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DESIGN PRIMER GENE *TET*(M) *LACTOBACILLUS PLANTARUM*

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ABSTRACT
Tetracycline is an antibiotic that reported have resistance to some infectious bacteria. One of the mechanisms of tetracycline antibiotic resistance is the ribosomal protection through the *tet*(M) gene. The *tet*(M) gene has also been identified in the probiotic bacteria Lactobacillus plantarum. Nucleotide *tet*(M) is used to design primers to be an important element in the technique of DNA amplification in the Polymerase Chain Reaction (PCR) method, it can be a useful diagnostic before using tetracycline antibiotics in therapy. The method used for designing primer *tet*(M) gene from Lactobacillus plantarum is by using nucleotide sequence data obtained from NCBI, which then obtained primer candidates using Primer3plus, and primer candidates analyzed of primer criteria contained on it with OlygoAnalyzer 3.1. The results obtained, there are five primer candidates and produce one best primer is primer number 4 with primer forward - GAC ACG CCA GGA CAT ATG GA - and primer reverse - GAC GGA CCT CGA TGT GTT GA -.

KEYWORDS: Tetracycline, *tet*(M), *Lactobacillus plantarum,* Primer.

INTRODUCTION
Tetracyclines are broad-spectrum agents, exhibiting a wide range of gram-positive and gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasma, and rickettsiae, and protozoan parasites. The favorable anti-robotic properties of these agents and the absence of adverse side effects have been led to their extensive use of human and animal infections.[1] Research conducted at the Community Health Center, South Jakarta, Indonesia, from September 2011 to May 2013, Tetracycline has been reported to be resistance to several bacterial infections, showed considerably high resistance rate (25% - 100%).[2] There are many genes that cause resistance to tetracyclines, only one representative from each class has been sequenced, making comparisons easier to perform. One exception is the *tet*(M) gene, which has been sequenced from anumber of gram-positive and gram-negative species.[3] Binding of the Tet (M) protein is not affected by tetracycline but is inhibited by thiostrepton, which also inhibits binding of the EF-G protein. EF-G and the Tet (M) proteins compete for binding on ribosomes, with Tet (M) having a higher affinity than EF-G. This suggests that these products have overlapping binding sites and that Tet (M) must be released from the ribosome to allow EF-G to bind.[4]

tet(M) acting as an antibiotic resistance agent in tetracycline has also been identified in one of probiotic bacteria Lactobacillus plantarum which also contains the *tet*(M) gene.[5] Lactobacilllus plantarum includes probiotic bacteria that are inherently contained in food or dietary supplements, or added to the two substances intentionally, which are potential sources and also useful as determinants of antibiotic resistance.[6] The target DNA nucleotide sequence can be designed with one program, Prime3plus, which will provide several primer candidates that can be used to amplify specific genes in the PCR method.[7] Primer has certain criteria as a marker of whether or not the primer is used in the PCR method, the primer criterion parameters are: melting temperature (Tm), percentage of G and C (% GC), 3'dimer, and hairpins.[8] These criteria, can be analyzed by one program, namely OlygoAnalyzer 3. 1, which will bring up the primer criteria for primer candidates to get the best primer sequence.[9]

MATERIALS AND METHODS
This study begins with a search for the tet (M) gene nucleotide sequence data from the bacterium Lactobacillus Plantarum, this search was performed on RefSeqGene data at NCBI by searching for 'refseqgene [keyword]' or using the NCBI search menu at https://www. ncbi.nlm.nih.gov/refseq/rsg with the key word is tetM Lactobacillus plantarum.[10] The results of the nucleotide sequence data obtained at https://www.ncbi.nlm.nih.gov/gene/1450350, followed by the search for primer candidates in the Primer3plus program, specifically using subsections at https://primer3plus.com/cgi-bin/dev/primer3plus.cgi, the design is done through a website program so that some

primer candidates are obtained from the results of using the program.[7] The results of the primer candidates obtained are analyzed related to the primer criteria in the Integrated DNA Technologies' (IDT) Oligo Analyzer 3.1 program (http://www.idtdna.com/calc/Analyzer) by entering the primers into the main box and clicking on the Analyze, Hairpin, Self-Dimer and Hetero-dimer options in the right position.[9]

RESULTS AND DISCUSSION

Sequence Nucleotide Gene *tet*(M) Lactobacillus plantarum from NCBI

The nucleotide sequence used is the nucleotide sequence of the tet (M) Lactobacillus plantarum gene obtained through the NCBI site. The selection or search of nucleotide sequences through the NCBI website is done by going to www.ncbi.nlm.nih.gov and typing the keyword "tetM Lactobacillus plantarum" in the gene menu section to make the search more specific and accurate. The search for nucleotide sequence search through GenBank NCBI obtained the tet (M) Lactobacillus plantarum gene strain 5057 having 1920 base pairs of nucleotide, identity of protein code shown in **Fig. 1** and biological information shown in **Fig. 2**.

tetM tetracycline resistance protein TetM [*Lactobacillus plantarum*]

Gene ID: 1450350, updated on 29-Jan-2018

Summary

Gene symbol	tetM
Gene description	tetracycline resistance protein TetM
Locus tag	pMD5057_009
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Lactobacillus plantarum (strain: 5057)
Lineage	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus

Fig. 1: Identity of sequence nucleotide of *tet*(M) *L. plantarum.*

```
FEATURES             Location/Qualifiers
     source          1..1920
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                     /mol_type="genomic DNA"
                     /strain="5057"
                     /db_xref="taxon:1590"
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                     YPNMCVTNFTESEQWDTVIEGNDDLLEKYMSGKSLEALELEQEESIRFHNCSLFPVYH
                     GSAKNNIGIDNLIEVITNKFYSSTHRGPSELCGNVFKIEYTKKRQRLAYIRLYSGVLH
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                     QRKKIENPHPLLQTTVEPSKPEQREMLLDALLEISDSDPLLRYYVDSTTHEIILSFLG
                     KVQMEVISALLQEKYHVEIELKEPTVIYMERPLKNAEYTIHIEVRPNPFWASIGLSVS
                     PLPLGSGMQYESSVSLGYLNQSFQNAVMEGIRYGCEQGLYGWNVTDCKICFKYGLYYS
                     PVSTPADFRMLAPIVLEQVLKKAGTELLEPYLSFKIYAPQEYLSRAYNDAPKYCANIV
                     DTQLKNNEVILSGEIPARCIQEYRSDLTFFTNGRSVCLTELKGYHVTTGEPVCQPRRP
                     NSRIDKVRYMFNKIT"
CONTIG          join(AF440277.1:1..10877)
```

Fig. 2: Bioinformation of Nucleotide *tet*(M) *L. plantarum.*

Designing Primer Candidates using Primer3Plus

Gene nucleotide sequence that has been obtained can be directly conducted primer candidate search by opening the site https://primer3plus.com/cgi-bin/dev/primer3plus.cgi. Primers obtained through this site contain 5 pairs of primer candidates with each primer forward and reverse primer, as well as different product lengths (Table 1). The results of the search through

Primer3Plus this is only obtained primer candidates so it needs to do the analysis of primer criteria to get a specific primer.

Table 1: Result of primer candidates with Primer3plus.

Candidates primer	Primer F*orward*	Primer R*everse*	Amplicon (bp)
Primer 1	TCCGTCTGAACTTTGCGGAA	CAGAAAGGATTTGGCCGCAC	591
Primer 2	TCAACACATCGAGGTCCGTC	GCCGCACTTCGATGTGAATG	592
Primer 3	GTGCGGCCAAATCCTTTCTG	TCCGACTATTTGGACGACGG	577
Primer 4	GACACGCCAGGACATATGGA	GACGGACCTCGATGTGTTGA	521
Primer 5	CACATCGAGGTCCGTCTGAA	GATTTGGCCGCACTTCGATG	594

Analysis of Primer Criteria using OligoAnalyzer

A good primer is a primer that satisfies the parameter criteria of a primer, so an analysis is needed to obtain a specific primer according to the target DNA. Primer criteria analysis was performed with OligoAnalyzer which is a special program to find suitable primers based on primer criteria. Parameters analyzed included base length, melting temperature (Tm), % GC, hairpin, self dimer and cross dimer.

Table 2: Data on primer criteria analysis with OligoAnalyzer 3.1.

Candidate Primer		Length (bp)	Tm (^{o}C)	GC (%)	Self-dimer (ΔG)	Cross-dimer (ΔG)	Hairpin (ΔG)
1	F	20	56.8	50	-8.26	-11.77	-2.08
	R	20	57.1	55	-9.28		-0.1
2	F	20	56.9	55	-6.76	-13.49	-0.71
	R	20	57	55	-6.76		-1.53
3	F	20	57.1	55	-9.28	-10.39	0.44
	R	20	56.7	55	-6.68		-1.24
4	F	20	56.9	55	-7.82	-4.77	-1.22
	R	20	56.9	55	-6.76		-1.71
5	F	20	56.6	55	-6.76	-10.19	-0.71
	R	20	57.1	55	-9.28		-1.1

The length of the primer base indicates the number of nucleotide bases contained in the primer candidate. The results obtained after the analysis of primer candidates in Oligo Analyzer 3.1, all primer candidates have the same value that consists of 20 nucleotide bases (Table 2). The primer candidates have fulfilled the criteria of a primer, because they fulfill the criteria for the primer criteria, which is a base length of 18-24 nucleotides.[8] So that all these primer candidates can be said to be good primers for the PCR process, especially in terms of the number of nucleotide bases., the utilization of primers of a minimal length that ensures melting temperatures of 54^{o}C or higher will provide the best chance for maintenance of specificity and efficiency.[8]

The optimum tm for 20 base pair of primer, ranges from 56 - 62 °C.[8] The results of the analysis obtained from primer candidates 1 to 5 candidates meet the criteria of the temperature range Tm 56 - 62 ^{o}C (Table 2), so that all of these primer candidates in terms of primer criteria on Tm can be regarded as a good primer. Tm with the smallest temperature value that is 56,1°C owned by primer candidate 3 reverse and Tm with highest value that is 57,1°C which is owned by three primer candidate that is primer candidate 1 reverse, primer 3 forward and Primer 5 reverse.

The percentage analysis of GC is also the result of analysis on the ANALYZE option in Oligo Analyzer 3. 1. The results obtained from the GC percentage analysis are eligible from the primer criteria that are in the 35-65% range of GC content in a primer.[11] Almost all primer candidates, both forward and reverse, have a percentage percentage of 55% GC of only one primer candidate having different percentages of GC content, that is primer candidate 1 forward (Table 2), but still within the range of good primer criteria.

One of the things that should be avoided in primer design is the existence of secondary structure. The secondary structure in question includes Self dimer, Cross dimer and Hairpin. In order to prevent the formation of stable secondary structures like hairpin, self dimer or cross dimer, the primers were designed with ΔG values higher than −2 for hairpin, and higher than −5 for self dimer and cross dimer.[12] The analyzes performed on the primer secondary structure form using Oligo Analyzer 3.1 with using SELF-DIMER option, The results of the analysis show that all primer candidates have the possibility of the occurrence of the self-dimer secondary structure (Table 2), because the resulting ΔG value is smaller than the tolerance limit on the self dimer criteria. The value of the 3 reverse primer candidate is the highest primer

candidate with a value of ΔG = -6.68, while the lowest ΔG value is owned by the candidate 1 reverse primer, 3 forward primer and 5 reverse primer, with the value ΔG = - 9.28. It may cause spontaneous secondary structures resulting from a small free energy, which can not be a barrier to the occurrence of primer bonds with their own primers, leading to inhibition in the PCR process.

Cross dimer's primer criterion, analyzed using HETERO-DIMER option on Oligo Analyzer 3.1. Cross dimer analysis, analyzed by calculating free bond energy between forward and reverse primers, so as to predict the occurrence of secondary structure between forward and reverse primers. The result of the analysis shows that primer candidates 1, primer 2, primer 3 and primer 5 have cross dimer that is ΔG bond lower than -5, ΔG value of each candidate are (-11,77), (-13,49), (-10.39) and (-10.19). While the primer candidate 4 has a value greater than the criteria of ΔG = (-4.77) which can minimize the occurrence of secondary structures of the primer formed.

Hairpin is a secondary structure of the forming primer, the loop structure at the primer end or 3' primer. If a primer has a value of ΔG more than required, it will form a loop structure on the primer and may cause a primer to adhere to an inappropriate region (mismatches) 7. The analysis results using OligoAnalyzer 3.1 in the HAIRPIN option, indicates that it is not possible to construct a hairpin structure, because the resulting value is greater than the tolerance limit ΔG = -2 (Table 2).

The results of the analysis five primer candidates, both forward and reverse, can show good results. Five criteria tested on each primer candidate, there are two criteria that are not fulfilled by several primer candidates, specifically Self dimer and cross dimer. However, there is also a benefit in the presence of such secondary structures because it can inhibit the occurrence of bonding on unwanted single-stranded DNA.[13] The results of the primer criterion analysis, the primer candidate number 4 being the best primer. This is evident from the value of ΔG in Cross dimer, only primer candidate 4 fulfills the primer criteria. Primer 4 candidate with nucleotide sequence Primer forward - GAC ACG CCA GGA CAT ATG GA - and reverse Primer – GAC GGA CCT CGA TGT GTT GA - can be selected as the best primer, from the primer design of Gen *tet*(M) Bacteria Lactobacillus plantarum with using Primer3plus.

CONCLUSION

Based on analyse, there are five pairs of primer forward and reverse candidates *tet*(M) Lactobacillus plantarum bacteria designed using Primer3plus. The results of the primer criteria analysis were tested, yielding Primer number 4 forward and reverse as the best primers, with sequence of nucleotides Primer forward - GAC ACG CCA GGA CAT ATG GA - and Primer Reverse - GAC GGA CCT CGA TGT GTT GA –

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