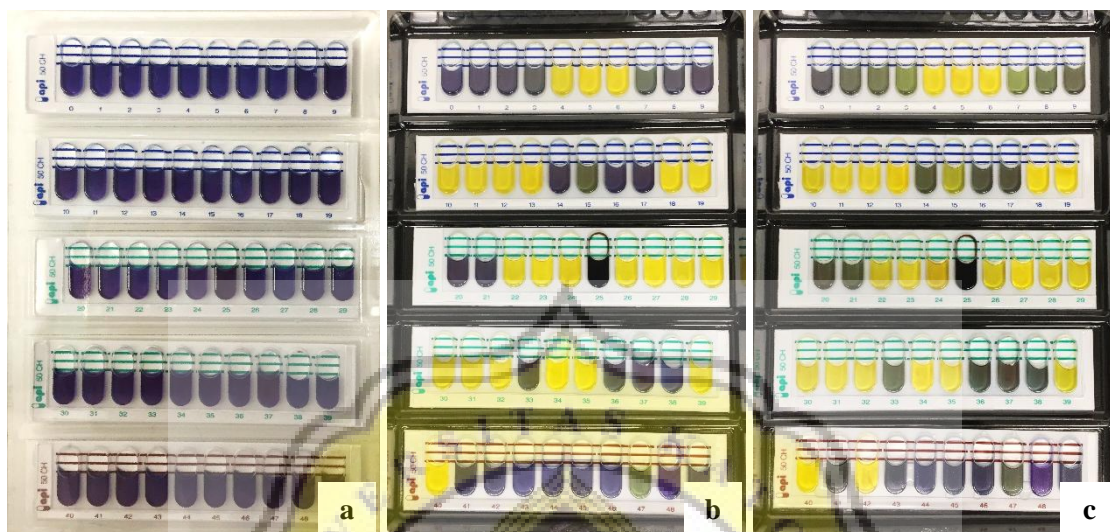


7. APPENDICES

Appendix 1. Species Identification of LAB Isolates using API 50 CHL

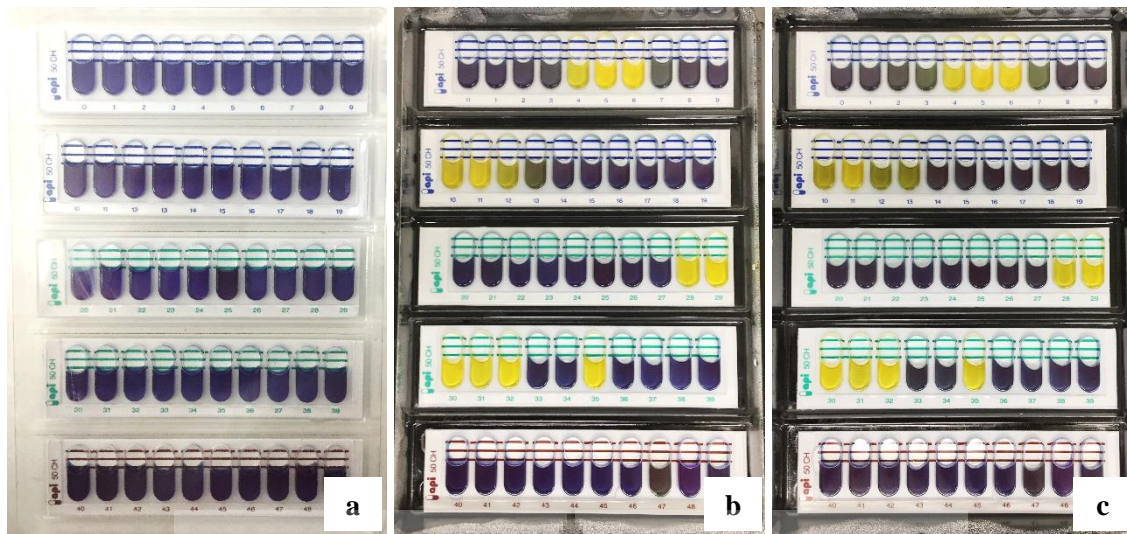


GOOD IDENTIFICATION						
Strip	API 50 CHL V5.2					
Profile	- - - - + + + + - - - - + + + + - - - - + + + + - - - - + + + + - - - - + + + + - - - -					
Note						
Significant taxa	% ID	T	Tests against			
Lactobacillus pentosus	97.3	0.91	GLY 75%	MLZ 25%		
Next taxon	% ID	T	Tests against			
Lactobacillus plantarum 1	2.6	0.8	DXYL 2%			

VERY GOOD IDENTIFICATION TO THE GENUS						
Strip	API 50 CHL V5.2					
Profile	- - - - + + + + - - - - + + + + - - - - + + + + - - - - + + + + - - - - + + + + - - - -					
Note						
Significant taxa	% ID	T	Tests against			
Lactobacillus pentosus	84.3	0.71	GLY 75%	MLZ 25%	TAG 1%	
Lactobacillus plantarum 1	15.5	0.69	DXYL 2%	TAG 7%		
Next taxon	% ID	T	Tests against			
Lactobacillus brevis 1	0.1	0.56	SOR 14%	MLZ 14%	TUR 14%	TAG
			GNT 85%			

Keys: a = before incubation; b = after 24 hours incubation; c = after 48 hours incubation

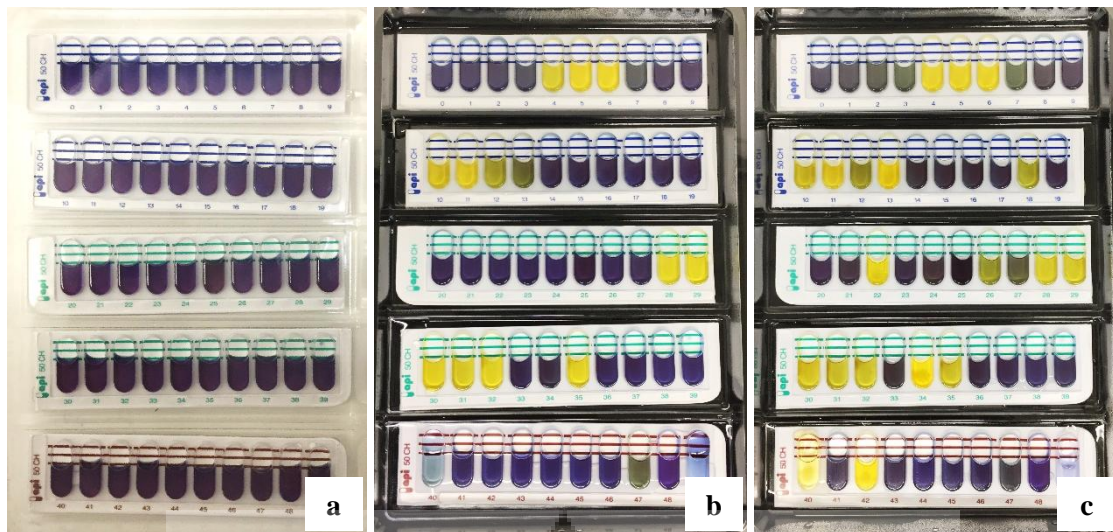
Figure 16. Species Identification of Isolate A8 using API 50 CHL



VERY GOOD IDENTIFICATION					
Strip	API 50 CHL V5.2				
Profile	- - - - + + - - + + - - + + + + - - - - -				
Note					
Significant taxa	% ID	T	Tests against		
Lactobacillus fermentum 1	99.8	0.79	FRU 80%	TRE 16%	
Next taxon	% ID	T	Tests against		
Lactobacillus fermentum 2	0.1	0.64	FRU 100%	ESC 81%	
					b
VERY GOOD IDENTIFICATION					
Strip	API 50 CHL V5.2				
Profile	- - - - + + - - + + - - + + + + - - - - -				
Note					
Significant taxa	% ID	T	Tests against		
Lactobacillus fermentum 1	99.1	0.69	GAL 92%	FRU 80%	TRE 16%
Next taxon	% ID	T	Tests against		
Lactobacillus fermentum 2	0.7	0.62	FRU 100%	ESC 81%	
					c

Keys: a = before incubation; b = after 24 hours incubation; c = after 48 hours incubation

Figure 17. Species Identification of Isolate A17 using API 50 CHL



VERY GOOD IDENTIFICATION						
Strip	API 50 CHL V5.2					
Profile	+++++-----					
Note						
Significant taxa	% ID	T	Tests against			
<i>Lactobacillus fermentum</i> 1	99.8	0.79	FRU 80%	TRE 16%		
Next taxon	% ID	T	Tests against			
<i>Lactobacillus fermentum</i> 2	0.1	0.64	FRU 100%	ESC 81%		
b						
UNACCEPTABLE PROFILE						
Strip	API 50 CHL V5.2					
Profile	-----+++++					
Note						
Significant taxa	% ID	T	Tests against			
<i>Leuconostoc mesenteroides</i> ssp <i>mesenteroides/dextranicum</i> 2			FRU 99%	MDG 99%	LAC 15%	MLZ 0%
<i>Lactobacillus brevis</i> 2			TAG 0%			
			FRU 99%	MNE 7%	NAG 7%	ESC 23%
<i>Leuconostoc lactis</i>			TRE 1%	TAG 1%	GNT 76%	
			LARA 15%	RIB 10%	DXYL 3%	FRU 82%
<i>Lactobacillus fermentum</i> 1			ESC 0%	MLZ 5%	TUR 5%	TAG 5%
			FRU 80%	NAG 6%	ESC 5%	TRE 16%
			MLZ 0%	TUR 4%	TAG 0%	
Next taxon	% ID	T	Tests against			
<i>Lactococcus raffinolactis</i>			RIB 16%	FRU 100%	ARB 83%	SAL
			CEL 100%	AMD 100%	TAG 1%	
c						

Keys: a = before incubation; b = after 24 hours incubation; c = after 48 hours incubation

Figure 18. Species Identification of Isolate B3 using API 50 CHL

Appendix 2. Media Used for Growth and Bacteriocin Inhibitory Activity Test

2.1. deMan Rogosa Sharpe Broth (MRS-B) Medium “Merck”

MRS-B medium was prepared by dissolving 52.2 g of powdered MRS-B into 1 liter of distilled water and stirring to dissolve the medium completely. MRS-B medium was sterilized using autoclave at 121°C for 15 minutes. Composition of MRS-B: 20 gL⁻¹ of D(+)-glucose, 10 gL⁻¹ of casein/meat peptone, 8 gL⁻¹ of meat extract, 5 gL⁻¹ of sodium acetate, 4 gL⁻¹ of yeast extract, 2 gL⁻¹ of dipotassium hydrogen phosphate, 2 gL⁻¹ of diammonium hydrogen citrate, 1 gL⁻¹ of Tween 80, 0.2 gL⁻¹ of magnesium sulfate, and 0.04 gL⁻¹ of manganese sulfate.

2.2. deMan Rogosa Sharpe Agar (MRS-A) Medium “Merck”

MRS-A medium was prepared by dissolving 68.2 g of powdered MRS-A into 1 liter of distilled water and stirring while boiled to dissolve the medium completely. MRS-A medium was sterilized using autoclave at 121°C for 15 minutes. Composition of MRS-A: 20 gL⁻¹ of D(+)-glucose, 14 gL⁻¹ of agar-agar, 10 gL⁻¹ of casein peptone, 10 gL⁻¹ of meat extract, 5 gL⁻¹ of sodium acetate, 4 gL⁻¹ of yeast extract, 2 gL⁻¹ of diammonium hydrogen citrate, 2 gL⁻¹ of dipotassium hydrogen phosphate, 1 gL⁻¹ of Tween 80, 0.2 gL⁻¹ of magnesium sulfate, and 0.04 gL⁻¹ of manganese sulfate.

2.3. Nutrient Broth (NB) Medium “Merck”

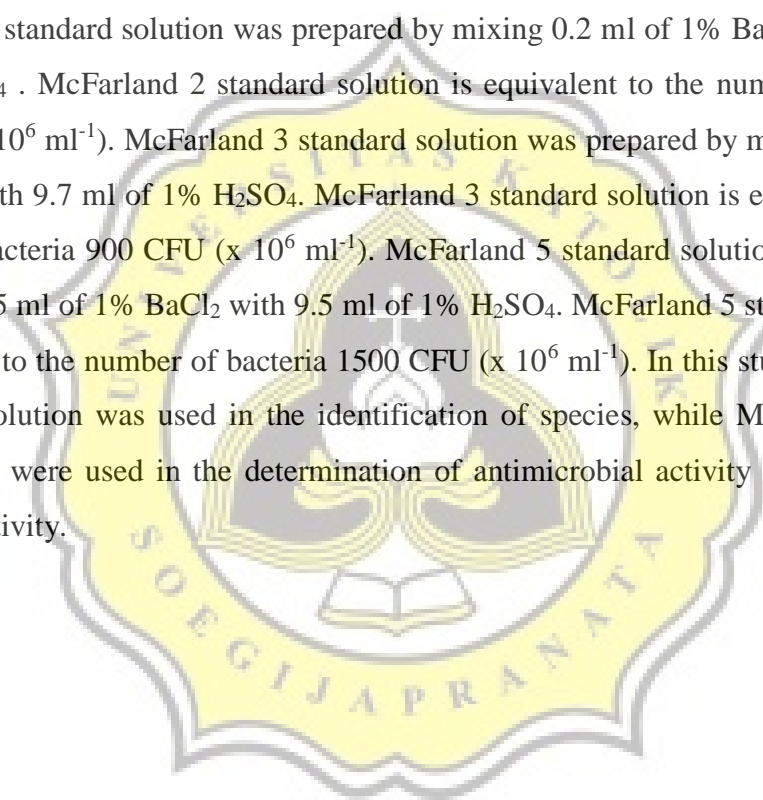
NB medium was prepared by dissolving 8 g of powdered NB into 1 liter of distilled water and stirring to dissolve the medium completely. NB medium was sterilized using autoclave at 121°C for 15 minutes. Composition of NB: 5 gL⁻¹ of peptone from meat and 3 gL⁻¹ of meat extract.

2.4. Nutrient Agar (NA) Medium “Merck”

NA medium was prepared by dissolving 20 g of powdered NA into 1 liter of distilled water and stirring while boiled to dissolve the medium completely. NA medium was sterilized using autoclave at 121°C for 15 minutes. Composition of NA: 12 gL⁻¹ of agar-agar, 5 gL⁻¹ of peptone from meat, and 3 gL⁻¹ of meat extract.

Appendix 3. Composition of Standard Solution McFarland 2, 3 and 5

McFarland 2 standard solution was prepared by mixing 0.2 ml of 1% BaCl₂ with 9.8 ml of 1% H₂SO₄. McFarland 2 standard solution is equivalent to the number of bacteria 600 CFU (x 10⁶ ml⁻¹). McFarland 3 standard solution was prepared by mixing 0.3 ml of 1% BaCl₂ with 9.7 ml of 1% H₂SO₄. McFarland 3 standard solution is equivalent to the number of bacteria 900 CFU (x 10⁶ ml⁻¹). McFarland 5 standard solution was prepared by mixing 0.5 ml of 1% BaCl₂ with 9.5 ml of 1% H₂SO₄. McFarland 5 standard solution is equivalent to the number of bacteria 1500 CFU (x 10⁶ ml⁻¹). In this study, McFarland 2 standard solution was used in the identification of species, while McFarland 3 and McFarland 5 were used in the determination of antimicrobial activity and bacteriocin inhibitory activity.



Appendix 4. Growth Capabilities of LAB at Different Temperature, pH, and NaCl Concentration

Table 7. Growth of LAB at Different Temperature (10°C and 45°C)

Isolate Code	Temperature 10°C				Temperature 45°C			
	24 hours		48 hours		24 hours		48 hours	
	1	2	1	2	1	2	1	2
A7	0.3866	0.3898	0.3176	0.3169	0.1948	0.1962	0.1999	0.2008
A8	0.3269	0.3286	0.2910	0.2950	0.2007	0.2019	1.9516	1.9431
A9	0.2649	0.2676	0.2703	0.2778	0.1876	0.1895	0.4573	0.4567
A10	0.3664	0.3699	0.2948	0.2997	0.1798	0.1794	0.1743	0.1748
A11	0.3354	0.3445	0.2720	0.2784	0.9848	0.9894	1.8666	1.8670
A12	0.3531	0.3559	0.2734	0.2735	0.6254	0.6239	2.0033	2.0032
A13	0.3793	0.3840	0.3056	0.3079	1.7217	1.7255	1.7507	1.7500
A14	0.3589	0.3554	0.2826	0.2857	1.4808	1.4723	1.6325	1.6312
A15	0.2924	0.2935	0.3128	0.3157	0.2880	0.2870	1.5964	1.6116
A16	0.3710	0.3719	0.2865	0.2870	0.2346	0.2321	1.4131	1.4149
A17	0.3399	0.3360	0.2627	0.2642	0.2094	0.2108	0.2155	0.2156
A18	0.3270	0.3304	0.3104	0.3106	0.2307	0.2314	0.2147	0.2186
A19	0.4243	0.4291	0.3472	0.3498	0.3409	0.3385	0.2439	0.2439
A20	0.3719	0.3741	0.2987	0.3011	0.3162	0.3161	1.3727	1.3636
A22	0.3465	0.3511	0.2764	0.2787	0.3027	0.3052	1.7437	1.7388
A24	0.3653	0.3619	0.2731	0.2752	0.2525	0.2543	0.2967	0.2973
B1	0.3628	0.3630	0.2822	0.2831	0.2895	0.2924	0.2078	0.2071
B2	0.3032	0.3035	0.2566	0.2573	0.2138	0.2147	0.2198	0.2211
B3	0.3807	0.3825	0.3004	0.3029	0.2855	0.2832	0.2168	0.2187
B4	0.4413	0.4470	0.3887	0.3906	1.3537	1.3750	1.7735	1.7888
B5	0.3565	0.3608	0.2669	0.2681	0.9470	0.9492	1.4514	1.4554
B6	0.3634	0.3644	0.2909	0.2932	0.3252	0.3256	0.2521	0.2514
B8	0.4891	0.4991	0.4807	0.4862	1.2535	1.2581	1.4893	1.4773
B9	0.5759	0.5923	0.5242	0.5273	0.5186	0.5218	0.4783	0.4794
B10	0.4819	0.4854	0.4154	0.4145	0.5351	0.5384	0.5658	0.5703
B11	0.4046	0.4088	0.3238	0.3252	0.3197	0.3230	0.2698	0.2688
B12	0.3119	0.3112	0.2548	0.2565	0.9523	0.9465	1.1960	1.2034
B13	0.3682	0.3747	0.2960	0.2971	0.3709	0.3726	1.6660	1.6792
B14	0.3734	0.3688	0.2779	0.2793	1.6062	1.6072	1.6389	1.6446
B15	0.4462	0.4460	0.3894	0.3897	0.3786	0.3813	0.4036	0.4079
B16	0.3576	0.3605	0.2880	0.2912	1.6940	1.6952	1.7246	1.7171
B19	0.3205	0.3261	0.3068	0.3066	1.5134	1.5158	1.6050	1.6026

Keys:

1, 2 = replication

Table 8. Growth of LAB at Different pH (4.4 and 9.6)

Isolate Code	pH 4.4				pH 9.6			
	24 hours		48 hours		24 hours		48 hours	
	1	2	1	2	1	2	1	2
A7	1.9330	1.9380	2.1420	2.1454	0.5776	0.5723	0.6147	0.6168
A8	2.0210	2.0176	2.3091	2.3090	0.4709	0.4754	0.5224	0.5244
A9	1.9646	1.9651	2.1319	2.1410	0.5252	0.5223	0.4476	0.4497
A10	1.9472	1.9517	2.1658	2.1695	0.4917	0.4949	0.5733	0.5754
A11	1.9622	1.9677	2.1158	2.1272	0.6037	0.6046	0.5945	0.5954
A12	1.9639	1.9693	2.1360	2.1385	0.6199	0.6181	0.6256	0.6251
A13	1.9660	1.9710	2.1185	2.1163	0.4756	0.4765	0.4782	0.4794
A14	2.0301	2.0210	2.1237	2.1357	0.4717	0.4689	0.5236	0.5220
A15	1.9885	1.9911	2.1355	2.1433	0.5449	0.5504	0.5392	0.5414
A16	1.9542	1.9434	2.1833	2.1884	0.5053	0.5060	0.5284	0.5303
A17	1.9347	1.9259	2.0983	2.1061	0.4492	0.4518	0.5029	0.5043
A18	1.9708	1.9764	2.1367	2.1337	0.5706	0.5477	0.5613	0.5503
A19	1.2508	1.2626	2.1052	2.0979	0.5430	0.5452	0.5506	0.5511
A20	1.9727	1.9838	2.1457	2.1501	0.5811	0.5819	0.6155	0.6163
A22	1.9809	1.9800	2.1431	2.1461	0.5459	0.5533	0.5903	0.5906
A24	1.9448	1.9506	2.1334	2.1310	0.4910	0.4984	0.5971	0.5971
B1	2.0170	2.0230	2.1385	2.1434	0.4985	0.5018	0.4982	0.4982
B2	1.0922	1.0917	2.0927	2.1030	0.5135	0.5196	0.5225	0.5252
B3	2.0219	2.0263	2.1225	2.1246	0.4541	0.4541	0.4618	0.4633
B4	2.0409	2.0443	2.1395	2.1478	0.5297	0.5242	0.6161	0.6210
B5	0.9118	0.9157	2.1236	2.1248	0.4862	0.4836	0.5347	0.5367
B6	1.9793	1.9788	2.1447	2.1508	0.4750	0.4730	0.5486	0.5497
B8	1.1810	1.1788	2.1559	2.1544	0.4127	0.4209	0.4708	0.4733
B9	0.6632	0.6605	1.1097	1.1067	0.5889	0.5979	0.6600	0.6505
B10	0.6472	0.6362	0.5973	0.6050	0.5028	0.5147	0.5578	0.5610
B11	1.4113	1.4092	2.1493	2.1565	0.4898	0.4886	0.4692	0.4712
B12	2.0224	2.0306	2.2013	2.2058	0.4933	0.4945	0.5989	0.5982
B13	1.7234	1.7252	2.1359	2.1466	0.5070	0.5058	0.5592	0.5617
B14	1.8928	1.8965	2.1762	2.1750	0.4064	0.4036	0.4371	0.4387
B15	0.4793	0.4824	0.5796	0.5868	0.6211	0.6242	0.5067	0.5132
B16	1.9335	1.9415	2.1816	2.1806	0.5716	0.5778	0.4835	0.4875
B19	1.9536	1.9489	2.1192	2.1219	0.4825	0.4822	0.4993	0.5028

Keys:

1, 2 = replication

Table 9. Growth of LAB at Different NaCl Concentration (6.5% and 18%)

Isolate Code	6.5% of NaCl				18% of NaCl			
	24 hours		48 hours		24 hours		48 hours	
	1	2	1	2	1	2	1	2
A7	0.3685	0.3734	0.5299	0.5337	0.3232	0.3195	0.2629	0.2640
A8	1.1423	1.1448	2.0915	2.0934	0.3282	0.3347	0.2548	0.2574
A9	0.3720	0.3702	0.8586	0.8644	0.2782	0.2816	0.3021	0.3017
A10	0.3931	0.3918	0.4032	0.4049	0.2748	0.2777	0.2184	0.2192
A11	0.3406	0.3409	0.3861	0.3898	0.2821	0.2894	0.2646	0.2652
A12	0.3576	0.3602	0.5683	0.5693	0.2418	0.2455	0.2477	0.2492
A13	0.3584	0.3626	0.3503	0.3518	0.2700	0.2702	0.2569	0.2571
A14	0.3695	0.3735	0.3257	0.3278	0.2727	0.2761	0.2306	0.2312
A15	0.2725	0.2735	0.3187	0.3191	0.2623	0.2694	0.2414	0.2444
A16	0.3773	0.3791	0.4297	0.4307	0.2746	0.2784	0.2683	0.2680
A17	0.2948	0.2934	0.5542	0.5551	0.2273	0.2370	0.2538	0.2550
A18	0.3127	0.3113	0.3693	0.3704	0.2506	0.2545	0.2636	0.2649
A19	0.3795	0.3764	0.4156	0.4159	0.3060	0.3154	0.3250	0.3273
A20	0.3724	0.3690	0.3665	0.3671	0.3298	0.3292	0.2421	0.2436
A22	0.3839	0.3727	0.3625	0.3637	0.2513	0.2521	0.2538	0.2539
A24	0.3100	0.3185	0.4294	0.4320	0.3450	0.3411	0.2525	0.2538
B1	0.3213	0.3267	2.0027	2.0038	0.2874	0.2975	0.2489	0.2497
B2	0.4370	0.4408	1.0261	1.0345	0.3037	0.3059	0.2339	0.2336
B3	0.3205	0.3211	0.4252	0.4263	0.3392	0.3480	0.2690	0.2702
B4	0.3735	0.3781	0.4386	0.4394	0.4094	0.4104	0.3404	0.3424
B5	0.3761	0.3803	0.4272	0.4297	0.3323	0.3336	0.2645	0.2657
B6	0.3821	0.3834	0.3202	0.3209	0.2709	0.2714	0.2415	0.2423
B8	0.2359	0.2334	0.3064	0.3083	0.3172	0.3214	0.2250	0.2272
B9	0.5650	0.5594	0.5533	0.5548	0.5346	0.5390	0.5545	0.5594
B10	0.5126	0.5144	1.8689	1.8704	0.4535	0.4530	0.4106	0.4110
B11	0.3581	0.3671	0.3590	0.3604	0.3811	0.3749	0.2973	0.2992
B12	0.4623	0.4522	2.0917	2.0925	0.3365	0.3389	0.2501	0.2519
B13	0.2995	0.3031	0.6882	0.6876	0.3516	0.3551	0.2669	0.2679
B14	0.3736	0.3697	0.5180	0.5185	0.3194	0.3216	0.2504	0.2522
B15	0.5853	0.5820	1.2789	1.2807	0.4814	0.4909	0.5056	0.5055
B16	0.3745	0.3790	0.5882	0.5899	0.3383	0.3391	0.2314	0.2327
B19	0.3640	0.3669	0.3135	0.3150	0.2351	0.2387	0.2323	0.2317

Keys:

1, 2 = replication