IDENTIFIKASI BAKTERI ASAM LAKTA BERBASIS MOLEKULER (DNA/RWA)

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KRITERIA IDENTIFIKASI YANG DIDASARKAN PADA MOL DNA/RNA :

Spesies yg sama

Genus yg sama

Famili yg sama

Similariti DNA > 70 % Sekuen rRNA > 98 % > 20-30 % > 93-95 %

> 89-93 %

TAHAPAN IDENTIFIKASI BAL



ISOLASI BAL



Isolat Murni

DNA Extraction

DNA isolat

PNH & ESR - PSPG UGM



TAHAPAN ISOLASI DNA (MANUAL METHOD) 1



PNH & ESR - PSPG UGM



TAHAPAN ISOLASI DNA (MANUAL METHOD) 2



X

Pemindahan pada kolom GD dengan tube

Sentrifugasi (13.000 RPM, 10 Menit)

Pembuangan larutan pada tube

Penambahan etanol 70% pada kolom GD

Sentrifugasi (13.000 RPM, 10 Menit)

Pembuangan larutan pada tube

Penambahan 50 µl TE

Sentrifugasi (13.000 RPM, 10 Menit)

Ekstrak DNA (Tube)

TAHAPAN ISOLASI DNA (MANUAL METHOD) 3

PNH & ESR - PSPG UGM

FUNGSI REAGENT

Reagent	Function
Buffer Lysis	Menjaga kondisi pH selama proses ektraksi/isolasi DNA sehingga DNA tidak mengalami kerusakan akibat perubahan pH
Proteinase-K	Mendigest kontaminan protein serta mendegradassi enzim nuklease yang dimungkinkan ada selama proses isolasi
Lysozym	Enzim yang dapat memecah dinding sel dan aktif di suhu sekitar 50-55°c
Fenol	Membuka dari lipatan protein sehingga DNA mudah untuk terdenaturasi
Chloroform	Meningkatkan efisiensi dari fenol dalam denaturasi protein dan memberikan pemisahan yang baik antara fasa organik dan fasa cair dan DNA tetap tertahan dengan baik pada fasa cair

FUNGSI REAGENT

Reagent	Function					
Ethanol Absolute	Mengendapkan DNA dalam bentuk pelet (presipitasi)					
Ethanol 70%	Membersihkan DNA dari debris garam yang timbul selama proses ekstraksi dari buffer					
TE Buffer	Melarutkan kembali DNA yang terpresipitasi					



Reference : https://geneticeducation.co.in/phenolchloroform-dna-extraction-basics-prepo

f-chemicals-and-protocol/

PCR (POLYMERASE CHAIN REACTION)

30x

- Proses penggandaan (copy) fragmen DNA spesifik.
- Terdiri atas beberapa tahap :
 - Predenaturasi
 - Denaturasi
 - Annealing
 - Extension
 - Last extension

Reagent	Jumlah
Nuclease Free Water	9,5 uL
Mix PCR (Biolane HS)	12,5 UL
Primer 27F	1 UL
Primer 1492R	1 UL
DNA Template	1 UL
Total	25 UL

Tahapan	Suhu	Waktu
Predenaturasi	96°C	4 Menit
Denaturasi	94°C	1 Menit
Annealing	51,5°C	1 Menit 30 detik
Extension	68°C	8 Menit
Last extention	68°C /	10 Menit

DNA SEQUENCING

- Setelah DNA si duplikasi menggunkana PCR DNA akan di sequencing pada alat DNA Sequencer.
- Pada tahap ini sampel diserahkan kepada teknisi di LPPT-UGM

IDENTIFIKASI BAL

Untuk melakukan identifikasi molekuler mikrobia dapat menggunakan beberapa software. Pada penjelasan ini menggunakan program DNA Baser Assembler dan MEGA X. Untuk database menggunakan web NCBI-BLAST.

IDENTIFIKASI BAL

 Setelah proses sequencing akan didapatkan data urutan genom dari isolat terpilih dalam bentuk file. Untuk mengolah file tersebut menggunakan program DNA Baser Assembler

DNA BASER

 Program tersebut untuk melakukan contig yaitu mengedit dengan cara memotong urutan genom
 File hasil sequencing forward dan reverse dilakukan proses assembly.

 Contoh pada file isolat 4 (4F dan 4R)

🗋 File 🕐 Tasks 📌 View 💼 External tools 🏽 🖓 Window 🍈 Info					
Project Manager - Sequence assembly					
DNA BASER - Project Ma	anager 🔥				
Project builder Project options Sequence processing options Vector removal Remaining time: 12 days 0h 1m 30s					
SAMPLE EXPLORER	_ JOB LIST				
Image: Browse All supported files Image: Browse All supported files Image: Browse 3F_A07_01_StdSeq50_POP7_Z.ab1 Image: Browse 3F_A07_01_StdSeq50_POP7_Z.ab1 Image: Browse 3F_A07_05_StdSeq50_POP7_Z.ab1 Image: Browse 3R_E07_05_StdSeq50_POP7_Z.ab1 Image: Browse 3R_E07_05_StdSeq50_POP7_Z.ab1 Image: Browse 3R_E07_05_StdSeq50_POP7_Z.ab1 Image: Browse 4F_C07_03_StdSeq50_POP7_Z.ab1 Image: Browse 4R_F07_06_StdSeq50_POP7_Z.ab1 Image: Browse 4R_F07_06_StdSeq50_POP7_Z.ab1 Image: Browse 5F_D07_04_StdSeq50_POP7_Z.ab1	Add D:\File Sequence Vita\4F_C07_03_StdSeq50_P0P7_Z.ab1 D:\File Sequence Vita\4R_F07_06_StdSeq50_P0P7_Z.ab1				
 SF_D07_04_StdSeq50_P0P7_Z.seq SR_G07_07_StdSeq50_P0P7_Z.ab1 SR_G07_07_StdSeq50_P0P7_Z.seq Contig - File Sequence Vita.FASTA Contig - Isolat4.FASTA File Sequence Vita.BaserProj Isolat4.BaserProj K1 F_D05_04_StdSeq50_P0P7_Z.ab1 K1 F_D05_04_StdSeq50_P0P7_Z.seq K1 R H05 08 StdSeq50 P0P7_Z.ab1 	Project name Save contig to: D:\File Sequence Vita\ Use current folder Browse Assemble to reference				
Constant of the second se	Reterence tile: Uraq your reference tile here				

DNA BASER

- Contoh hasil contig menggunakan DNA Baser Assembler
- File yang disimpan berupa fasta file (.fasta)

💫 DNA Baser Assembler v5.15.0 (32bit). Trial. Mem: 19.45 MB - [Sequence assembly: File Sequence Vita [25 mismatches]]	_ 0 _ ×
🜃 🗋 File 🖏 Tasks 📌 View 💼 External tools 📄 Project 📝 Edit 🗟 Search 🗾 Samples 脳 Chromatograms 🚍 Contig 🗔 Assembly grid 🕴 🍋 Window 🍈 Info	_ & ×
Assembly Log	
F 4F C07 03 StdSeq A C C A G T G G C G A A G G C G G C T C T C T G G T C T G T A A C T G A C G C T G A G G C T C G A A A G C G T G G G G A G C A A A C A G G A T T A G A T A C C R 4R F07 06 StdSeq A C C A G T G G C G A A G G C G G C T C T C T G G T Y T G T A A C T G A C G C T G A G G C T C G A A A G C G T G G G G A G C A A A C A G G A T T A G A T A C C C T G G T A G T C C C T G G T G G G A G C A A A C A G G A T T A G A T A C C C T G G T A G C T C G A A G C G T G G G G G G G G G G A G C A A C A G G A T T A G A T A C C C T G G T A G T C G A G C C T G G G G A G C A A C A G G A T T A G A T A C C C T G G T A G T C C C T G G T G G G G A G C A A C A G G A T T A G A T A C C C T G G T A G T C C C T G G T G G G A G C A A C A G C G T G G G G A G C A A C A G C G T G G G G A G C A A C A G C G T G G G A G C A A C A G C G T G G G G A G C A A C A G C G T G G G A G C A A C A G C G T G G G A G C A A C A G C G T G G G A G C A A C A G G A T T A G A T A C C C T G G T A G T C C C T G G T A G T C C C T G G T G G G G A G C A A C A G C G C G C G C T C T G G T G G G G C A C C A G T G G G G A G C A A C A G C G T G G G G A G C A A C A G G C G C T C T C T G G T G G T G G C G C	с м с s с с с т
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BLAST (BASIC LOCAL ALIGNMENT SEARCH TOOLS)

Merupakan web based database yang menyimpan database mikrobia di seluruh dunia.

NIH U.S. National Library of M	dicine NCBI National Center for Biotechnology Information				Sign i	in to NCBI
BLAST [®]			Home	Recent Results	Saved Strategies	Help
Q	COVID-19 is an emerging, rapidly evolving situa Get the latest public health information from CDC: <u>https://www</u> . Get the latest research from NIH: <u>https://www.nih.gov/</u>	ion. <u>v.coronavirus, gov</u> . <u>ioronavirus</u> .				
	BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.	tacoronavirus Database :reated a new BLAST database focused on the SARS ry syndrome coronavirus 2) Sequences. For further o IBank. eb 2020 10:00:00 EST	6-CoV-2 (Sever detail please More BLAS	re acute visit ST news		
r - pspg ugm	Web BLAST Image: State of the state of	Protein BLAS				

- Untuk proses BLAST digunakan option Nucleotide BLAST
- Digunakan File fasta dari hasil Contig untuk memasukkan sequence yang akan di BLAST

	de BLAST
	Standard Nucleotide BLAST
blastn <u>blastp</u> blast	x tblastn tblastx
Enter Query Se	BLASTN programs search nucleotide databases using a nucleotide query. more
Enter accession nu	umber(s), gi(s), or FASTA sequence(s) 😡 <u>Clear</u> Query subrange 😡
>Isolat4 GKGAACGSTTCTTTTCMC(GTGGGTAACCTGCCCATCA/ CGCATGGTTTTGATTTGAA/ GGTGAGGTAACGGCTCACC/	CGGGAAGCTTGCTCCACCGGAAAAAGAGGAGTGKYKAACGGGTGAGTAACAC SAAGGGGATAACACTTGGAAACAAGTGCTAATACCGTATAACAATCRAAAC AGGCGCTTTCGGGTGTCGCTGATGGATCGGACCCGCGGTGCATTAGCTAGTT AAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACT
Or, upload file	Choose File No file chosen
Job Title	Isolat4
	Enter a descriptive title for your BLAST search 😣
🗆 Align two or mo	re sequences 😡
Choose Search	Set
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	Nucleotide collection (nr/nt)
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	Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown @
Exclude Optional	□ Models (XM/XP)
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Optional	Enter an Entrez query to limit search 😡

Contoh hasil Blast pada isolat no 4

BLAST [®] »	blastn suite » results for RID-9GTN0V1J014	Home Recent Results Saved Strategies Help
< Edit Se	arch Save Search Search Summary 🛩	How to read this report? I BLAST Help Videos DBack to Traditional Results Page
f You	search is limited to records that exclude: uncultured/environmental sample	sequences
Job Title	Isolat4	Filter Results
RID	<u>9GTN0V1J014</u> Search expires on 04-17 22:07 pm Download All 💙	
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	erococcus faecium strain CAU3862 16S ribosomal RNA gene, partial sequence	2507 2507 99% 0.0 98.23% MF429683.1
	erococcus faecium strain 7-10 16S ribosomal RNA gene, partial sequence	2507 2507 99% 0.0 98.23% KJ702545.1
	erococcus durans strain CAU6810 16S ribosomal RNA gene, partial sequence	2505 2505 99% 0.0 98.23% <u>MF583035.1</u>
	erococcus taecium strain CAU9113 16S ribosomal RNA gene, partial sequence	2505 2505 99% 0.0 98.22% <u>MF424050.1</u>
	erococcus taecium strain D1 165 ribosomal RNA gene, partial sequence	2505 2505 99% 0.0 98.22% <u>KJ702551.1</u>

Cari isolat yang memiliki query cover 100% atau mendekati 100%

 Klik bagian Acsession number untuk membuka informasi mengenai isolat tersebut

Sec	luences producing significant alignments	mana	ge coi	uiiiis	. 31		
	select all 100 sequences selected	<u>Gen</u>	<u>Bank</u>	<u>Graph</u>	ics D	istance t	ree of results
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
~	Enterococcus faecium strain CAU3862 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	MF429683.1
	Enterococcus faecium strain 7-10 16S ribosomal RNA gene, partial sequence	2507	2507	99%	0.0	98.23%	KJ702545.1
	Enterococcus durans strain CAU6810 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.23%	MF583035.1
	Enterococcus faecium strain CAU9113 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	MF424050.1
	Enterococcus faecium strain D1 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.22%	KJ702551.1
	Enterococcus faecium strain TW5-3 16S ribosomal RNA gene, partial sequence	2505	2505	99%	0.0	98.16%	KJ026572.1
	Enterococcus faecium strain IGM5-9 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MT197265.1
	Enterococcus faecium strain IGM4-6 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	<u>MT197247.1</u>
	Enterococcus faecium strain VVEswe-R chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP041261.2
	Enterococcus faecium strain VVEswe-S chromosome, complete genome	2503	15014	99%	0.0	98.15%	CP041270.2
	Enterococcus faecium strain SRR24 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP038996.1
	Enterococcus faecium strain ZY2 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP039729.1
	Enterococcus faecium strain ZY11 chromosome, complete genome	2503	15019	99%	0.0	98.15%	CP038995.1
	Enterococcus faecium strain ME3 chromosome	2503	10013	99%	0.0	98.15%	CP043865.1
	Enterococcus faecium strain 4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MH236305.2
	Enterococcus faecium strain AHC4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.15%	MN749966.1
	Enterococcus faecium strain VRE chromosome, complete genome	2503	15014	99%	0.0	98.15%	CP046077.1
~	Enterococcus durans strain APED4 16S ribosomal RNA gene, partial sequence	2503	2503	99%	0.0	98.22%	MN595072.1
	Enterococcus faecium strain LAC7.2 chromosome, complete genome	2503	15001	99%	0.0	98.15%	CP045012.1

Managa Columna V Show 100 V

- Pada bagian "send to" klik dan pilih file lau pilih format fasta lalu download/send file
- File tersebut digunakan dalam proses elanjutnya menggunakan program MEGA X
- Pilih setidaknya 5-6 database yang mirip (query mendekati 100%)

GenBank 🗸			Send to: -	- n shown	
		Choose Destin	ation	TSHOWN	
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FASTA Gra	aphics PopSet	Download 1 iter	m.		
<u>Go to:</u> ⊘ LOCUS	MF429683 1423 bp DNA linear BCT 10-AUG-2017	Format GenBank Summary	•	equence	· · · · · · · · · · · · · · · · · · ·
DEFINITION	Enterococcus faecium strain CAU3862 165 ribosomal RNA gene, partial sequence.	GenBank GenBank (full)		ce Features	
ACCESSION	MF429683	FASTA			
VERSION	MF429683.1	ASN.1		ence	
KEYWORDS SOURCE ORGANISM	Enterococcus faecium <u>Enterococcus faecium</u> Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae; Enterococcus.	XML INSDSeq XML TinySeq XML Feature Table	Related int	formation	
REFERENCE AUTHORS TITLE	1 (bases 1 to 1423) Ren,F. and Zhao,L. Direct Submission	GI List GFF3	PopSet		
JOURNAL	Submitted (04-JUL-2017) College of Food Science & Nutritional Engineering, China Agricultural University, No. 17 Qinghua East Road, Beijing 100083, China		LinkOut to	external resources	
COMMENT	##Assembly-Data-START## Sequencing Technology :: Sanger dideoxy sequencing ##Assembly-Data-END##		SILVA SSU	Database	[SILVA]
FEATURES	Location/Qualifiers				
source	11423 /organism="Enterococcus faecium"		Recent act	livity	
	/ 1 / 1 / 2001				

MEGA (MOLECULAR EVOLUTIONARY GENETIC ANALYSIS)

 Tahapan selanjutnya menggunakan software MEGA (Molecular Evolutionary Genetic Analysis). Di sini digunakan MEGA versi 10 (MEGA X)

- Untuk membuat pohon filogeni dilakukan pengaturan sequences agar sequence menjadi teratur
- Tahapan pertaman dapat dilihat dari slide

 Masukkan semua sequence yang telah di unduh dari BLAST dan sequence isolat 4 (Fasta File)

🖂 MX: Al	ignment Explorer			and the Statements	Sec. 1	Constitution of the second
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1. Seque	Y Delete					
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	Select Sequence(s)					
	Select All					
	 Allow Base Editing 					
	Modify All Bases To Upper Case					
		-				

 Masukkan semua sequence yang telah di unduh dari BLAST dan sequence isolat 4 (Fasta File)

 Highlight semua sequence lalu pilih alignment – align by ClustalW

MX: Alignment Explorer		
Data Edit Search	and the second	cer Display Help
1 🛥 🖪 📽 🗐 🕪	W Align by ClustalW	□ X □ X 4 + 2
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1. KJ026572.1_Enter T G G C A G		C A C C G G A G C T T G C T C C A C C G G A A A A A G A G G A G T G G C G A A C G G G T G A G C A C C T G C C C A T C A G A A G G G G A T ,
2. KJ702551.1_Enter T G C A A G	Mark/Unmark Site	A ¹ C G G A G C T T G C T C C A C C G G A A A A A G A G A G T G G C G A A C G G G G T A A C A C G T G G G T A A C C T G C C C A T C A G A A G G G G A T A /
3. MF424050.1_Ente G C A G T C	Align Marked Sites	C G G A G C T T G C T C C A C C G G A A A A A G A G G A G G G C G A A C G G G T G A G C A C C T G C C C A T C A G A A G G G G A T A A C ,
4. MF429683.1_Ente C A G T C G	Unmark All Sites	G G A G C T T G C T C C A C C G G A A A A A G A G A G T G G C G A A C G G G T G A G C A C G T G G G T A A C A I
5. KJ702545.1_Enter T G C A A G	Delete Gan-Only Sites	A C C G G A G C T T G C T C C A C C G G A A A A A G A G G A G T G G C G A A C G G G T G A G T A C C C G G C C A T C A G A A G G G G G A T A /
6. ContigIsolat4 GKGAAC	 Auto-Fill Gaps 	A G C T T G C T C C A C C G G A A A A A G A G G A G T G K Y K A A C G G G T G A G T A A C A C G T G G G T A A C C T G C C C A T C A G A A G G G G A T A A C A C T

 Sequence yang sudah di align di simpan dalam format mega untuk pembuatan pohon filogeni

 File disimpan dalam file berekstensi .meg

- Untuk pembuatan pohon filogeni menggunakan menu phylogeny – construct/test neighbor joining tree
- Lalu pilih file mega yang sudah disimpan tadi

 Untuk preferences mengikuti option sepeti gambar di samping

MX: Analysis Preferences	and some the second sec
Phylogeny Reconstruction	
Option	Setting
ANALYSIS	
$Scope \to$	All Selected Taxa
Statistical Method $ ightarrow$	Neighbor-joining
PHYLOGENY TEST	
Test of Phylogeny $ ightarrow$	Bootstrap method
No. of Bootstrap Replications $ ightarrow$	100
SUBSTITUTION MODEL	
Substitutions Type $ ightarrow$	Nucleotide
Genetic Code Table $ ightarrow$	Not Applicable
Model/Method $ ightarrow$	Kimura 2-parameter model 📃 💌
Fixed Transition/Transversion Ratio $ ightarrow$	Not Applicable
Substitutions to Include $ ightarrow$	d: Transitions + Transversions
RATES AND PATTERNS	
Rates among Sites $ ightarrow$	Uniform Rates
Gamma Parameter 🔶	Not Applicable
Pattern among Lineages $ ightarrow$	Same (Homogeneous)
DATA SUBSET TO USE	
Gaps/Missing Data Treatment $ ightarrow$	Pairwise deletion
Site Coverage Cutoff (%) $ ightarrow$	Not Applicable
Select Codon Positions $ ightarrow$	▼ 1st ▼ 2nd ▼ 3rd ▼ Noncoding Sites
SYSTEM RESOURCE USAGE	
Number of Threads $ ightarrow$	3
? Help	🗴 Cancel 📿 ОК

 Contoh hasil pembuatan pohon filogeni

KJ702551.1 Enterococcus faecium strain D1 16S ribosomal RNA gene partial sequence KJ702545.1 Enterococcus faecium strain 7-10 16S ribosomal RNA gene partial sequence Contig - Isolat4

KJ026572.1 Enterococcus faecium strain TW5-3 16S ribosomal RNA gene partial sequence MF429683.1 Enterococcus faecium strain CAU3862 16S ribosomal RNA gene partial sequence MF424050.1 Enterococcus faecium strain CAU9113 16S ribosomal RNA gene partial sequence

SEQUENCE SIMILARITY

- Untuk mengetahui tingkat kemiripan antara strain isolat dengan isolat lain dapat menggunakan distances menu
- Dari menu distances pilih compute pairwise distance
- Gunakan file hasil align (.meg)

SEQUENCE SIMILARITY

 Preferensi dalam model penghitungan tingkat similarity seperti ditunjukkan dalam gambar

IX: Analysis Preferences	
Distance Estimation	
Option	Setting
ANALYSIS	
$_{\rm Scope} \rightarrow$	Pairs of taxa
ESTIMATE VARIANCE	
Variance Estimation Method $ ightarrow$	None
No. of Bootstrap Replications $ ightarrow$	Not Applicable
SUBSTITUTION MODEL	
Substitutions Type $ ightarrow$	Nucleotide
Genetic Code Table $ ightarrow$	Not Applicable
Model/Method $ ightarrow$	Kimura 2-parameter model
Fixed Transition/Transversion Ratio $ ightarrow$	Not Applicable
Substitutions to Include $ ightarrow$	d: Transitions + Transversions
RATES AND PATTERNS	
Rates among Sites $ ightarrow$	Uniform Rates
Gamma Parameter →	Not Applicable
Pattern among Lineages $ ightarrow$	Same (Homogeneous)
DATA SUBSET TO USE	
Gaps/Missing Data Treatment $ ightarrow$	Pairwise deletion
Site Coverage Cutoff (%) $ ightarrow$	Not Applicable
Select Codon Positions $ ightarrow$	▼ 1st ▼ 2nd ▼ 3rd ▼ Noncoding Sites
? Help	× Cancel ✓ OK

SEQUENCE SIMILARITY

Hasil kalkulasi akan ditunjukkan seperti tabel di bawah

MX: Pairwise Distances (Isolat 4 Vita Baru.meg)				frank (
File Display Average Caption Help							
	1	2	3	4	5	6	
1. Contig - Isolat4							
2. KJ026572.1 Enterococcus faecium strain TW5-3 16S ribosomal RNA gene partial sequence	0.0014445656	> A	ngka ini m	nenunjukko	an similarita	as sebesar '	99,86%
3. KJ702551.1 Enterococcus faecium strain D1 16S ribosomal RNA gene partial sequence	0.0007229352	0.0021235206					
4. MF424050.1 Enterococcus faecium strain CAU9113 16S ribosomal RNA gene partial sequence	0.0014456098	0.0021126775	0.0028362544				
5. MF429683.1 Enterococcus faecium strain CAU3862 16S ribosomal RNA gene partial sequence	0.0014444349	0.0007036061	0.0007085918	0.0014088232			
6. KJ702545.1 Enterococcus faecium strain 7-10 16S ribosomal RNA gene partial sequence	0.0014444349	0.0021100782	0.000000000	0.0028182694	0.0007036061		

► Hasil identifikasi isolat 4 adalah sebagai berikut :

Strain	Related Species Name	Acc. No	Identity	Identification by 16S rRNA Gene	
Isolat 4	Enterococcus faecium TW5-3	KJ026572	99,86%		
	Enterococcus faecium D1	KJ702551	99,93%		
	Enterococcus faecium CAU9113	MF424050	99,86%	Enterococcus faecium	
	Enterococcus faecium CAU 3862	MF 429683	99,86%	group	
	Enterococcus faecium 7-10	KJ702545	99,86%		