

Effects of *Bifidobacterium animalis* subsp. *lactis* BI-04 on Skin Wrinkles and Dryness: A Randomized, Triple-Blinded, Placebo-Controlled Clinical Trial

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Table S1. Participants' self-evaluated lifestyle and skin characteristics in *Bifidobacterium animalis* subsp. *lactis* BI-04 (BI-04) and placebo groups in the per-protocol (PP) population.

Item	Classification	BI-04 (N = 61) N (%) ¹	Placebo (N = 67) N (%) ¹	Total (N = 128) N (%) ¹
Average daily sleep	Fewer than 5 h	2 (3.3)	2 (3.0)	4 (3.1)
	5–8 h	51 (83.6)	58 (86.6)	109 (85.2)
	More than 8 h	8 (13.1)	7 (10.4)	15 (11.7)
Average daily smoking	No	61 (100.0)	66 (98.5)	127 (99.2)
	Fewer than 10 pieces	0 (0.0)	1 (1.5)	1 (0.8)
	More than 10 pieces	0 (0.00)	0 (0.00)	0 (0.00)
Sensitivity of skin	Yes	7 (11.5)	5 (7.5)	12 (9.4)
	No	54 (88.5)	62 (92.5)	116 (90.6)
Tendency to feel stinging within 30 minutes after applying cosmetics	Yes	1 (1.6)	2 (3.0)	3 (2.3)
	No	60 (98.4)	65 (97.0)	125 (97.7)
Adverse reaction to cosmetics in the last 12 months	Yes	0 (0.00)	0 (0.00)	0 (0.00)
	No	61 (100.0)	67 (100.0)	128 (100.0)

¹ N (Frequency) = Number of answers, % (Percentage) = Number of answers/Total number of subjects (61 or 67) × 100.

Table S2. Transepidermal water loss (TEWL) from the skin in the PP population. TEWL was measured from three areas (cheek, forearm, and hand) every 4 weeks from baseline to Week 12.

Location	Time point	BI-04 (N = 61)		Placebo (N = 67)		<i>p</i> -Value ²
		Mean (SD) ⁵	<i>p</i> -Value ¹	Mean (SD) ⁵	<i>p</i> -Value ¹	
Cheek, g/hm ²	Baseline	15.39 (4.48)	-	15.80 (3.79)	-	0.569
	4 weeks	15.21 (4.26)	0.652	15.40 (3.58)	0.377	0.710
	8 weeks	14.49 (3.98)	0.047*	14.23 (3.23)	0.002*	0.308
	12 weeks	13.85 (3.42)	<0.001*	13.88 (3.48)	<0.001*	0.525
Forearm, g/hm ²	Baseline	7.51 (2.34)	-	6.93 (1.86)	-	0.275 ⁴
	4 weeks	6.27 (1.63)	<0.001* ³	6.43 (2.14)	0.030*	0.920 ⁴
	8 weeks	6.12 (1.55)	<0.001* ³	6.20 (2.28)	0.007*	0.728 ⁴
	12 weeks	6.08 (1.42)	<0.001* ³	5.85 (1.13)	<0.001*	0.532 ⁴
Hand, g/hm ²	Baseline	13.10 (3.36)	-	12.94 (2.67)	-	0.769
	4 weeks	12.10 (3.26)	0.005*	11.57 (2.70)	<0.001*	0.428
	8 weeks	11.42 (2.71)	<0.001*	10.96 (2.84)	<0.001*	0.521
	12 weeks	10.54 (2.29)	<0.001*	10.39 (2.07)	<0.001*	0.987

¹ Within-group comparisons to baseline by Repeated measures analysis of variance (RM-ANOVA). Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ² Between-group comparisons at baseline by independent t-test and at Weeks 4, 8, and 12 by RM-ANOVA. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ³ Wilcoxon Signed-rank test with Bonferroni correction was applied when values were not normally distributed. ⁴ Mann-Whitney U-test was applied when values were not normally distributed. ⁵ Values represented as mean ± standard deviation (SD).

Table S3. Skin elasticity according to the R2 parameter (E/mm) in the PP population. Skin elasticity was measured from the cheek every 4 weeks from baseline to Week 12.

Time point	BI-04 (N = 61)		Placebo (N = 67)		<i>p</i> -Value ²
	Mean (SD)	<i>p</i> -Value ¹	Mean (SD)	<i>p</i> -Value ¹	
Baseline	0.6679 (0.0359)	-	0.6734 (0.0410)	-	0.420
4 weeks	0.6810 (0.0337)	<0.001*	0.6809 (0.0365)	0.028*	0.249
8 weeks	0.6727 (0.0372)	0.147	0.6812 (0.0365)	0.008*	0.490
12 weeks	0.6766 (0.0356)	0.007*	0.6793 (0.0373)	0.067	0.536

¹ Within-group comparisons to baseline by RM-ANOVA. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ² Between-group comparisons at baseline by independent t-test and at Weeks 4, 8, and 12 by RM-ANOVA. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*).

Table S4. Skin gloss values in skin gloss units (SGUs), measured from the cheek in the PP population. Skin gloss was measured from the cheek every 4 weeks from baseline to Week 12.

Time point	BI-04 (N = 61)		Placebo (N = 67)		<i>p</i> -Value ²
	Mean (SD)	<i>p</i> -Value ¹	Mean (SD)	<i>p</i> -Value ¹	
Baseline	63.77 (6.74)	-	64.34 (6.52)	-	0.629
4 weeks	62.70 (5.72)	0.053	63.41 (5.91)	0.036*	0.835
8 weeks	62.96 (5.33)	0.165	63.68 (5.52)	0.162	0.843
12 weeks	63.09 (5.54)	0.188	63.65 (5.19)	0.158	0.994

¹ Within-group comparisons to baseline by RM-ANOVA. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ² Between-group comparisons at baseline by independent t-test and at Weeks 4, 8, and 12 by RM-ANOVA. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*).

Table S5. Subjective evaluation of investigational product efficacy and usability in the PP population. Subjective efficacy assessment was performed at Weeks 4, 8, and 12, and usability was assessed at Week 12.

Parameter	Time point	BI-04 (N = 61) N (%) ¹	Placebo (N = 67) N (%) ¹	p-Value ²
Efficacy Assessment				
Q1. I feel like my skin became more hydrated/moistened after intake of the test product.	4 weeks	34 (55.74)	33 (49.25)	0.195
	8 weeks	38 (62.30)	49 (73.13)	0.302
	12 weeks	40 (65.57)	44 (65.67)	0.101
Q2. I feel like my skin became brighter after intake of the test product.	4 weeks	27 (44.26)	23 (34.33)	0.288
	8 weeks	32 (52.46)	38 (56.72)	0.719
	12 weeks	37 (60.66)	36 (53.73)	0.940
Q3. I feel like my skin became firmer after intake of the test product.	4 weeks	15 (24.59)	19 (28.36)	0.975
	8 weeks	26 (42.62)	27 (40.30)	0.811
	12 weeks	23 (37.70)	31 (46.27)	0.438
Q4. I feel like the skin wrinkles at the corner of the eye decreased after intake of the test product.	4 weeks	13 (21.31)	14 (20.90)	1.000
	8 weeks	15 (24.59)	21 (31.34)	0.449
	12 weeks	24 (39.34)	26 (38.81)	0.632
Q5. I feel like my skin dryness decreased after intake of the test product.	4 weeks	34 (55.74)	34 (50.75)	0.407
	8 weeks	42 (68.85)	51 (76.12)	0.288
	12 weeks	40 (65.57)	51 (76.12)	0.473
Q6. I feel like my overall skin condition and appearance improved after intake of the test product.	4 weeks	26 (42.62)	32 (47.76)	0.856
	8 weeks	41 (67.21)	47 (70.15)	0.813
	12 weeks	37 (60.66)	46 (68.66)	0.805
Q7. I feel like my skin gloss increased after intake of the test product.	4 weeks	23 (37.70)	26 (38.81)	1.000
	8 weeks	28 (45.90)	38 (56.72)	0.569
	12 weeks	31 (50.82)	31 (46.27)	0.208
Q8. I feel like my fine lines and wrinkles decreased after intake of the test product.	4 weeks	16 (26.23)	15 (22.39)	0.778
	8 weeks	17 (27.87)	21 (31.34)	0.350
	12 weeks	22 (36.07)	22 (32.84)	0.260
Usability Assessment				
Q1. Was it easy to take the test product?	12 weeks	55 (90.16)	64 (95.52)	0.525
Q2. What was your satisfaction in using the test product?	12 weeks	46 (75.41)	58 (86.57)	0.229
Q3. Would you like to purchase the test product?	12 weeks	44 (72.13)	50 (74.63)	0.774
Q4. Would you recommend this product to others?	12 weeks	43 (70.49)	46 (68.66)	0.411

¹ N (Frequency) = Number of positive answers (4. Somewhat agree/satisfied/easy or 5. Agree/satisfied/easy), % (Percentage) = Number of positive answers / Total number of subjects × 100. Answers "1. Difficult/Dissatisfied/Disagree," "2. Somewhat difficult/dissatisfied/disagree," and "3. No opinion" not shown in this table. ²Between-group comparison by chi-square test and Fisher's exact test. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*).

Table S6. Body composition of the participants in the PP population. The body composition was measured at baseline and at Week 12.

Parameter	Time point	BI-04 (N = 61) Mean (SD)	p-Value ¹	Placebo (N = 67) Mean (SD)	p-Value ¹	Total (N = 128) Mean (SD)	p-Value ³
Body fat mass, kg	Baseline	20.21 (6.796)		18.73 (5.178)		19.44 (6.026)	0.169
	12 weeks	19.60 (6.973)		18.39 (5.255)		18.97 (6.139)	0.275
	Change from baseline	-0.61 (1.258)	<0.001*	-0.33 (1.411)	0.009* ²	-0.47 (1.343)	

Body mass index, kg/m ²	Baseline	23.67 (3.721)		22.86 (2.767)		23.24 (3.269)	0.166
	12 weeks	23.36 (3.808)		22.63 (2.810)		22.98 (3.330)	0.220
	Change from baseline	-0.31 (0.505)	<0.001*	-0.23 (0.552)	0.001*	-0.27 (0.529)	
Percent body fat, %	Baseline	32.52 (5.555)		32.00 (5.382)		32.25 (5.450)	0.590
	12 weeks	31.89 (5.994)		31.71 (5.200)		31.80 (5.571)	0.856
	Change from baseline	-0.63 (1.902)	0.012*	-0.29 (1.730)	0.180	-0.45 (1.815)	
Waist-hip ratio, AU	Baseline	0.862 (0.0477)		0.853 (0.0456)		0.857 (0.0467)	0.271
	12 weeks	0.851 (0.0480)		0.845 (0.0412)		0.848 (0.0445)	0.469
	Change from baseline	-0.011 (0.0197)	<0.001*	-0.008 (0.0202)	<0.001* ²	-0.010 (0.0200)	
Weight, kg	Baseline	60.98 (9.793)		57.69 (8.346)		59.26 (9.178)	0.042*
	12 weeks	60.18 (9.974)		57.11 (8.479)		58.57 (9.313)	0.062
	Change from baseline	-0.81 (1.316)	<0.001*	-0.58 (1.440)	0.002*	-0.69 (1.381)	

¹ Within-group comparisons to baseline by paired t-test. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ² Wilcoxon Signed-rank test was applied when values were not normally distributed. ³ Between-group comparisons by t-test. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*).

Table S7. Blood safety test values of the participants in the safety population. Blood test values were measured at the screening visit and at Week 12.

Parameter	Time point	BI-04 (N = 74)		Placebo (N = 73)	
		Mean (SD)	<i>p</i> -Value ¹	Mean (SD)	<i>p</i> -Value ¹
Glucose, mg/dL	Screening	97.15 (11.84)		96.40 (9.54)	
	12 weeks	101.85 (14.03)	<0.001* ²	102.15 (12.20)	<0.001* ²
Hemoglobin, g/dL	Screening	13.18 (1.06)		13.28 (0.93)	
	12 weeks	13.19 (1.14)	0.951	13.28 (1.00)	0.944
GPT, U/L	Screening	18.50 (7.81)		22.03 (18.87)	
	12 weeks	18.26 (9.39)	0.107 ²	19.15 (12.22)	0.012* ²
GOT, U/L	Screening	23.14 (4.91)		27.12 (20.42)	
	12 weeks	22.41 (5.66)	0.045*	23.15 (8.77)	<0.001* ²
γ-GTP, U/L	Screening	21.88 (43.29)		19.33 (15.70)	
	12 weeks	21.26 (43.30)	0.013* ²	16.42 (11.61)	<0.001* ²
Cholesterol, mg/dL	Screening	199.64 (33.84)		203.36 (34.24)	
	12 weeks	214.05 (35.08)	<0.001*	218.12 (36.65)	<0.001*
Triglyceride, mg/dL	Screening	101.62 (54.24)		103.99 (56.27)	
	12 weeks	113.24 (72.13)	0.115 ²	112.78 (62.29)	0.258 ²
LDL-Cholesterol, mg/dL	Screening	123.78 (26.57)		129.01 (26.75)	
	12 weeks	127.15 (26.96)	0.094	130.93 (26.91)	0.313
HDL-Cholesterol, mg/dL	Screening	68.46 (13.24)		67.95 (13.23)	
	12 weeks	64.27 (13.36)	<0.001*	63.75 (11.59)	<0.001*
Creatinine, mg/dL	Screening	0.79 (0.08)		0.79 (0.10)	
	12 weeks	0.82 (0.08)	<0.001*	0.82 (0.10)	<0.001*

¹ Comparisons to baseline by paired t-test. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*). ² Wilcoxon Signed-rank test with Bonferroni correction was applied when values were not normally distributed.

Table S8. Descriptive statistics of 7-day average daily mean temperature (°C), minimum temperature (°C), maximum temperature (°C), and mean relative humidity (%) by treatment and visit for the PP population.

Parameter	Time point	BI-04 (N = 61)	Placebo (N = 67)	Total (N = 128)
		Mean (SD)	Mean (SD)	Mean (SD)
Mean temperature, °C	Baseline	-0.82 (4.234)	-1.18 (4.315)	-1.01 (4.264)
Minimum temperature, °C		-5.08 (4.381)	-5.47 (4.430)	-5.29 (4.394)
Maximum temperature, °C		3.47 (4.048)	3.17 (4.164)	3.31 (4.096)
Mean relative humidity, %		59.46 (6.358)	58.86 (6.291)	59.15 (6.305)
Mean temperature, °C	4 weeks	0.44 (3.070)	0.40 (2.969)	0.42 (3.005)
Minimum temperature, °C		-4.42 (2.805)	-4.47 (2.830)	-4.45 (2.807)
Maximum temperature, °C		5.72 (3.355)	5.72 (3.249)	5.72 (3.287)
Mean relative humidity, %		53.50 (6.963)	54.33 (7.473)	53.94 (7.218)
Mean temperature, °C	8 weeks	9.85 (1.769)	9.66 (1.776)	9.75 (1.768)
Minimum temperature, °C		4.50 (1.511)	4.33 (1.525)	4.41 (1.515)
Maximum temperature, °C		16.13 (1.916)	15.96 (1.920)	16.04 (1.912)
Mean relative humidity, %		57.50 (4.893)	58.01 (4.962)	57.77 (4.916)
Mean temperature, °C	12 weeks	14.10 (2.184)	14.16 (2.498)	14.13 (2.344)
Minimum temperature, °C		8.95 (2.405)	9.05 (2.646)	9.00 (2.524)
Maximum temperature, °C		19.79 (2.556)	19.75 (2.882)	19.77 (2.721)
Mean relative humidity, %		52.71 (7.264)	52.09 (7.715)	52.38 (7.480)

Table S9. Descriptive statistics of 7-day mode of ultraviolet (UV) index and 7-day maximum UV index for the PP population.

Parameter	Time point	Value	BI-04 (N = 61)	Placebo (N = 67)	Total (N = 128)
			N (%)	N (%)	N (%)
7-day mode of UV index	Baseline	Low	42 (68.9)	40 (59.7)	82 (64.1)
		Moderate	19 (31.1)	27 (40.3)	46 (35.9)
	4 weeks	Low	7 (11.5)	10 (14.9)	17 (13.3)
		Moderate	54 (88.5)	57 (85.1)	111 (86.7)
	8 weeks	Moderate	48 (78.7)	53 (79.1)	101 (78.9)
		High	13 (21.3)	14 (20.9)	27 (21.1)
7-day maximum UV index	12 weeks	Moderate	8 (13.1)	15 (22.4)	23 (18.0)
		High	53 (86.9)	52 (77.6)	105 (82.0)
	Baseline	Moderate	52 (85.2)	56 (83.6)	108 (84.4)
		Extreme	9 (14.8)	11 (16.4)	20 (15.6)
	4 weeks	Moderate	61 (100.0)	67 (100.0)	128 (100.0)
	8 weeks	Moderate	32 (52.5)	40 (59.7)	72 (56.3)
		High	29 (47.5)	27 (40.3)	56 (43.8)
	12 weeks	High	61 (100.0)	67 (100.0)	128 (100.0)

Table S10. Analysis of 7-day average daily mean temperature (°C), minimum temperature (°C), maximum temperature (°C), mean relative humidity (%), and maximum UV index for the PP population between time points and groups.

Comparison, p-values ¹	7-day temperature			7-day average daily mean relative humidity	7-day maximum UV index
	Mean	Minimum	Maximum		
BI-04 at 4 weeks <i>vs</i> baseline	0.050*	0.307	0.002*	<0.001*	0.004*
BI-04 at 8 weeks <i>vs</i> baseline	<0.001*	<0.001*	<0.001*	0.114	0.328
BI-04 at 8 weeks <i>vs</i> 4 weeks	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
BI-04 at 12 weeks <i>vs</i> baseline	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

BI-04 at 12 weeks <i>vs</i> 8 weeks	<0.001*	<0.001*	<0.001*	0.003*	<0.001*
Placebo at 4 weeks <i>vs</i> baseline	0.002*	0.110	<0.001*	0.002*	0.001*
Placebo at 8 weeks <i>vs</i> baseline	<0.001*	<0.001*	<0.001*	0.492	0.911
Placebo at 8 weeks <i>vs</i> 4 weeks	<0.001*	<0.001*	<0.001*	0.004*	<0.001*
Placebo at 12 weeks <i>vs</i> baseline	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Placebo at 12 weeks <i>vs</i> 8 weeks	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Overall BI-04 <i>vs</i> Placebo	0.670	0.582	0.770	0.845	0.610
BI-04 <i>vs</i> Placebo at baseline	0.704	0.648	0.804	0.598	0.799
BI-04 <i>vs</i> Placebo at 4 weeks	0.950	0.962	0.943	0.364	1.000
BI-04 <i>vs</i> Placebo at 8 weeks	0.609	0.616	0.672	0.604	0.413
BI-04 <i>vs</i> Placebo at 12 weeks	0.916	0.932	0.981	0.609	1.000

¹ Comparisons were made by Wilcoxon Signed-rank test or Mann-Whitney U-test. Significant *p*-values (*p* < 0.05) are marked with an asterisk (*).

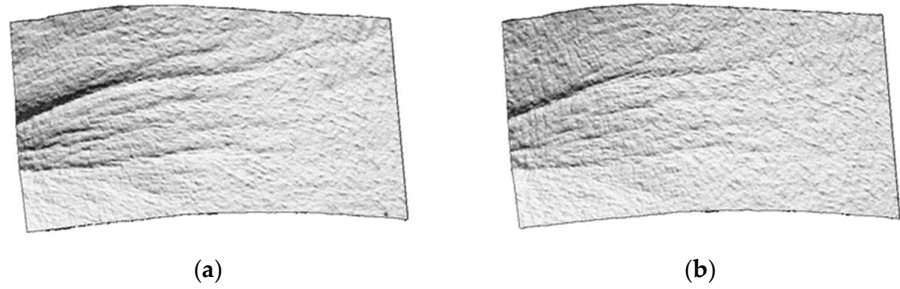


Figure S1. Three-dimensional (3D) image of the corner of the eye area of a participant in the BI-04 group. Corner of the eye area of Participant DEF-20107-077 at (a) baseline and (b) Week 4, taken with the PRIMOS® premium imaging system.