, such as sewage ponds and eutrophic lakes. These bacteria is a mesophilic bacterium that has a habitat in freshwater with a neutral water pH and without salts, such as NaCl, and reproduce by binary fission and budding are taxonomically members of the phylum Proteobacteria,

Abstract

Rhodobacter is one of the purple phototrophic bacteria widely used in aquaculture activity. It is particularly difficult in identifying and distinguishing Rhodobacter from other non-sulfur purple bacteria. This study aimed to evaluate primer pairs designed based on the 16S rRNA gene from Rhodobacter as an effective and efficient molecular identification that can be performed in the laboratory using conventional PCR techniques. The 16S rRNA sequences from Rhodobacter and other bacteria were aligned and analyzed for the high-variation region to capture the desired target amplicon. Forward and reverse primer pairs were designed based on the appropriate target amplicon. The primer pairs were also assessed for their feasibility based on ten assessment criteria for the quality of the PCR primers. The PCR optimization results obtained showed a specific band with a size of 548 bp according to the amplicon target size in the Rhodobacter samples tested and did not show any cross-reaction with other comparator bacteria, and no dimers or other non-specific bands were found. Based on the results obtained, it can be concluded that the primer pairs designed in terms of quality and specificity are good and can be used as alternative specific primers for the genus Rhodobacter sp.

class Alphaproteobacteria, order Rhodobacterales, and family Rhodobacteraceae (Imhoff, 2015).

Rhodobacter is a phototrophic purple bacterium that uses bacteriochlorophylls α and carotenoid pigments in its photosynthesis process. Aerobic culture conditions will cause colonies of *Rhodobacter* in liquid media to be pink to red. Photoheterotrophic growth can occur when conditions contain high levels of organic matter, which provide carbon and electron sources in waters and under no oxygen conditions. Hydrogen, sulfide, or thiosulfate can be source of electron donors when the photoautotrophic mechanism occurs. Aerobic, low oxygen and dark conditions can cause chemotrophic processes such as denitrification, fermentation, and oxidant-linked metabolism (Imhoff, 2015). Species of *Rhodobacter* sp. have many uses–*R. sphaeroides* can denitrify nitrite in the environment, so they can be used as a probiotic that can reduce nitrite levels in dark conditions and with little oxygen (Satoh et al., 1976; Shimizu et al., 2018). Brown and Herbert (1977) stated that *R. capsulatus* and *R. sphaeroides* use ammonia as a nitrogen source assimilated through the glutamine synthase or glutamate synthase reaction (NADPH-dependent).

The denitrification ability of the Rhodobacter is the main factor of using these bacteria in aquaculture activities as probiotics which function in bioremediation to break down inorganic nitrogen compounds in the aquaculture environment. Aside from being bioremediation of inorganic nitrogen compounds, Rhodobacter is reported to have a function in breaking down heavy metals. Li et al. (2016) stated that using R. sphaeroides, although with low activity, can reduce lead (Pb) levels in the environment. Bai et al. (2008) reported similar findings and that R. sphaeroides can help the cadmium (Cd) bioremediation process.

The process of identifying Rhodobacter to date has been guided by conventional identification, chemotaxonomy using lipopolysaccharide (LPS), and molecular using sequencing of the 16S rRNA gene (Weckesser et al., 1995; Imhoff, 2015). However, this requires quite a long time, and not all probiotic development laboratories have the resources to conduct the sequencing process for identification. The objective of this study was aimed to design alternative specific primer pairs to detect the genus Rhodobacter because there is still limited specific polymerase chain reaction (PCR) primer pairs that can specifically detect the genus Rhodobacter.

Materials and Methods

Preparation of Forward and Reverse Primer Design of *Rhodobacter* sp.

The preparation of the primer design assembled from the 16S rRNA gene sequence was carried out by analyzing the 16S rRNA sequence of Rhodobacter sp. beforehand and comparing it with 16S rRNA from several other bacterial species, such as Streptococcus sp., Aeromonas sp., Vibrio sp., Bacillus sp., and Staphylococcus sp. The preparation of unique sequences with high variation of 16S rRNA from Rhodobacter sp. was performed using MEGA software version 11.0 (Tamura et al., 2021). We used Rhodobacter capsulatus strain ATCC 11166 *16S rRNA* gene (accession number NR_043407) (Hiraishi & Ueda, 1994) as reference data in comparing unique sequences with high variation from GeneBank's database (https://www.ncbi.nlm.nih.gov/nuccore/NR_043407.1?report=genbank) (NCBI, 1988).

After obtaining a high variation of the 16S rRNA gene sequence from *Rhodobacter* sp, then a primer design search was performed using the primer design web (https://www.bioinformatics.nl/cgi-bin/primer3pl us/primer3plus.cgi) (Untergasser, 2007) and primer quality checking was carried out on the OligoAnalyzer[™] Tool (Owczarzy et al., 2008)(https://sg.idtdna.com/pag es/tools/oligoanalyzer?returnurl=%2Fcalc%2Fanalyzer), and primer specificity on the NCBI BLAST Primer (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (NCBI, 1988).

Primer Design Assessment Criteria

The primer design assessment was carried out following the ten criteria that must be possessed by forward and reverse primer pairs and meets the requirements. Primers were ordered to Integrated DNA Technologies, Inc. (Coralville, Iowa, USA) as a third party. The ten criteria are primer length 18–22 bases, forward and reverse primer melting temperature (Tm) must be the same or nearly the same, primer annealing temperature (Ta) must be comparable to primer Tm, the difference in primer melting temperature (Δ Tm) <5°C, GC content 40-60%, secondary structure like hairpin $(\Delta G = maximum - 2 \text{ to } - 3 \text{ kcal/mol}); \text{ self-dimer} (\Delta G = < -5)$ kcal/mol); and cross-dimer ($\Delta G = \langle -6 \text{ kcal/mol} \rangle$, selfcomplementary and pair-complementary <4 bases, repeats and runs no more than five base repeats or two base pairs more than two times, specificity high primer BLAST yield against target sequences, and amplicon product length <2000 bp (Lowe et al., 1990; Burpo, 2001; Lin et al., 2005; Sasmito et al., 2014).

Bacterial Sample DNA Extraction

The extraction method was performed according to the manufacturer's instructions in the IQ 2000 DNA Extraction kit (GeneReach Biotechnology Corp., Taiwan). Briefly, one mL of bacterial culture from both two *Rhodobacter* sp. archive isolates (PT. Marindolab Pratama, Serang, Banten, Indonesia) in Sistrom's minimal medium (SMM) was pelleted by centrifugation at 12,000 rpm or 11,269 ×g for three minutes. The pellet resuspended with Dodecyltrimethylammonium Bromide (DTAB) solution incubated at 75°C for five

Table 1. PCR amplification cycles used for primer design in the study

PCR Steps	Temperature (°C)	Duration	Annotation	
Initial denaturation	95	3 minutes		
Denaturation	95	1 minute		
Annealing	50	1 minute	35 cycles	
Elongation	72	1 minute		
End elongation	72	5 minutes		

minutes. After cooled to room temperature, the suspension was then homogenized by vortex and spun down for a few seconds to mix, and then 0.7 mL of chloroform was added and homogenized again by vortex for 20 seconds and centrifuged at 12,000 rpm or 13,800 ×g for five minutes. The top layer of the suspension was transferred 200 µL into a 1.5 mL microtube containing a mixture of 100 µL of Cetyltrimethylammonium Bromide (CTAB) solution and 900 μ L of ddH₂O. The mixture was then homogenized with a rapid vortex and incubated at 75°C for five minutes. The suspension was cooled to room temperature and centrifuged at 12,000 rpm or 13,800 ×g for ten minutes. The supernatant discarded and, 150 μ L of dissolve solution (patent material extraction kit) was added to the pellet, and homogenized. The suspension was incubated at 75°C for five minutes, cooled to room temperature, and then centrifuged at 12,000 rpm or 13,800 ×g for five minutes. A total of 130 μ L of the supernatant was transferred into a new 1.5 mL microtube containing 300 µL of 96% ethanol, then homogenized by vortex quickly and centrifuged at 12,000 rpm or 13,800 ×g for five minutes, and the supernatant was removed. The pellet was washed with 200 µL 75% ethanol, centrifuged at 12,000 rpm or 13,800 ×g xg for five minutes, then the supernatant was discarded, and the pellet was dried and dissolved with Diethylpyrocarbonate (DEPC) H2O solution according to the size of the pellet.

Polymerase Chain Reaction (PCR) Method

The target bacteria was *Rhodobacter* sp., with the primer forward (Rh_c being 5'-F) ATCTGAAAGTCAGAGGTGAA-3' and the reverse primer (Rh c R) being 5'-TGATGGCAACTGAAAGTGTG-3'. The total reaction volume was 25 µL with the premix composition used being 8.5 µL nuclease-free water (NFW), 1 µL each of forward and reverse primers (10 μM), 12.5 μL GoTaq[®] Green Master Mix (Promega, Wisconsin, USA), and 2 µL DNA template. The target amplicon product was 548 bp, with the target gene being 16S rRNA at the 505th to 1056th base position. Pure isolates from Streptococcus sp., Aeromonas sp., Vibrio sp., Bacillus sp., and Staphylococcus sp. in tryptic soy broth (TSB) medium (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) were used as a comparison control in the PCR process. The universal gene of 16S rRNA was also used in PCR as quality control of genomic DNA (gDNA) samples which were examined with forward primer (27F) 5'-AGAGTTTGATCCTGGCTCAG-3', reverse primer (1492R) 5'-GGTTACCTTGTTACGACTT-3'., and the amplicon length of 1465 bp (Lane, 1991; Rizkiantino *et al.* 2020). The total reaction volume was 50 μ L with the premix composition used being 13 μ L nuclease-free water (NFW), 5 μ L each of forward and reverse primers (10 μ M), 25 μ L GoTaq[®] Green Master Mix (Promega, Wisconsin, USA), and 2 μ L DNA template.

The amplification cycles used for primer design and universal gene of 16S rRNA were presented in Table 1. and Table 2. The PCR products were electrophoresed on agarose gel with a concentration of 1.5% (BIORON, Römerberg, Germany) in $1 \times$ Tris-Borate-Ethylenediaminetetraacetic acid (EDTA) (TBE) buffer solution at an electric voltage of 100 V with a duration of 35 minutes. Gel was visualized with the UVITEC Cambridge Gel Documentation System (Uvitec Ltd, Cambridge, UK). NEB N3200L 1 kb Plus DNA Ladder (New England Biolabs Ltd., UK) was used as the standard base size. The PCR product results were sent for sequencing (1st BASE, Singapore) for reconfirmation. The sequencing results were analyzed using MEGA software version 11.0 (Tamura et al., 2021), and BLAST was performed GeneBank on (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=bl astn&PAGE TYPE=BlastSearch&LI151 NK_LOC=blasthome).

Results

Preparation and Evaluation of Primer Designs from *Rhodobacter* sp.

Based on the unique gene sequences with hypervariation in the 16S rRNA gene from Rhodobacter capsulatus strain ATCC 11166, target sequences at the 505th to 1056th base position was obtained (Figure 1. and Figure 2.). The sequence was then analyzed further to carry out the forward and reverse primer designs by searching the primer using the primer design web (https://www.bioinformatics.nl/cgi-bin/primer3plus/pr imer3plus.cgi). The primer design results obtained for forward primers was 5'-ATCTGAAAAGTCAGAGGTGAAprimer 3', and the reverse was 5'-TGATGGCAACTGAAAAGTGTG-3'. The primer pairs were

Table 2. PCR amplification cycles used for universal gene of 16S rRNA.

PCR Steps	Temperature (°C)	Duration	Annotation
Initial denaturation	95	3 minutes	
Denaturation	95	30 seconds	
Annealing	50	30 seconds	35 cycles
Elongation	72	1 minute	
End elongation	72	5 minutes	

assessed and evaluated for primer quality on the OligoAnalyzer[™] Tool and primer specificity on the NCBI BLAST Primer. The forward and reverse primer assessments obtained the following results, which are presented in Table 3.

Polymerase Chain Reaction (PCR) Results

Based on the results of PCR examination, it was found that only samples from Rhodobacter sp. showed a specific band at 548 bp (Figure. 3). Different isolates, such as Vibrio sp., Aeromonas sp., Streptococcus sp., Staphylococcus sp., and Bacillus sp. did not show a crossreaction when amplified using a designed primer pair. Dimer results were also not seen in all wells, which showed well-optimized primers. Based on sequencing analysis found Rhodobacter capsulatus in well 1 with query coverage 100%, E-value 0, and percentage identity 100.00%; and Rhodobacter sphaeroides in well 2 with query coverage 100%, E-value 0, and percentage identity 99.67%.

ORIGIN							
	1	aacgaacgct	ggcggcaggc	ctaacacatg	caagtcgagc	gagaccttcg	ggtctagcgg
	61	cggacgggtg	agtaacgcgt	gggaacgtgc	cctttgctac	ggaatagccc	cgggaaactg
	121	ggagtaatac	cgtatgtgcc	cttcggggga	aagatttatc	ggcaaaggat	cggcccgcgt
	181	tggattaggt	agttggtggg	gtaatggcct	accaagccga	cgatccatag	ctggtttgag
	241	aggatgatca	gccacactgg	gactgagaca	cggcccagac	tcctacggga	ggcagcagtg
	301	gggaatctta	gacaatgggg	gaaaccctga	tctagccatg	ccgcgtgagc	gatgaaggcc
	361	ttagggttgt	aaagctcttt	caggtgggaa	gataatgacg	gtaccaccag	aagaagcccc
	421	ggctaactcc	gtgccagcag	ccgcggtaat	acggaggggg	ctagcgttgt	tcggaattac
	481	tgggcgtaaa	gcgcacgtag	gcgg atctga	aagtcagagg	tgaaatccca	gggeteaace
	541	ttggaactgc	ctttgaaact	cctggtcttg	aggtcgagag	aggtgagtgg	aatteegagt
	601	gtagaggtga	aattcgtaga	tattcggagg	aacaccagtg	gcgaaggcgg	ctcactggct
	661	cgatactgac	gctgaggtgc	gaaagcgtgg	ggagcaaaca	ggattagata	ccctggtagt
	721	ccacgccgta	aacgatgaat	gccagtcgtc	ggcaggcatg	cctgtcggtg	acacacctaa
	781	cggattaagc	attecgeetg	gggagaacgg	tcgcaagatt	aaaactcaaa	ggaattgacg
	841	ggggcccgca	caagcggtgg	agcatgtggt	ttaattogaa	gcaacgcgca	gaacettace
	901	aaccettgae	atcgggatcg	cggttaccgg	agacggtttc	cttcagttcg	getggatece
	961	agacaggtgc	tgcatggctg	tcgtcagete	gtgtcgtgag	atgttgggtt	aagteeggea
1	021	acgagcgcaa	cccacacttt	cagttgccat	<pre>cattcagttg</pre>	ggcactctgg	aagaactgcc
1	081	gatgataagt	cggaggaagg	tgtggatgac	gtcaagtcct	catggccctt	acgggttggg
1	141	ctacacacgt	gctacaatgg	tggtgacaat	gggccaatcc	caaaaagcca	tctcagttcg
1	201	gattggggtc	tgcaactcga	ccccatgaag	tcggaatcgc	tagtaatcgc	gtaacagcaa
1	261	gacgcggtga	atacgttccc	gggccttgta	cacaccgccc	gtcacaccat	gggaattggg
1	321	tctaccctaa	gatggtgcgc	caacccgcaa	gggaggcagc	cagccacggt	aggctcagtg
1	381	actggggtg					
11							

Figure 1. Analysis of unique sequences with high variation in the 16S rRNA gene of the Rhodobacter sp. Unique sequences were at the 505th–1056th base position (red colour).

Species/Abbrv	
1. NR 043407.1 Rhodobacter capsulatus strain ATCC 11166	AATTACTCCCCCGTAAACCCCCACCTAC-CCCCACCTAC-CCCACCCCAC
2. FR731160.1 Rhodobacter sphaeroides strain CNT-2A	AATTACTGGGCGTAAAGCGCACGTAG-GCGCATCGGAAAGTCAGAGGTGAAATCCCCAGGGCTCAACCCTGGAACTGC
3. NR 042644.1 Rhodobacter aestuarii strain JA296	AATTACTGGGCGTAAAGCGCGCGTAG-GCGCACTATTAAGTCGGGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC
4 NR 042629 1 Rhodobacter maris strain JA276	AATTACTGGGCGTAAAGCGCACGTAG-GCGCATATTTAAGTCGGGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGC
5. NR 119042.1 Aeromonas salmonicida strain ATCC 33658	AATTACTGGGCGTAAAGCGCACGCAG-GCGCTTGGATAAGTTAGATGTGAAAGCCCCCGGGCTCAACCTGGGAATTGC
6. NR 119040.1 Aeromonas jandaei strain ATCC 49568	AATTACTGGGCGTAAAGCGCACGCAG-GCGCTTGGATAAGTTAGATGTGAAAGCCCCCGGGCTCAACCTGGGAATTGC
7. NR 119039.1 Aeromonas hydrophila strain ATCC 7966	AATTACTGGGCGTAAAGCGCACGCAG-GCGCTTGGATAAGTTAGATGTGAAAGCCCCCGGGCTCAACCTGGGAATTGC
8. NR 118947.1 Aeromonas veronii by, veronii strain ATCC 35624	AATTACTGGGCGTAAAGCGCACGCAG-GCGCTTGGATAAGTTAGATGTGAAAGCCCCCGGGCTCAACCTGGGAATTGC
9. NR 102783.2 Bacillus subtilis subsp. subtilis strain 168	AATTATTGGGCGTAAAGGGCTCGCAG-GCGCTTTCTTAAGTCTGATGTGAAAGCCCCCCGGCTCAACCGGGGGGGTC
10. NR 149175.1 Bacillus mesophilus strain SA4	AATTATTGGGCGTAAAGCGCGCGCGCGCGCGCGCGCTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
11. NR 157732.1 Bacillus nitratireducens strain MCCC 1A00732	AATTATTGGGCGTAAAGCGCGCGCGCGG-GTGCTTTCTTAAGTCTGATGTGAAAGCCCCACGGCTCAACCGTGGAGGGTC
12. NR 115714.1 Bacillus cereus strain CCM 2010	AATTATTGGGCGTAAAGCGCGCGCGCGG-GTGCTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
13. NR 036955.1 Staphylococcus haemolyticus strain SM 131	AATTATTGGGCGTAAAGCGCGCGTAG-GCGCTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
14. NR 118997.2 Staphylococcus aureus strain ATCC 12600	AATTATTGGGCGTAAAGCGCGCGTAG-GCGCTTTTTTAAGTCTGATGTGAAAGCCCCACGGCTCAACCGTGGAGGGTC
15. NR 113957.1 Staphylococcus epidermidis strain NBRC 100911	AATTATTGGGCGTAAAGCGCGCGTAG-GCGCTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
16. NR 112035.1 Staphylococcus piscifermentans strain ATCC 51136	AATTATTGGGCGTAAAGCGCGCGTAG-GCGCTTTTTTTAAGTCTGATGTGAAAGCCCCACGGCTCAACCGTGGAGGGTC
17. NR 117503.1 Streptococcus agalactiae ATCC 13813	ATTTATTGGGCGTAAAGCGAGCGCAG-GCGCTTCTTTAAGTCTGAAGTTAAGGCAGTGG-CTTAACCATTGTACGCT
18. NR 115802.1 Streptococcus ictaluri 707-05	ATTTATTGGGCGTAAAGCGAGCGCAG-GCGCTTCTTTAAGTCTGAAGTTAAAGGCATTGGCTCAACCAATGTACGCT
19. NR 115731.1 Streptococcus iniae strain ATCC 29178	ATTTATTGGGCGTAAAGCGAGCGCAG-GCGCTTCTATAAGTCTGAAGTAAAGGCAGTGGCTCAACCATTGTATGCT
20. NR 043001.1 Streptococcus parauberis strain DSM 6631	ATTTATTGGGCGTAAAGCGAGCGCAG-GCGCTTATTTAAGTCTGAAGTTAAAGGCCGTGGCTCAACCATGGTTCGCT
21. NR 119058.1 Vibrio parahaemolyticus strain ATCC 17802	AATTACTGGGCGTAAAGCGCATGCAG - GTGCTTTGTTAAGTCAGATGTGAAAGCCCCGGGG - CTCAACCTCGGAATTGC
21. NR 115050.1 VIDIO paranaemolyticus strain A100 11002	AATTACTGGGCGTAAAGCGCATGCAG-GTGCTTTGTTAACTCAGATGTGAAAGCCCCGGGGCTCAACCTCGGAATAGC
22 ND 110054 1 Vibrio hanvois strain ATCC 14126	
22. NR 119054.1 Vibrio harveyi strain ATCC 14126	
23. NR 119050.1 Vibrio campbellii CAIM 519 = NBRC 15631 = ATCC 25 24. NR 119049.1 Vibrio alginolyticus strain ATCC 17749	592 A A T T A C T G G G C G T A A A G C G C A T G C A G - G T G C T T T G T T A A G T C A G A T G T G A A A G C C C G G G G C T C A A C C T C G G A A T T G C
23 NR 119050 1 Vibrio campbelli CAIM 519 = NBRC 15631 = ATCC 25 24 NR 119049 1 Vibrio alginopticus strain ATCC 17749 Reverse Primer Target	592 A TTACTGGGCGTAAAGCGCATGCAG.GTGG TTTGTTAAGTCAGATGTGAAAGCCCGGGGCTCAACCTCGGAATTGC AATTACTGGGCGTAAAGCGCATGCAG.GTGG TTTGTTAAGTCAGATGTGAAAGCCCGGGGCTCAACCTCGGAATAGC
23 NR 119050 1 Vibro campbelli CAM 519 = NBRC 15031 = ATCC 22 24 NR 119049 1 Vibro alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abby	522 A TTAC TGGGCGTAAAGCGCA TGCAG, STGC TTTGTTAAGTCAGA TGTGAA AGCCCGGGG CTCAACCTCGGAA TGCAAATGCAGA TGTGAAAGCCGGGG CTCAACCTCGGAA TGCAACCTGGGGG CTCAACCTCGGAA TGCAACCTGGGGG CTCAACCTCGGAA TGCAACCTGGGGGG CTCAACCTCGGAA TGCAAGCCGGGG CTCAACCTCGGAA TGCAAGCCGGGG CTCAACCTCGGAA TGCAGGCGGGG CTCAACCTCGGAA TGCAGGCGGGG CTCAACCTCGGAA TGCAGGCGGGG CTCAACCTCGGAA TGCAGGCGGGG CTCAACCTCGGGGGG CTCAACCTCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
23 NR 119050.1 Vibrio campbellii CAM 519 = NBRC 15831 = ATCC 28 24 NR 119049.1 Vibrio alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abbry 1. NR 043407.1 Rhodobacter capsulatus strain ATCC 11166	522 A TTAC TG GGCG TAAAGCGCA TGCAG, GTGC TTTG TTAAGTCAGA TG TGAA AGCCCGGGG CTCAACCTCGGAA TTG C AATTAC TG GGCG TAAAGCGCA TGCAG - GTGC TTTG TTAAGTCAGA TG TGAA AGCCCGGGG CTCAACCTGGGAA TAGC
23 NR 119950 1 Vibro campbelli CAM 519 = NBRC 15031 = ATCC 22 24 NR 119049 1 Vibro alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abbry 1 NR 043407 1 Rhodobacter capsulatus strain ATCC 11166 2 FR731160 1 Rhodobacter sphaeroides strain CNT-2A	522 A TT AC TG G G C G TA A G C G C A TG C A G C G C G TT G TT
23 NR 119050 1 Vibrio campbelli CAM 519 = NBRC 15831 = ATCC 25 24 NR 119049 1 Vibrio alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abby 1. NR 043407 1 Rhodobacter capsulatus strain ATCC 11166 2. FR731160 1 Rhodobacter spheeroides strain CMT-2A 3. NR 042644 1 modobacter spheeroides strain CMT-2A	522 A TT A C TG G G C G TA A A G C G C A TG C A G G C G TG G TT TG TT A A G TC A C A TG TC A A A G C C C G G G - C TC A A C T C G G A A TG C A A TT A C TG G G C G TA A A G C G C A TG C A G C G G G - G TG TT G TT A A G TC A G A TG TG A A A G C C C G G G C - C T C A A C C T C G G A A TA C A TT A C TG G G C G TA A A G C C C A TG C A G C G C A TG C A A C T C A A C T C A A G C C C G G G C - C T C A A C C T C G G A A TA C TG TT G G G TT A A G T C C G C A A C G A G C G C A A C C C A C C T TC A TT C A - C TT C A - C TT C G C A C C C C G C A C C C C A C C C A C C C A C C C A C C
23 NR 119950 1 Vibro campbelli CAM 519 = NBRC 15031 = ATCC 22 24 NR 119949 1 Vibro alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abbry 1 NR 04307 1 Rhodobacter capsulatus strain ATCC 11166 2 FR731160 1 Rhodobacter asphaeroides strain CNT-2A 3 NR 042644 1 Rhodobacter assiuni strain A2266 4 NR 042629 1 Hodobacter assiuni strain A2276	522 A TT AC TG G G C G TA A G C G C A TG C A G C G C G TT G TT
23 NR 119050 1 Vibrio campbelli CAM 519 = NBRC 15031 = ATCC 25 24 NR 119049 1 Vibrio alginolyticus strain ATCC 17749 Reverse Primer Target Species/Abbv 1 NR 043007 1 Rhodobacter capsulatus strain ATCC 11166 2 FR731160 1 Rhodobacter sphaeroides strain CNT-2A 3 NR 042644 1 Rhodobacter mains strain JA2290 4 NR 042729 1 Rhodobacter mains strain JA2290 4 NR 042729 1 Rhodobacter mains strain JA278 5 NR 119042 1 Aeronomas salmonicida strain ATCC 3958	522 A TT A C TG GG CG TA A A GC GC A TG CA G, G TG C TT G TT A A G TC A CA TG TG A A A GC CC GG GG C TC A A C C TC G G A A TT G C A A TT A C TG GG CG TA A A GC GC A TG CA G, G TG C TT G TT A A G TC A GA TG TG A A A GC CC GG GG C TC A A C C TC G G A A TG C A A TT A C TG GG CG TA A A GC GC A TG CA G, G G G TT G TT G TT TG TT A A G TC A GA TG TG A A A GC CC GG GG C TC A A C C TC G G A A TA GC TG TT GG G TT A A G TC CG G C A C G A GC G C A C C C A C C TT CG TT G G TG CC A C C TT CG TT A G TC CG GC CA C C C A GA GC G C A C C C A C C A C C TT G G TG CC A C C A TC A T
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Forward Primer Target

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24. NR 119049.1 Vibrio alginolyticus strain ATCC 17749	TGTTGGGT	TAAGTCCCG	CAACGAG	CGCAACO	CTTATCCTTGT	TTGCCAGCG	AGTAATG	TCGGGA	ACTCCAGG
23. NR 119050.1 Vibrio campbellii CAIM 519 = NBRC 15631 = ATCC 2592	TGTTGGGT	TAAGTCCCG	CAACGAG	CGCAACO	CTTATCCTTGT	TTGCCAGCG	AGTAATG	TCGGGAA	ACTCCAGG
22. NR 119054.1 Vibrio harveyi strain ATCC 14126	TGTTGGGT	TAAGTCCCG	CAACGAG	CGCAACO	CTTATCCTTGT	TTGCCAGCG	AGTAATG	TCGGGA	ACTCCAGG

Figure 2. Target sequence in the annealing process for the designed forward and reverse primer pairs (yellow box).

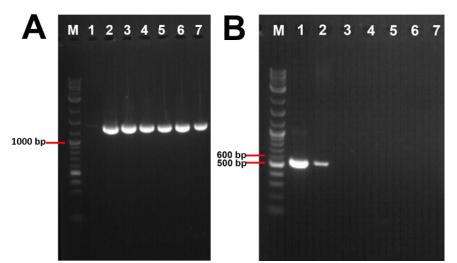


Figure 3. Results of PCR examination of the *16S rRNA* universal gene (A) and the primers that had been designed (B). The results showed a specific band only on isolates from the *Rhodobacter* sp. and did not show any cross-reaction with the *16S rRNA* gene from other bacteria as a comparison. M. Marker. 1. *Rhodobacter capsulatus*. 2. *Rhodobacter sphaeroides*. 3. *Vibrio* sp.. 4. *Aeromonas* sp.. 5. *Streptococcus* sp.. 6. *Staphylococcus* sp.. 7. *Bacillus* sp..

Table 3. The results of the assessment of the ten criteria for designed primer pairs

No. Assessment criteria		Primer						
NO.		Forward	Reverse					
	Oligonucleotides	5'-ATCTGAAAGTCAGAGGTGAA-3'	5'-TGATGGCAACTGAAAGTGTG-3'					
1.	Primer length	20 bases	20 bases					
	Ideal: 18 – 22 bases							
2.	Primer melting temperature	53,59 °C	56,55 °C					
	(Tm)							
	Ideal: Tm forward and							
	reverse must be the same or							
	nearly the same							
3.	Primer annealing	50),07 °C					
	temperature (Ta)	Gradient annealing optimization temperature suggestion: 49,00–53,00 °C						
	Ideal: Comparable to primer							
	Tm							
4.	The difference in primer	(ΔTm) = 56,55 °C	C − 53,59 °C = 2,96 °C					
	melting temperature (Tm)							
	Ideal: <5 °C							
5.	GC content	40%	45%					
	Ideal: 40–60%							
6.	Secondary structure							
	 Hairpin (ΔG: maximum 	-2.16 to -1.88 kcal/mol	–0,65 kcal/mol					
	−2 to −3 kcal/mol)							
	- Self-Dimer (ΔG : <—5							
	kcal/mol)	−1,47 to −6,70 kcal/mol	−1,34 to −3,14 kcal/mol					
	 Cross-dimer (ΔG : <-6 							
	kcal/mol)	−1,37 to −5,13 kcal/mol	−1,34 to −5,13 kcal/mol					
7.	Self-complementary and	7,00 & 0,00	3,00 & 0,00					
	pair-complementary							
	Ideal: <4 bases							
8.	Repeats and runs	None	None					
	Ideal: No more than five							
	base repeats or two base							
	pairs more than two times							
9.	Specificity	Species of the genus Rhodobacter sp.	dominate the BLAST results of the target					
	Ideal: High primer BLAST	sequence						
	yield against target							
	sequences							
10.	Product length (amplicon)	54	48 bp					
	Ideal: <2000 bp							

Discussion

The morphological characteristics possessed by Rhodobacter species and also possessed by Rhodovulum species are rod-shaped cells, an internal vesicular membrane, and carotenoids. The thing that can distinguish between the two genera is that Rhodobacter species can grow very optimally if there is no presence of NaCl salts due to their characteristics in a freshwater environment. The chemotaxonomy of the two genera (Rhodobacter and Rhodovulum) also have similarities, such as glucosamine content in lipopolysaccharides that are owned and can assimilate sulfate using the 3'phosphoadenosine-5'-phosphosulfate pathway (Imhoff, 1982). Biochemical test such as NaCl requirements, sulfide oxidation end products, polar lipid composition, and tolerance to sulfides, take a long time to conduct because the colony of genus Rhodobacter, such as R. sphaeroides, will only grow on the day 4 of the incubation period (Paustian and Kurtz 1994; Suresh et al., 2019).

The most likely difference between the genus *Rhodobacter* and other purple phototrophic bacteria to be analyzed is based on their *16S rRNA* sequence. Unfortunately, time-consuming the *16S rRNA* gene sequencing methods and limitations require specific primers that can characterize the genus *Rhodobacter* without having to conduct the sequencing(Weckesser et al., 1995; Imhoff, 2015). The existing primers still have cluster targets in the genera *Rhodobacter* and *Pseudorhodobacter*, while the two of these genera are different in terms of having complete genes for photosynthesis, where genus *Rhodobacter* has bacteriochlorophyll a and carotenoids (Imhoff et al., 1984; Bräuer et al., 2011; Kim and Lee, 2019).

Therefore, it is necessary to have specific primers that can be used to detect genus Rhodobacter quickly, effectively and efficiently to accelerate the molecular identification process in the initial isolation purpose. The primer could be assembled from a unique sequence in the 16S rRNA gene of a bacterium. Identical to each individual, can be used as an evolutionary chronometer, has conservative sequences, and has nine hypervariable regions (V1-V9) that make it easy to identify a type of bacteria are some of the advantages of using the 16S rRNA gene in molecularly identifying bacterial species (Pangastuti, 2006). These hypervariable regions can indicate the presence of considerable sequence diversity among different bacterial species. These species-specific or genus-specific sequences in the hypervariable region are valuable targets for diagnostic tests and other scientific investigation purposes (Chakravorty et al., 2007).

The current study showed potential primer pairs that can be used to identify the genus of *Rhodobacter* molecularly. The results of testing the primer design based on the unique sequence of the *16S rRNA* gene of *Rhodobacter* showed the potential to identify this bacterium. Cross-reactions against Gram-positive bacteria (Bacillus sp., Staphylococcus sp., and Streptococcus sp.) and other Gram-negative bacteria (Aeromonas sp. and Vibrio sp.), as well as dimer bands, were not found during observation on the electrophoresis gel results. The results indicate that there is sufficient specificity and high potential to be able to detect the target amplicon of the designed primer pair. However, there is a difference in the band thickness found between the two samples of Rhodobacter used in this study. It is suspected that species differences cause less than optimal levels of primer attachment on the template DNA, thus affecting the resulting amplification products. Based on the results of the sequencing analysis performed on the PCR products from the two *Rhodobacter* samples, it was found that both are members of the *Rhodobacter* genus with the species of Rhodobacter capsulatus and Rhodobacter sphaeroides. R. capsulatus band was thicker and brighter, while the R. sphaeroides tend to be thinner when compared to R. captulatus. This is presumably because the primer pair was designed from the R. capsulatus 16S rRNA gene sequence, so it showed the greatest band on the *R. capsulatus* sample in well 1. The sequencing results were obtained to strengthen the primer design's ability to detect Rhodobacter specifically. However, primer pairs in the current study should be tested on other genera in the Rhodobacteraceae family that are more closely related, such as Tabrizicola, Paenirhodobacter, and Thioclava, should also be explored in future studies (Hördt et al., 2020).

Conclusion

Based on the results of the evaluation of the ten criteria for primer pairs, it can be concluded that the primer pairs designed in terms of quality and specificity are good and have the potential to be used as specific primers in detecting bacteria from the genus *Rhodobacter* sp.

References

- Bai, H.J., Zhang, Z.M., Yang, G.E., & Li, B.Z. (2008). Bioremediation of cadmium by growing *Rhodobacter* sphaeroides: kinetic characteristic and mechanism studies. *Bioresource Technology*, 99(16), 7716–7722. https://doi.org/10.1016/j.biortech.2008.01.071.
- Bräuer, S.L., Adams, C., Kranzler, K., Murphy, D., Xu, M., Zuber, P., Simon, H.M., Baptista, A.M., & Tebo, B.M. (2011). Culturable *Rhodobacter* and *Shewanella* species are abundant in estuarine turbidity maxima of the Columbia Riveremi. *Environmental Microbiology*,13(3), 589–603. https://doi.org/10.1111/j.1462-2920.2010.02360.x.
- Brown, C.M., & Herbert, R.A. (1977). Ammonia assimilation in members of Rhodospirillaceae. *FEMS Microbiology Letters*, 1(1), 3–45. https://doi.org/10.1111/j.1574-6968.1977.tb00576.x.
- Bryant, D.A., & Frigaard, N.U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. *Trends in Microbiology*, 14(11), 488–496.

https://doi.org/10.1016/j.tim.2006.09.001.

- Burpo, F.J. (2001). A critical review of PCR primer design algorithms and cross-hybridization case study. *Biochemistry*, 218, 1–12. https://biochem218.stanford.edu/Projects%202001/Bu rpo.pdf.
- Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods*, 69(2), 330–339. https://doi.org/10.1016/j.mimet.2007.02.005.
- Cohen-Bazire, G., Sistrom, W.R., & Stanier, R.Y. (1957). Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *Journal of Cellular and Comparative Physiology*, 49(1), 25–68. https://doi.org/10.1002/jcp.1030490104.
- Hiraishi, A., & Ueda, Y. (1994). Intragenetic structure of the genus Rhodobacter: Transfer of Rhodobacter sulfidophilus and related marine species to the genus Rhodovulum gen. nov. International Journal of Systematic and Evolutionary Microbiology, 44(1), 15–23. https://doi.org/10.1099/00207713-44-1-15.
- Hördt, A., López, M.G., Meier-Kolthoff, J.P., Schleuning, M., Weinhold, L.M., Tindall, B.J., Gronow, S., Kyrpides, N.C., Woyke, T., & Göker, M. (2020). Analysis of 1,000+ typestrain genomes substantially improves taxonomic classification of Alphaproteobacteria. *Frontiers in Microbiology*, 11:468. https://doi.org/10.3389/fmicb.20 20.00468.
- Imhoff, J.F. (1982). Occurrence and evolutionary significance of two sulfate assimilation pathways in the Rhodospirillaceae. Archives of Microbiology, 132, 197– 203. https://doi.org/10.1007/BF00508731.
- Imhoff, J.F., Trüper, H.G., & Pfennig, N. (1984). Rearrangement of the species and genera of the phototrophic "purple nonsulfur bacteria". *International Journal of Systematic* and Evolutionary Microbiology, 34:340–343. https://doi.org/10.1099/00207713-34-3-340.
- Imhoff, J.F. (2015). Rhodobacter. Bergey's Manual of Systematics of Archaea and Bacteria. New Jersey (US): John Wiley & Sons, Inc., in association with Bergey's Manual Trust.

https://doi.org/10.1002/9781118960608.gbm00862.

- Kim, S.C., & Lee, H.J. (2019). Draft genome sequence of ureaseproducing *Pseudorhodobacter* sp. strain E13, isolated from the Yellow Sea in Gunsan, South Korea. *Microbiology Resource Announcements*, 8:e00189-19. https://doi.org/10.1128/MRA.00189-19.
- Lane, D.J. (1991). 16S/23S rRNA Sequencing. In E. Stackebrandt, & M. Goodfellow (Eds.), Nucleic Acid Techniques in Bacterial Systematic (pp. 115–175). John Wiley and Sons, New York.
- Li, X., Peng, W., Jia, Y., Lu, L., & Fan, W. (2016). Bioremediation of lead contaminated soil with *Rhodobacter sphaeroides*. *Chemosphere*, 156, 228-235. https://doi.org/10.1016/j.chemosphere.2016.04.098.
- Lin, F-M., Huang, H-D., Huang, H-Y., & Horng, J-T. (2005, June 25-29), Primer design for multiplex PCR using a genetic algorithm. Conference: Genetic and Evolutionary Computation Conference, GECCO 2005, Proceedings, Washington DC, USA, pp. 475–476. https://doi.org/10.1145/10000001000007

https://doi.org/10.1145/1068009.1068087.

Lowe, T., Sharefkin, J., Yang, S. Q., & Dieffenbach, C. W. (1990). A computer program for selection of oligonucleotide primers for polymerase chain reactions. *Nucleic Acids Research*, 18(7), 1757–1761. https://doi.org/10.1093/nar/18.7.1757.

National Center for Biotechnology Information (NCBI). (1988). (2017, April 06), Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information.

https://www.ncbi.nlm.nih.gov/tools/primer-blast/.

- Owczarzy, R., Tataurov, A.V., Wu, Y., Manthey, J.A., McQuisten, K.A., Almabrazi, H.G., Pedersen, K.F., Lin, Y., Garretson, J., McEntaggart, N.O., Sailor, C.A., Dawson, R.B., & Peek, A.S. (2008). IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Research*,1(36)(Web Server issue), W163169. https://doi.org/10.1093/nar/gkn198.
- Pangastuti, A. 2006. Species definition of procaryotes based on 16S rRNA and protein coding genes sequence. *Biodiversitas*, 7(3), 292–296.

https://doi.org/10.13057/biodiv/d070319.

- Paustian, T.D., & R.S. Kurtz. (1994). Transposon mutagenesis of *Rhodobacter sphaeroides*. In C. A. Goldman (Ed), Tested Studies for Laboratory Teaching (Volume 15, pp. 45–61). Proceedings of the 15th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 390 pages.
- Rizkiantino, R., Wibawan, I.W.T., Pasaribu, F.H., Soejoedono, R.D., Arnafia, W., Ulyama, V., & Wibowo, D.B. (2020). Isolation and characterisation of the *Enterococcus faecalis* strain isolated from red tilapia (*Oreochromis* hybrid) in Indonesia: A preliminary report. *Journal of Survey in Fisheries Sciences*, 7(1), 27–42. http://sifisheriessciences.com/index.php/journal/article /view/143.
- Sasmito, D.E.K., Rahadian Kurniawan, R., & Muhimmah, I. (2014, December 06). Primer characteristics of Polymerase Chain Reaction (PCR) for DNA sequencing: Mini review [Section articles]. National Seminar on Medical Informatics, Islamic University of Indonesia, Yogyakarta, Indonesia. https://journal.uii.ac.id/snimed/ article/view/6329.
- Satoh, T., Hoshino, Y., & Kitamura, H. (1976). Rhodopseudomonas sphaeroides forma sp. denitrificans, a denitrifying strain as a subspecies of Rhodopseudomonas sphaeroides. Archives of Microbiology, 108, 265–269.

https://doi.org/10.1007/BF00454851.

- Shimizu, T., Horiguchi, K., Hatanaka, Y., Masuda, S., Shimada, K., Matsuura, K., & Haruta, S. (2018). Nitrite-reducing ability is related to growth inhibition by nitrite in *Rhodobacter sphaeroides* f. sp. *denitrificans. Bioscience, Biotechnology, and Biochemistry*, 82(1), 148–151. https://doi.org/10.1080/09168451.2017.1412247.
- Suresh, G., Lodha, T.D., Indu, B., Sasikala, C., & Ramana, C.V. (2019). Taxogenomics resolves conflict in the genus *Rhodobacter*: A two and half decades pending thought to reclassify the genus *Rhodobacter*. *Frontiers in Microbiology*, 10:2480. doi: 10.3389/fmicb.2019.02480.
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120.
- Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., & Jack, A.M. (2007). Leunissen: Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research*, 35, W71-W74. https://doi.org/10.1093/nar/gkm306.
- Weckesser, J., Mayer, H., & Shulz, G. (1995). Anoxygenic phototrophic bacteria: model organisms for studies on cell wall macromolecules. In Blankenship. Madigan and