BAB V
PENUTUP

A. Kesimpulan

Berdasarkan hasil penelitian dapat disimpulkan bahwa :

Pertama, di dalam produk Rusip terdapat BAL.

Kedua, supernatan BAL produk Rusip memiliki aktivitas sebagai antibakteri terhadap bakteri *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* ATCC 25923.

Ketiga, konsentrasi paling aktif dari supernatan BAL produk rusip yang memiliki aktivitas antibakteri terhadap *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* ATCC 25923 adalah 800 ppm.

Konsentrasi hambat minimum (KHM) dari supernatan BAL produk Rusip terhadap *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* ATCC 25923 adalah 200 ppm. Konsentrasi Bunuh Minimum (KBM) dari supernatan BAL produk Rusip terhadap *Escherichia coli* ATCC 25922 adalah 400 ppm, sedangakan terhadap *Staphylococcus aureus* ATCC 25923 adalah 800 ppm.

B. Saran

Dalam penelitian ini masih banyak kekurangan, maka perlu dilakukan penelitian lebih lanjut mengenai:

Pertama, perlu dilakukan penelitain lebih lanjut untuk mengetahui aktivitas antibakteri supernatan BAL produk Rusip terhadap mikroorganisme lainnya.

Kedua, perlu dilakukan sentrifugasi dengan kecepatan yang lebih tinggi agar diperoleh supernatan yang lebih murni.

Ketiga, perlu dilakukan penelitian lebih lanjut pada tahap formulasi supernatan agar dapat digunakan masyarakat.

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LAMPIRAN

Lampiran 1. Daftar Komposisi Media MRS (Man Rogosa Sharpe) Agar

Enteric Pathogens, Central Public Health Laboratory, Colindale, London. Dr. B. Rowe, Personal Communication, 1988.)

Technique

1 Inoculate three drops (ca. 0.1ml) of the pre-enrichment culture (after incubation for 16–20 hours) in separate spots on the surface of the MSRV Medium plates.

2 Incubate the plates in an upright position at 42 °C for up to 24 hours. (Care should be taken not to exceed 24 hours.)

3 Examine the plates for motile bacteria which will be shown by a halo of growth originating from the inoculation spot.

4 Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

De Smedt[6] reported that if MSRV medium is contained in test tubes and incubation is carried out under anaerobic conditions, visible migration zones are produced in 6 hours enabling *Salmonella* in foods to be detected in 24 hours.

Storage conditions and Shelf life

Store the dehydrated medium below 25 °C and use before the expiry date on the label.

Store the selective supplement in the dark at 2 °C to 8 °C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2 °C to 8 °C in the dark.

Quality Control

Positive control:

Salmonella typhimurium ATCC® 14028 – Straw colonies at site of inoculation surrounded by halo of growth.

Salmonella enteritidis ATCC® 13076 – Straw colonies at site of inoculation surrounded by halo of growth.

Negative control:

Citrobacter freundii ATCC® 8090 – Restricted or no growth.

Precautions

The basal medium is very hygroscopic. When handling the powder a face mask and gloves must be worn.

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MRS AGAR

(DE MAN, ROGOSA, SHARPE)

Code: CM361

A solidified version of MRS Broth for the culture of 'lactic acid bacteria'.

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	8.0
Yeast extract	4.0
Glucose	20.0
Sorbitan mono-oleate	1ml
Dipotassium hydrogen phosphate	2.0
Sodium acetate $3H_2O$	5.0
Triammonium citrate	2.0
Magnesium sulphate $7H_2O$	0.2
Manganese sulphate $4H_2O$	0.05
Agar	14.0
pH 6.2 ± 0.2	

Directions

Suspend 62 grams in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes, bottles or flasks and sterilise by autoclaving at 121 °C for 15 minutes.

Description

The MRS formulation was developed by de Man, Rogosa and Sharpe[1] to replace a variable product (tomato juice) and at the same time to provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media.

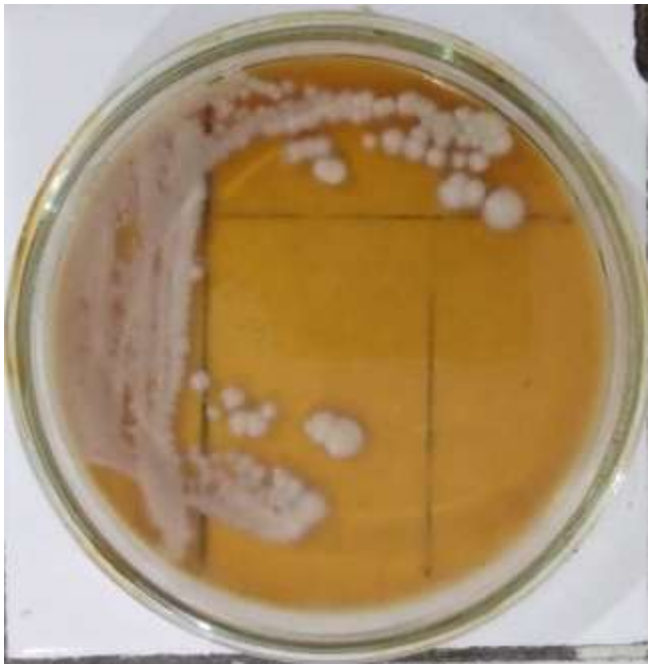
MRS medium is superior to the tomato juice medium of Briggs[2] and the meat extract tomato juice medium of de Man. It gives more profuse growth of all strains of lactobacilli, especially the difficult and slow growing strains of *L. brevis* and *L. fermenti*.

MRS Agar and Broth were designed to encourage the growth of the 'lactic acid bacteria' which includes species of the following genera: *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc*. All these species can produce lactic acid in considerable amounts. They are Gram +ve, catalase and oxidase -ve and are fastidious in their nutritional requirements. Growth is enhanced considerably by micro aerophilic conditions.

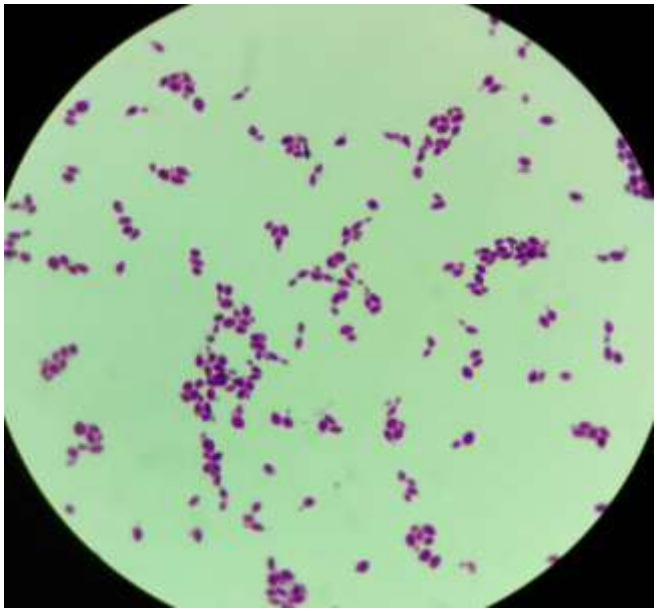
Generally the 'lactic acid bacteria' show delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if incubation is required for 2–4 days.

Selection can be made by pH adjustment, thus lactobacilli will tolerate lower pH levels than streptococci (pH 5.0–6.5) with pediococci and leuconostocs growing best within this range. Inhibitors of the main groups of competitor microflora include thallous acetate, sodium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin. These substances can be used at varying concentrations and combinations but inevitably a compromise has to be reached between selectivity and productivity of the organism sought[3].

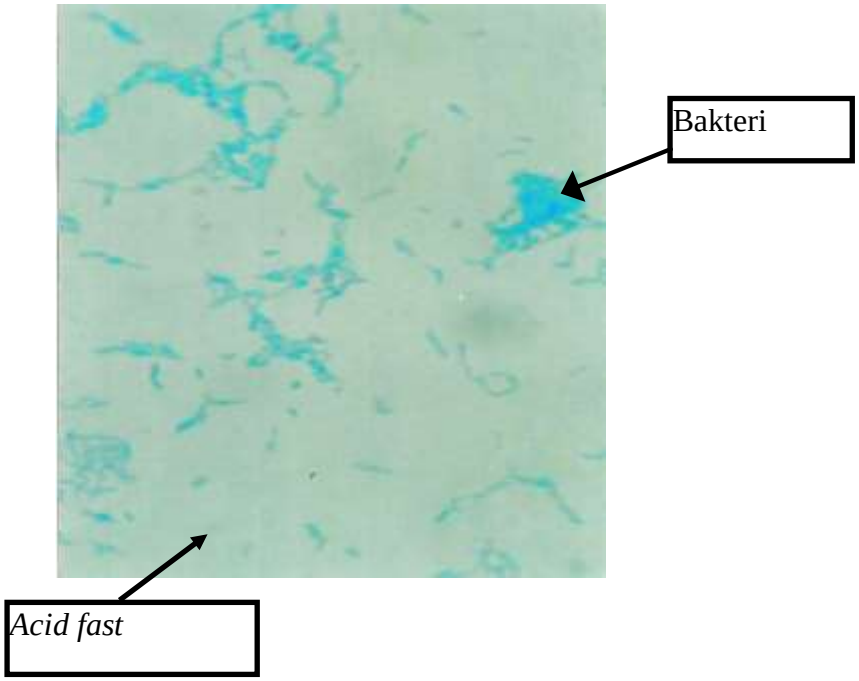
Lampiran 2. Hasil Morfologi Koloni Isolat Rusip dalam media MRSA

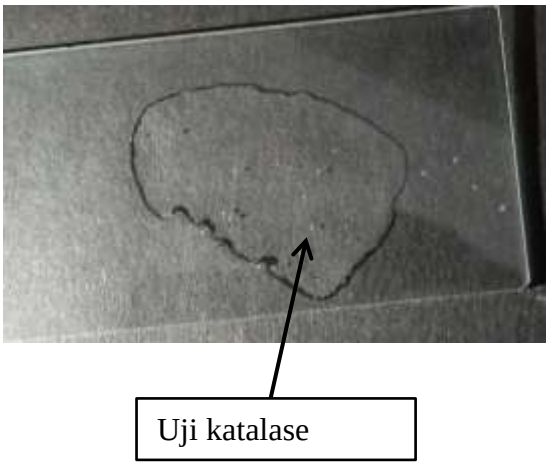


Lampiran 3. Gambar Hasil Identifikasi Mikroskopis BAL dalam Rusip dengan Pewarnaan Gram

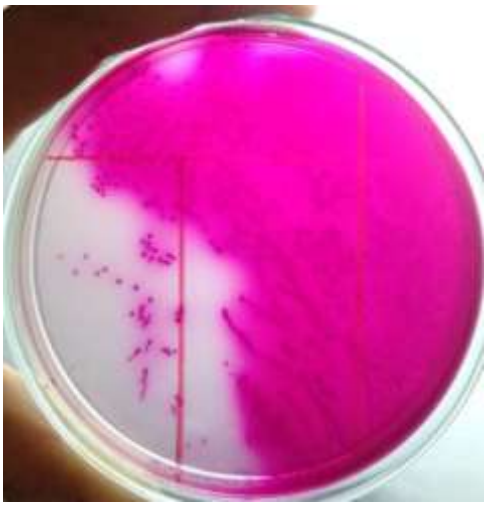


Lampiran 4. Gambar hasil mikroskopis dengan pewarnaan acid fast dan uji katalase pada bakteri BAL

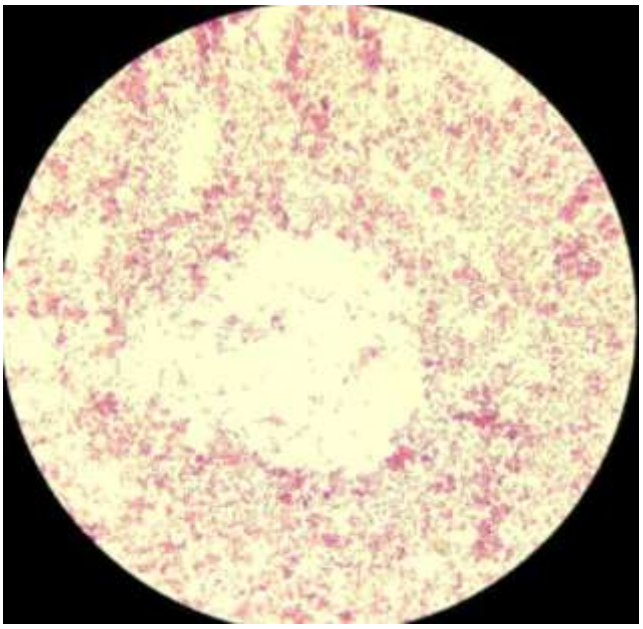




Lampiran 5. Gambar hasil identifikasi makroskopis bakteri *Escherichia coli* ATCC 25922 pada media *Endo Agar* (EA)



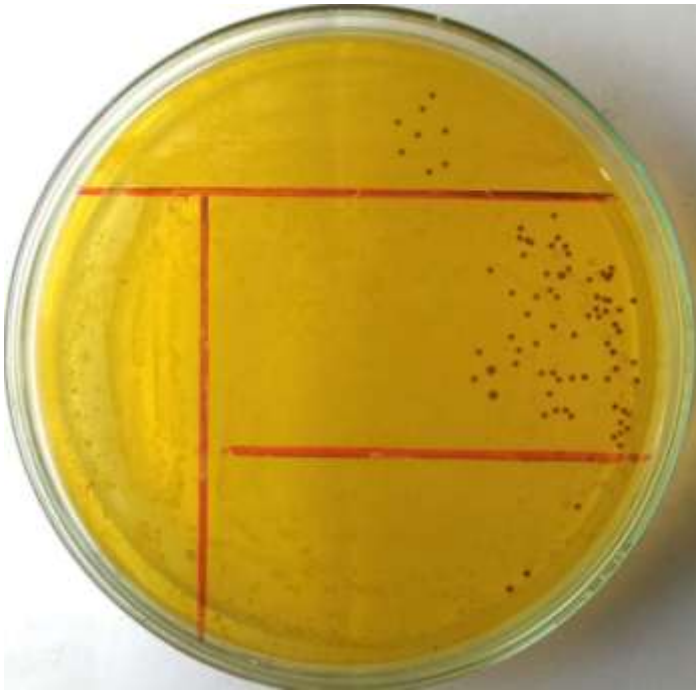
Lampiran 6. Gambar hasil identifikasi mikroskopis bakteri *Escherichia coli* ATCC 25922 dengan pewarnaan gram



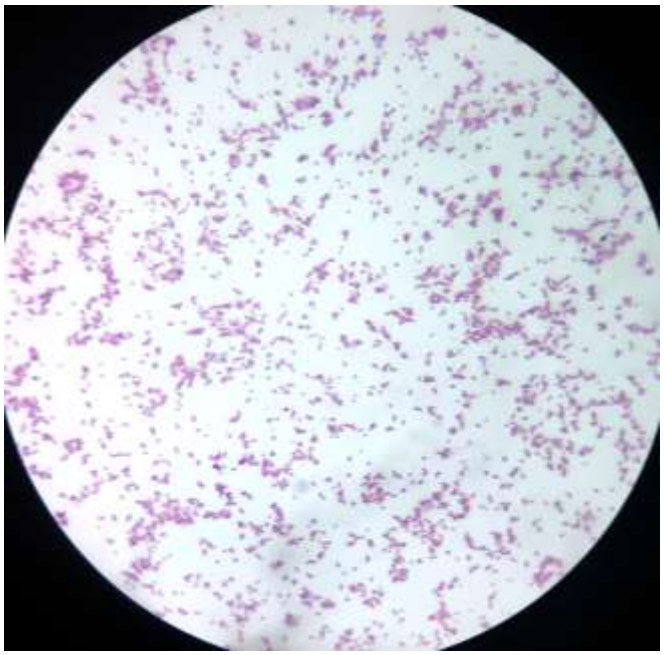
Lampiran 7. Hasil Uji Biokimia

No	Jenis Bakteri	Medium	Gambar	Hasil	Keterangan
1	*E. Coli*	SIM		Sulfida : (-) Indol : (+) Motilitas : (+)	• Uji sulfida : uji positif jika pada media terbentuk warna hitam • Uji indol : media ditambahkan reagen Erlich A (3tetes) dan Erlich B (3tetes), jika terbentuk indol maka akan terbenrtuk warna merah. • Uji motilitas : uji positif jika terjadi pertumbuhan pada seluruh media dan uji negatif jika ada pertumbuhan hanya di bekas inokulas (seraput-seraput berwarna putih)
		KIA		A/A $^{S-}$ $_{G+}$	• Bagian lereng : jika berwarna merah maka ditulis K • Bagian dasar : jika berwarna kuning maka ditulis A • Adanya gas : jika media pecah atau terangkat keatas maka ditulis G+ , jika media tetap maka ditulis G- • Sulfida : jika media berwarna hitam maka ditulis S+, jika media tidak terbentuk warna hitam maka ditulis S-
		LIA		K/K $^{S-}$ $_{G-}$	• Bagian lereng : jika berwarna merah maka ditulis K • Bagian dasar : jika berwarna kuning maka ditulis A • Adanya gas : jika media pecah atau terangkat keatas maka ditulis G+ , jika media tetap maka ditulis G- • Sulfida : jika media berwarna hitam maka ditulis S+, jika media tidak terbentuk warna hitam maka ditulis S-
		CITRAT		-	• Uji positif : jika media berwarna biru

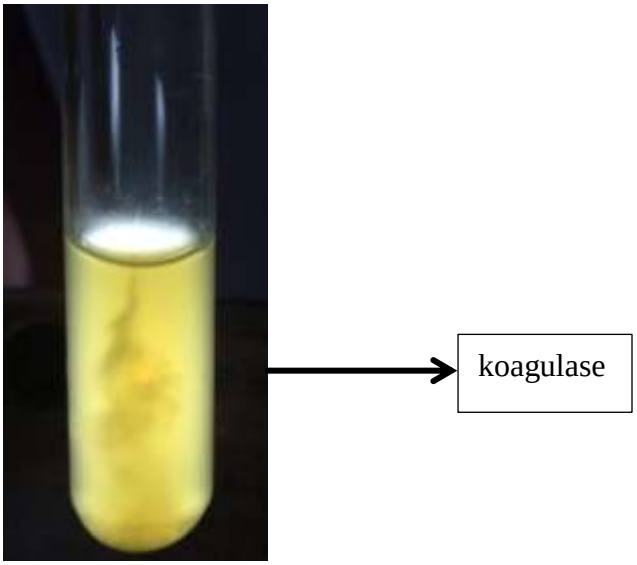
Lampiran 8. Gambar hasil identifikasi makroskopis bakteri *Staphylococcus aureus* ATCC 25923 pada media VJA

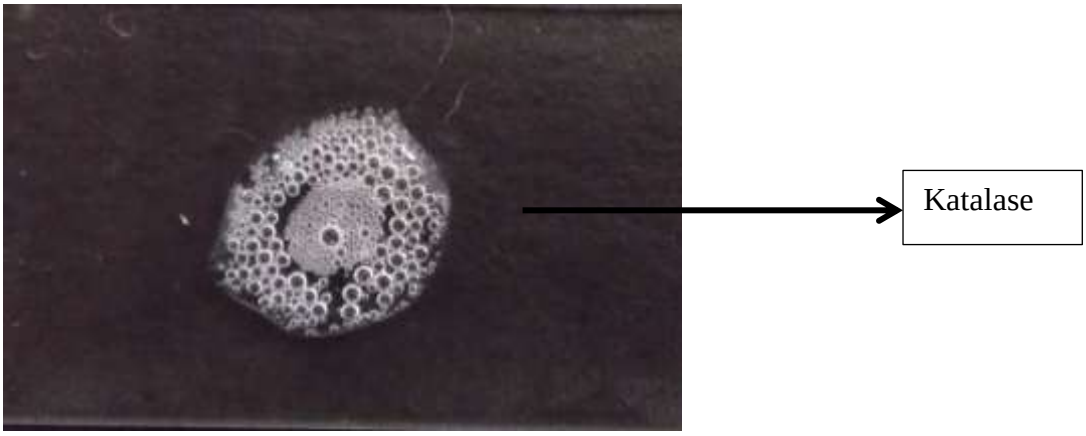


Lampiran 9. Gambar hasil identifikasi mikroskopis bakteri *Staphylococcus aureus* ATCC 25923 dengan pewarnaan gram

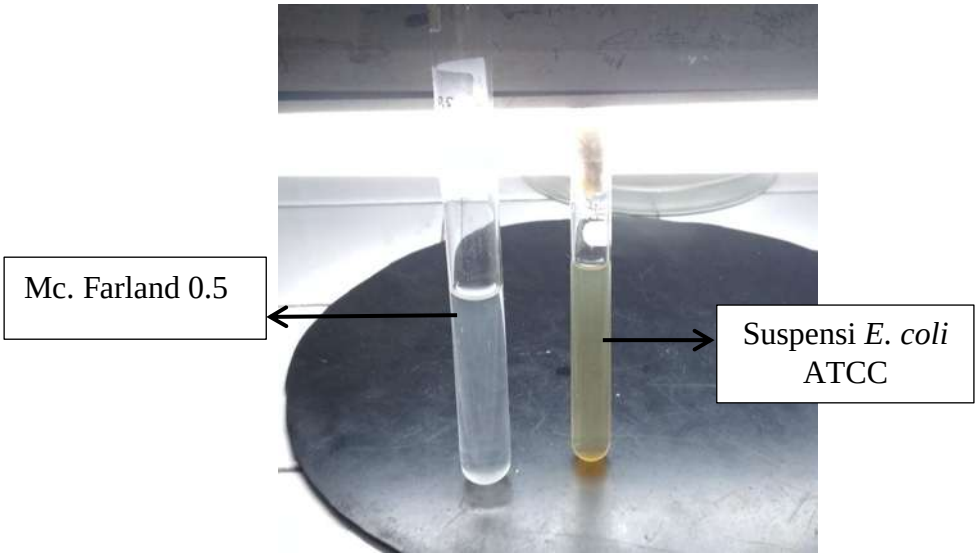


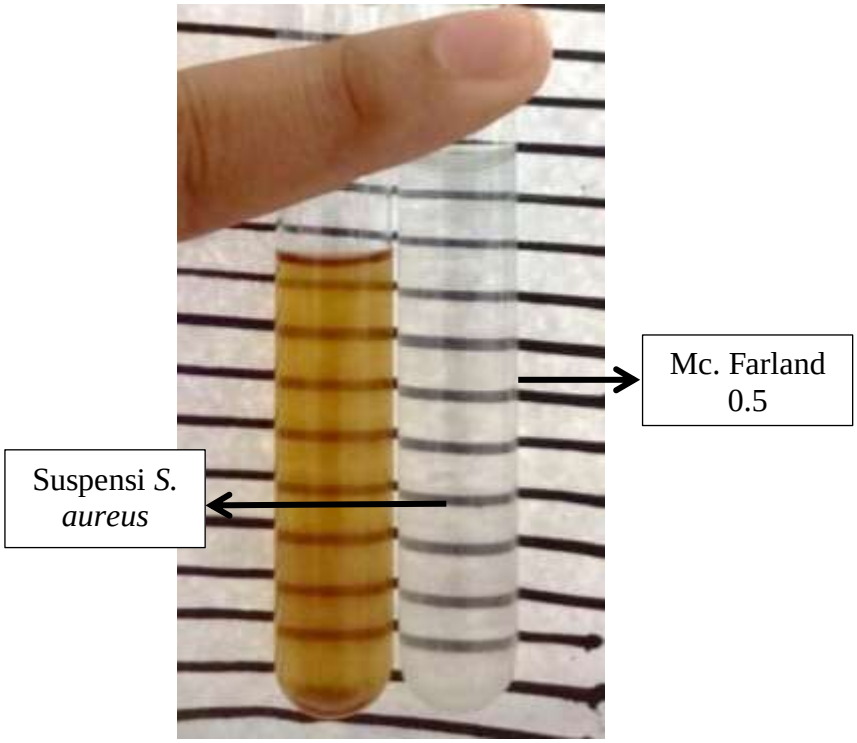
Lampiran 9. Hasil Identifikasi Biokimia *Staphylococcus aureus* ATCC 25923





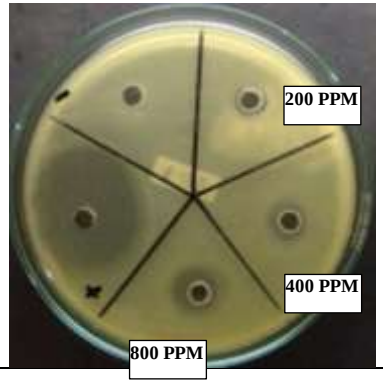
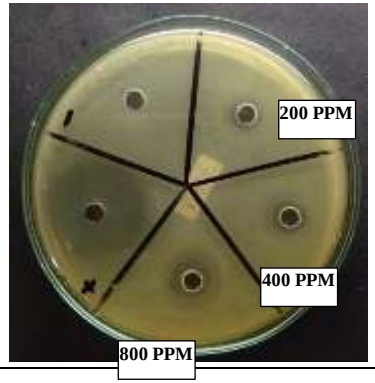
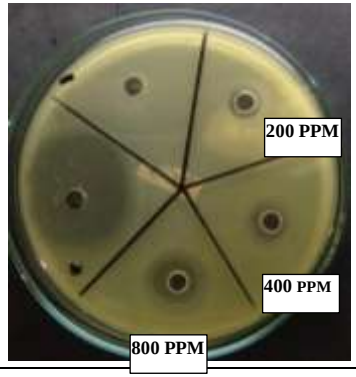
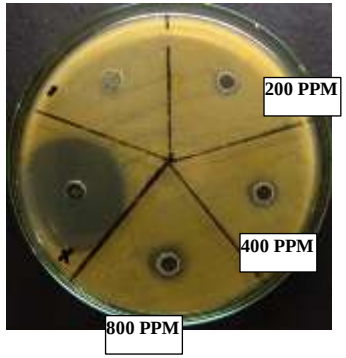
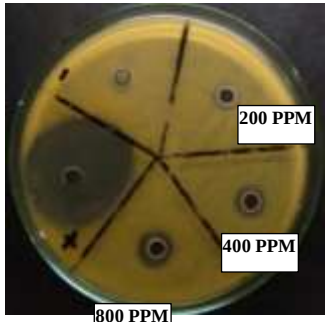
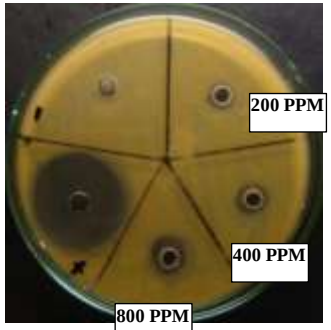
Lampiran 10. Pembuatan suspensi bakteri *Escherichia coli* ATCC 25922 dan *Staphylococcus aureus* ATCC 25923 pada media BHI





Lampiran 11. Supernatan Produk Rusip

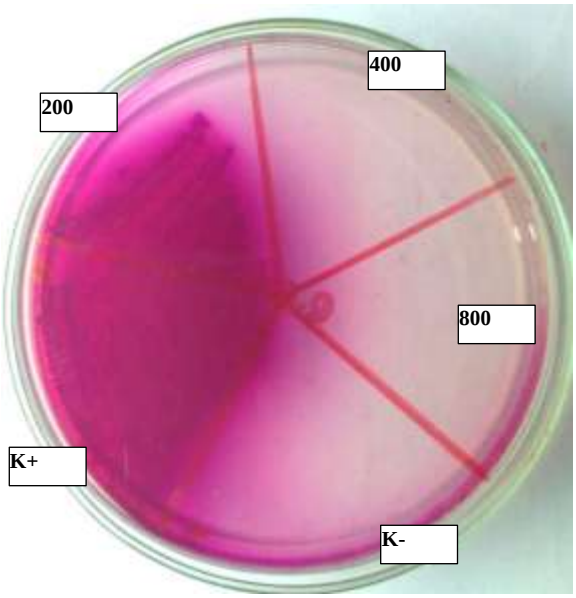
Lampiran 12. Foto Hasil Uji Aktivitas Antibakteri Secara Difusi Sumuran

Bakteri uji	Replikasi		
	1	2	3
***E. coli* ATCC 25922**			
***S. aureus* ATCC 25923**			

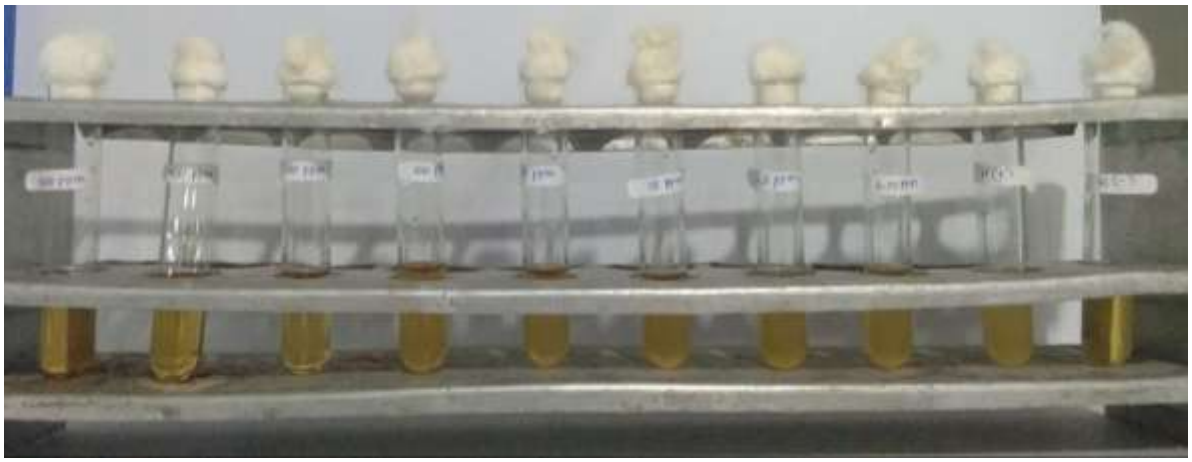
Lampiran 13. Foto Hasil Uji Aktivitas Antibakteri Secara Dilusi

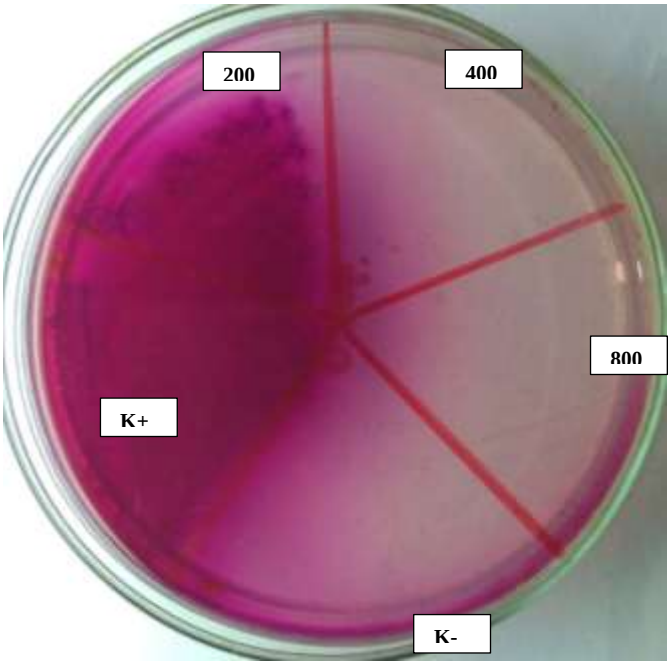
***E. coli* ATCC 25922**

Replikasi I



Replikasi II





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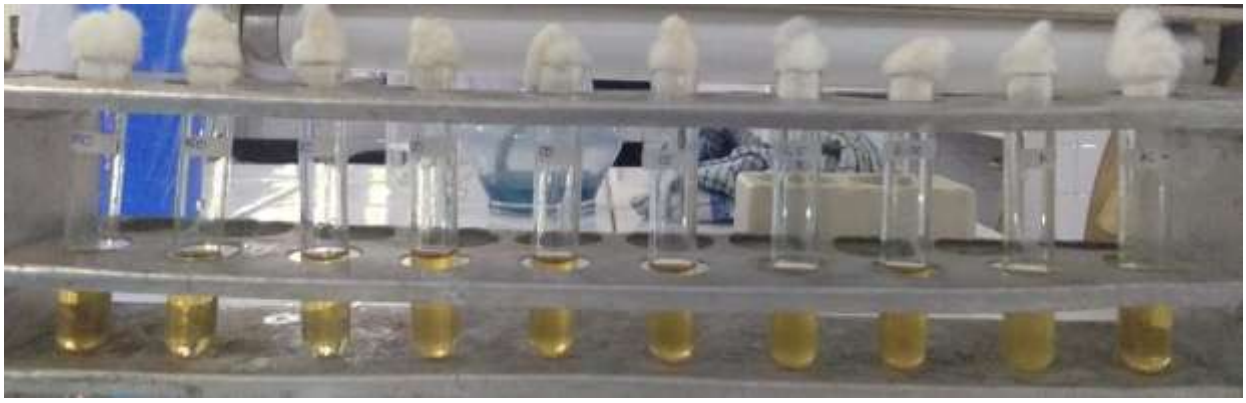
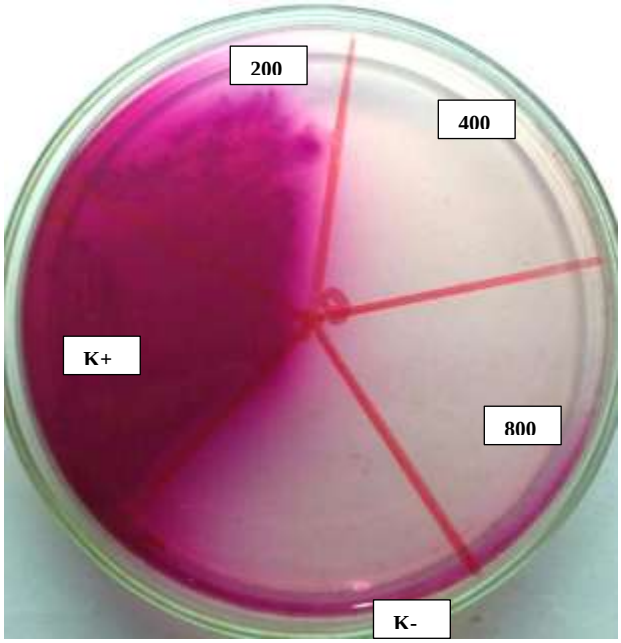
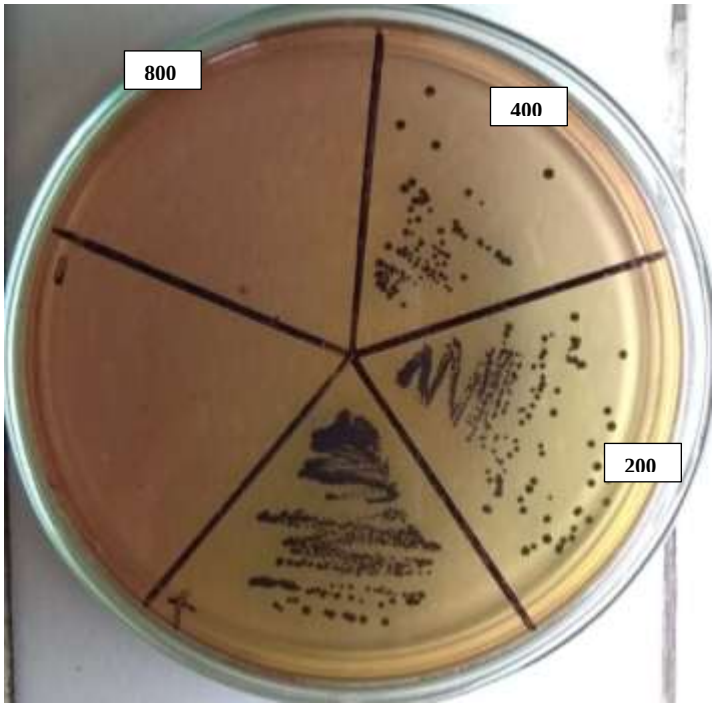




S. aureus ATCC 25922

Replikasi I



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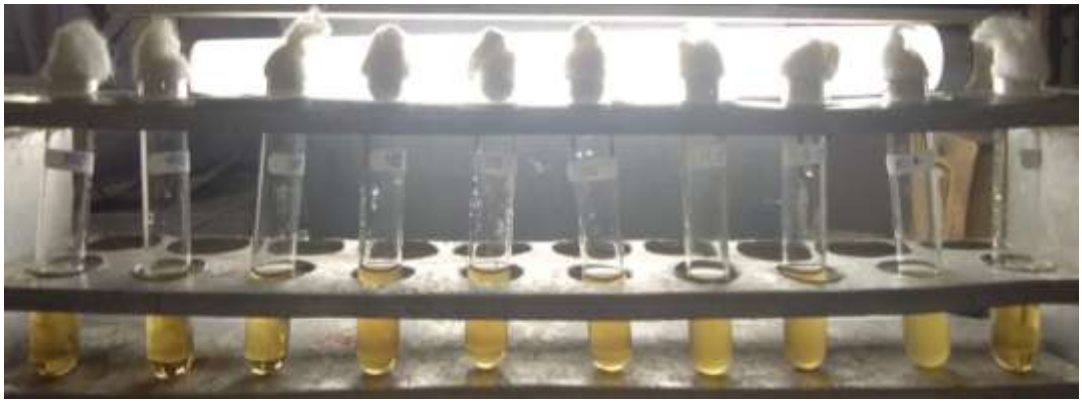

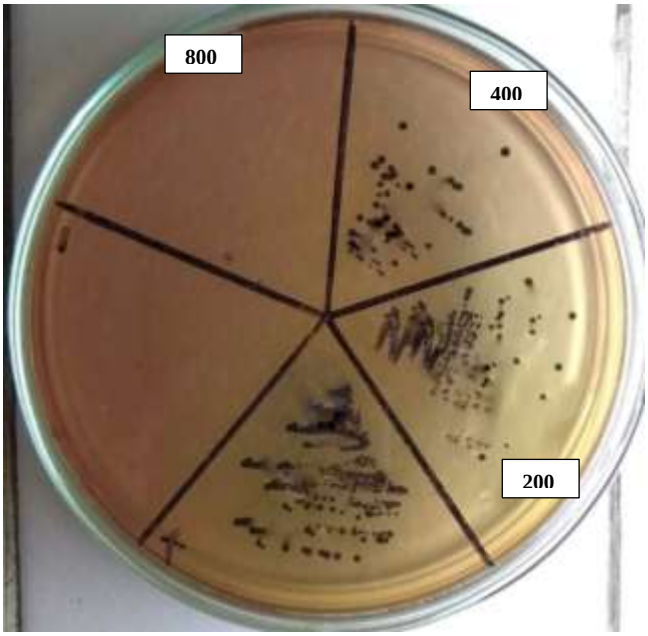

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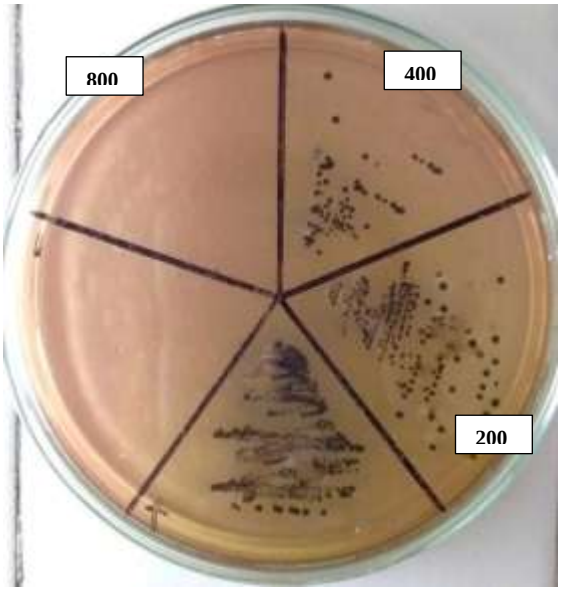
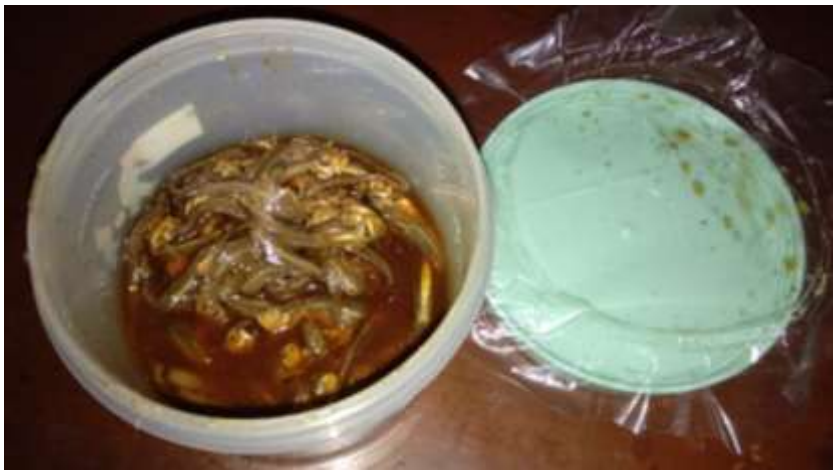


Lampiran 14. Produk Rusip



Lampiran 15. Hasil Analisis Statistik

One-Sample Kolmogorov-Smirnov Test

		Ecoli	Scoccus
N		12	12
Normal Parameters[a,,b]	Mean	14.25	12.08
	Std. Deviation	8.884	9.709
Most Extreme Differences	Absolute	.306	.335
	Positive	.306	.335
	Negative	-.189	-.188
Kolmogorov-Smirnov Z		1.060	1.160
Asymp. Sig. (2-tailed)		.211	.135

a. Test distribution is Normal.

b. Calculated from data.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
Ecoli	800	3	12.33	.577	.333	10.90	13.77	12	13
	400	3	10.67	.577	.333	9.23	12.10	10	11
	200	3	5.67	.577	.333	4.23	7.10	5	6
	kontrol (+)	3	28.33	.577	.333	26.90	29.77	28	29
	Total	12	14.25	8.884	2.565	8.61	19.89	5	29
Scoccus	800	3	9.67	.577	.333	8.23	11.10	9	10
	400	3	7.67	.577	.333	6.23	9.10	7	8
	200	3	3.33	.577	.333	1.90	4.77	3	4
	kontrol (+)	3	27.67	.577	.333	26.23	29.10	27	28
	Total	12	12.08	9.709	2.803	5.91	18.25	3	28

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Ecoli	.000	3	8	1.000
Scoccus	.000	3	8	1.000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ecoli	Between Groups	865.583	3	288.528	865.583	.000
	Within Groups	2.667	8	.333		
	Total	868.250	11			
Scoccus	Between Groups	1034.250	3	344.750	1034.250	.000
	Within Groups	2.667	8	.333		
	Total	1036.917	11			

Multiple Comparisons

Dependent Variable		(I) kelompok	(J) kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Ecoli	Tukey HSD	800	400	1.667*	.471	.031	.16	3.18
			200	6.667*	.471	.000	5.16	8.18
			kontrol (+)	-16.000*	.471	.000	-17.51	-14.49
		400	800	-1.667*	.471	.031	-3.18	-.16
			200	5.000*	.471	.000	3.49	6.51
			kontrol (+)	-17.667*	.471	.000	-19.18	-16.16
		200	800	-6.667*	.471	.000	-8.18	-5.16
			400	-5.000*	.471	.000	-6.51	-3.49
			kontrol (+)	-22.667*	.471	.000	-24.18	-21.16
		kontrol (+)	800	16.000*	.471	.000	14.49	17.51
			400	17.667*	.471	.000	16.16	19.18
			200	22.667*	.471	.000	21.16	24.18
	LSD	800	400	1.667*	.471	.008	.58	2.75
			200	6.667*	.471	.000	5.58	7.75
			kontrol (+)	-16.000*	.471	.000	-17.09	-14.91
		400	800	-1.667*	.471	.008	-2.75	-.58
			200	5.000*	.471	.000	3.91	6.09
			kontrol (+)	-17.667*	.471	.000	-18.75	-16.58
		200	800	-6.667*	.471	.000	-7.75	-5.58
			400	-5.000*	.471	.000	-6.09	-3.91
			kontrol (+)	-22.667*	.471	.000	-23.75	-21.58
		kontrol (+)	800	16.000*	.471	.000	14.91	17.09
			400	17.667*	.471	.000	16.58	18.75
			200	22.667*	.471	.000	21.58	23.75
Scoccus	Tukey HSD	800	400	2.000*	.471	.012	.49	3.51
			200	6.333*	.471	.000	4.82	7.84
			kontrol (+)	-18.000*	.471	.000	-19.51	-16.49

		400	800	-2.000*	.471	.012	-3.51	-.49
			200	4.333*	.471	.000	2.82	5.84
			kontrol (+)	-20.000*	.471	.000	-21.51	-18.49
		200	800	-6.333*	.471	.000	-7.84	-4.82
			400	-4.333*	.471	.000	-5.84	-2.82
			kontrol (+)	-24.333*	.471	.000	-25.84	-22.82
		kontrol (+)	800	18.000*	.471	.000	16.49	19.51
			400	20.000*	.471	.000	18.49	21.51
			200	24.333*	.471	.000	22.82	25.84
	LSD	800	400	2.000*	.471	.003	.91	3.09
			200	6.333*	.471	.000	5.25	7.42
			kontrol (+)	-18.000*	.471	.000	-19.09	-16.91
		400	800	-2.000*	.471	.003	-3.09	-.91
			200	4.333*	.471	.000	3.25	5.42
			kontrol (+)	-20.000*	.471	.000	-21.09	-18.91
		200	800	-6.333*	.471	.000	-7.42	-5.25
			400	-4.333*	.471	.000	-5.42	-3.25
			kontrol (+)	-24.333*	.471	.000	-25.42	-23.25
		kontrol (+)	800	18.000*	.471	.000	16.91	19.09
			400	20.000*	.471	.000	18.91	21.09
			200	24.333*	.471	.000	23.25	25.42

*. The mean difference is significant at the 0.05 level.

Ecoli

			Subset for alpha = 0.05			
	Kelompok	N	1	2	3	4
Tukey HSD[a]	200	3	5.67			
	400	3		10.67		
	800	3			12.33	
	kontrol (+)	3				28.33
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Scoccus

			Subset for alpha = 0.05			
	Kelompok	N	1	2	3	4
Tukey HSD[a]	200	3	3.33			
	400	3		7.67		
	800	3			9.67	
	kontrol (+)	3				27.67
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.