別紙様式（Ⅴ）－１【添付ファイル用】　※複数の機能性に関する表示を行う等、必要な場合使用する。

機能性の科学的根拠に関する点検表

１．製品概要

商品名	ライラック乳酸菌　スタンダード（分包）
機能性関与成分名	有胞子性乳酸菌（*Bacillus coagulans*）lilac-01
表示しようとする機能性	本品には、生きた有胞子性乳酸菌（*Bacillus coagulans*）lilac-01が含まれています。便秘傾向の方の便の状態（便の色、臭い、量、形）を整え、お通じ（回数、残便感）を改善します。便通が気になる方に適した食品です。

２．科学的根拠

【臨床試験及び研究レビュー共通事項】

□（主観的な指標によってのみ評価可能な機能性を表示しようとする場合）当該指標は日本人において妥当性が得られ、かつ、当該分野において学術的に広くコンセンサスが得られたものである。

☑（最終製品を用いた臨床試験又は研究レビューにおいて、実際に販売しようとする製品の試作品を用いて評価を行った場合）両者の間に同一性が失われていないことについて、届出資料において考察されている。

☑最終製品を用いた臨床試験

（研究計画の事前登録）

☑UMIN臨床試験登録システムに事前登録している[注1]。

□（海外で実施する臨床試験の場合であってUMIN臨床試験登録システムに事前登録していないとき）WHOの臨床試験登録国際プラットフォームにリンクされているデータベースへの登録をしている。

（臨床試験の実施方法）

☑「特定保健用食品の表示許可等について」（平成26年10月30日消食表第259号）の別添２「特定保健用食品申請に係る申請書作成上の留意事項」に示された試験方法に準拠している。

□科学的合理性が担保された別の試験方法を用いている。

→□別紙様式（Ⅴ）－２を添付

（臨床試験の結果）

☑国際的にコンセンサスの得られた指針に準拠した論文を添付している[注1]。

☑査読付き論文として公表されている論文を添付している。

□（英語以外の外国語で書かれた論文の場合）論文全体を誤りのない日本語に適切に翻訳した資料を添付している。

☑研究計画について事前に倫理審査委員会の承認を受けたこと、並びに当該倫理審査委員会の名称について論文中に記載されている。

□（論文中に倫理審査委員会について記載されていない場合）別紙様式（Ⅴ）

－３で補足説明している。

☑掲載雑誌は、著者等との間に利益相反による問題が否定できる。

☐最終製品に関する研究レビュー

☐機能性関与成分に関する研究レビュー

☐（サプリメント形状の加工食品の場合）摂取量を踏まえた臨床試験で肯定的な結果が得られている。

☐（その他加工食品及び生鮮食品の場合）摂取量を踏まえた臨床試験又は観察研究で肯定的な結果が得られている。

☐海外の文献データベースを用いた英語論文の検索のみではなく、国内の文献データベースを用いた日本語論文の検索も行っている。

☐（機能性関与成分に関する研究レビューの場合）当該研究レビューに係る成分と最終製品に含有されている機能性関与成分の同等性について考察されている。

☐（特定保健用食品の試験方法として記載された範囲内で軽症者等が含まれたデータを使用している場合）疾病に罹患していない者のデータのみを対象とした研究レビューも併せて実施し、その結果を、研究レビュー報告書に報告している。

☐（特定保健用食品の試験方法として記載された範囲内で軽症者等が含まれたデータを使用している場合）疾病に罹患していない者のデータのみを対象とした研究レビューも併せて実施し、その結果を、別紙様式（Ｉ）に報告している。

☐表示しようとする機能性の科学的根拠として、査読付き論文として公表されている。

☐当該論文を添付している。

☐（英語以外の外国語で書かれた論文の場合）論文全体を誤りのない日本語に適切に翻訳した資料を添付している。

☐PRISMA 声明（2009 年）に準拠した形式で記載されている。

☐（PRISMA 声明（2009 年）に照らして十分に記載できていない事項がある場合）別紙様式（Ｖ）－３で補足説明している。

☐（検索に用いた全ての検索式が文献データベースごとに整理された形で当該論文に記載されていない場合）別紙様式（Ｖ）－５その他の適切な様式を用いて、全ての検索式を記載している。

☐（研究登録データベースを用いて検索した未報告の研究情報についてその記載が当該論文にない場合、任意の取組として）別紙様式（Ｖ）－９その他の適切な様式を用いて記載している。

☐食品表示基準の施行前に査読付き論文として公表されている研究レビュー論文を用いているため、上記の補足説明を省略している。

☐各論文の質評価が記載されている[注2]。

☐エビデンス総体の質評価が記載されている[注2]。

□研究レビューの結果と表示しようとする機能性の関連性に関する評価が記載されている[注2]。

□表示しようとする機能性の科学的根拠として、査読付き論文として公表されていない。

研究レビューの方法や結果等について、

□別紙様式（Ｖ）－４を添付している。

□データベース検索結果が記載されている[注3]。

□文献検索フローチャートが記載されている[注3]。

□文献検索リストが記載されている[注3]。

□任意の取組として、未報告研究リストが記載されている[注3]。

□参考文献リストが記載されている[注3]。

□各論文の質評価が記載されている[注3]。

□エビデンス総体の質評価が記載されている[注3]。

□全体サマリーが記載されている[注3]。

□研究レビューの結果と表示しようとする機能性の関連性に関する評価が記載されている[注3]。

注１　食品表示基準の施行後１年を超えない日までに開始（参加者１例目の登録）された研究については、必須としない。

注２　各種別紙様式又はその他の適切な様式を用いて記載（添付の研究レビュー論文において、これらの様式と同等程度に詳しく整理されている場合は、記載を省略することができる。）

注３　各種別紙様式又はその他の適切な様式を用いて記載（別紙様式（Ｖ）－４において、これらの様式と同等程度に詳しく整理されている場合は、記載を省略することができる。）

別紙様式（Ｖ）－３【添付ファイル用】

表示しようとする機能性の科学的根拠に関する補足説明資料

１．製品概要

商品名	ライラック乳酸菌 スタンダード(分包)
機能性関与成分名	有胞子性乳酸菌（*Bacillus coagulans*）lilac-01
表示しようとする機能性	本品には、生きた有胞子性乳酸菌（*Bacillus coagulans*）lilac-01 が含まれています。便秘傾向の方の便の状態（便の色、臭い、量、形）を整え、お通じ（回数、残便感）を改善します。便通が気になる方に適した食品です。

２．補足説明

(1) 届出しようとする最終製品と臨床試験で使用した製品の同一性に関する説明

届出しようとする最終製品の機能性は、有胞子性乳酸菌（*Bacillus coagulans*）lilac-01 を含む製品を摂取する臨床試験により評価しました*1。臨床試験で使用した試験食は、届出しようとする最終製品の製造所（別紙様式(III)-1 で示した製造所）にて製造されたものではありませんが、以下の理由から、最終製品と試験食の同一性は失われていないと判断しました。

①形状が同一（粉末剤）であること。
②最終製品と試験食の原材料が同一であること。
③最終製品と試験食の機能性関与成分は、有胞子性乳酸菌（*Bacillus coagulans*）lilac-01 のみであり、一日摂取目安量あたりの機能性関与成分量（有胞子性乳酸菌（*Bacillus coagulans*）lilac-01 数）が同量であること。
④機能性関与成分量（有胞子性乳酸菌（*Bacillus coagulans*）lilac-01）を届出後も定量し、機能性関与成分量を保証すること。

(2) 便の状態（便の色、臭い、量、形）に関する説明

便の状態（便性：色、臭い、量、形）は、腸内環境の指標のため、腸内フローラに係る臨床試験の評価指標として用いられています。本品は、便の状態を改善することが目的で、疾病の診断、治療又は予防に使用されることや、身体の構造又は機能に影響を及ぼすことを目的とするものではありませんので、医薬品医療機器等法には触れません。

(3) 論文の層別解析に関する説明

論文の層別解析は、便秘傾向のある健康成人男女の被験者を、ROMEIII 診断基準に基づいた独自のアンケートにより、機能性便秘傾向群と非機能性便秘傾向群に分けて実施されたものなので、「特定保健用食品の表示許可等について」の別添２「特定保健用食品申請に係る申請書作成上の留意事項」の「第 2―5―(7)―エの対象被験者」から逸脱していません。

*1 Minamida K, Nishimura M, Miwa K, &Nishihira J. Effects of dietary fiber with *Bacillus coagulans* lilac-01 on bowel movement and fecal properties of healthy volunteers with a tendency for constipation. Biosci Biotechnol Biochem. 2015;79:300-306.

添付書類６

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Effects of dietary fiber with *Bacillus coagulans* lilac-01 on bowel movement and fecal properties of healthy volunteers with a tendency for constipation

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To elucidate the effects of Lilac LAB (*Bacillus coagulans* lilac-01 and okara [soy pulp] powder) on bowel movements/fecal properties, we conducted a double-blind placebo-controlled randomized trial with healthy Japanese volunteers with a tendency for constipation ($n = 297$). The subjects ingested 2 g/d placebo (okara powder) or test food (Lilac LAB, 1×10^8 CFU) once a day for 2 weeks. In the test group of functionally constipated subjects, the changes in the average scores of self-reported fecal size, sensation of incomplete evacuation, and defecation frequency were significantly improved compared to the placebo group ($p < 0.05$), and fecal color and odor tended to improve ($p = 0.07$). In the test food group of all subjects and among the non-functionally constipated subjects, the fecal size tended to improve compared to the placebo group ($p = 0.06$, $p = 0.07$, respectively). Lilac LAB was effective in improving bowel movements and fecal properties in functionally constipated persons.

Key words: *Bacillus coagulans*; okara; human trial; synbiotics; constipation

In general, constipation is the state resulting from a disruption of the normal rhythm of defecation, whereby the stool remains in the colon for a long time. Constipation has been evaluated on the basis of the Rome III diagnostic criteria[1]; that is, straining, fecal hardness, sensation of incomplete evacuation, sensation of anorectal obstruction/blockage, and manual maneuvers to facilitate and defecation frequency.

Relief from these symptoms of constipation may be achieved by consuming probiotics, prebiotics, or synbiotics. Probiotics are redefined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[2] Probiotics contain lactic acid bacteria and yogurt. Prebiotics are "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health."[3] Prebiotics contain oligosaccharides (fructo-oligosaccharides [FOS], raffinose, and others) and resistant carbohydrates (inulin and others). Synbiotics are defined as "the combination of both probiotics and prebiotics."[4]

As a probiotic, *Bacillus coagulans* is a spore-forming lactic acid bacterium.[5,6] As part of its ability to form spores, *B. coagulans* has tolerance to heat, oxygen, drying, acid, and bile acid.[7] Because almost 100% of consumed *B. coagulans* reaches the intestinal tract and produces lactic acid in the intestines, *B. coagulans* is widely used in probiotic formulations around the world.[8–10] In Japan, Dr. O. Nakayama isolated *B. coagulans* from green malt in 1949,[6] and a strain of it (SANK 70258) is used as a medicine (Panlacmin Tablet, Daiichi-Sankyo, Tokyo) and in a food additive (Lacris®-S, Mitsubishi-kagaku Foods, Tokyo). A clinical trial tested the effects of Lacris®-S (1×10^8 CFU/d for 2 weeks) on fecal properties and defecation frequency in 20 healthy adults, and it revealed that the ingestion of Lacris®-S improved the subjects' intestinal environment, defecation frequency, and fecal properties.[7]

The strain *B. coagulans* lilac-01 (NITE P-1102) was isolated from the petals of *Syringa vulgaris* (lilac; the symbol flower of the city of Sapporo, Japan) by Arterio Bio Co (Hokkaido, Japan). Their product, Lilac LAB, is composed of *B. coagulans* lilac-01 and okara powder.

Okara, also known as soy pulp, is residue from the production of tofu (soybean curd). It has healthy properties (non-cholesterol and low calorie) and many nutrient and functional constituents (vitamin E, calcium, soy protein, isoflavone, and saponin). Okara also contains oligosaccharides (raffinose and stachyose)[11] and dietary fiber, and thus, okara is often used in Japan to prevent constipation. Because Lilac LAB contains okara powder (a prebiotic) and *B. coagulans* lilac-01 (a probiotic), it can be considered a synbiotic.

Here, we conducted a randomized double-blind placebo-controlled trial to evaluate the effects of Lilac LAB on bowel movements and fecal properties in healthy volunteers with a tendency for constipation.

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Materials and methods

Study food. The test food (Lilac LAB, Arterio Bio) was a mixture of *B. coagulans* lilac-01 and okara powder (Kikkoman Soyfoods, Tokyo), and the viable count of strain lilac-01 was 1×10^8 CFU/d. The placebo food was okara powder only. The nutritional composition of the placebo (okara powder) per 100 g was protein (31.4 g), fat (18.6 g), ash (4.4 g), carbohydrate (6.9 g), dietary fiber (35.6 g), moisture (3.1 g), and calories (392 kcal). The nutritional composition of the test food (Lilac LAB) was protein (34.8 g), fat (16.8 g), ash (4.6 g), carbohydrate (5.4 g), dietary fiber (35.1 g), moisture (3.3 g), and calories (382 kcal) per 100 g (Japan food Research Laboratories, Tokyo, Japan).

Subjects. The subjects were 297 healthy volunteers (20–80 years) who reported having a tendency to experience constipation. None had a history of recent gastrointestinal illness, pregnancy, significant disease, surgery, or severe allergic reactions to food (especially soy bean), or current use of any medications. For our division of the subjects into a group of functionally constipated subjects and a group of non-functionally constipated subjects (functionally constipated subjects have no underlying organic cause), each subject answered a questionnaire based on the Rome III diagnostic criteria (Table 1) before this study.

The protocol for this study was approved by the Ethics Committee for Human Health of Hokkaido Information University, and written informed consent was obtained from all subjects. The study was carried out in accord with the ethical principles that have their origin in the Declaration of Helsinki.

Study design. This study was conducted with a double-blind placebo-controlled randomized design (Fig. 1). The subjects were evenly assigned to the placebo and test food groups in terms of gender, age, and scores on the questionnaire based on the Rome III diagnostic criteria. As a result, the placebo group was 149 subjects and the test food group was 148 subjects. The subjects ingested 2 g/d placebo (okara powder) or test food (Lilac LAB) once a day for 2 weeks. We instructed the subjects to continue with their usual diets and avoid taking new medicines and supplements. The subjects kept a diary (their food intake and health status) and completed a daily questionnaire (defecation frequency, fecal properties, and symptoms of constipation) with scores during the entire study period (Table 2). The subjects' assessment of fecal properties (color, shape, and size) used a color chart card and a size model (2 cm dia. × 5 cm length) as a reference. Fecal shape was determined in reference to the Bristol stool form scale.[12] Except for fecal shape, the scores went from "worse" to "better," and for fecal shape, the scores were from 1 (softer) to 7 (harder) in incremental steps.

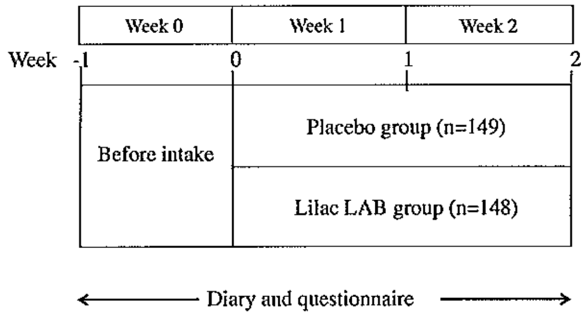

Fig. 1. Experimental schedule.

Statistical analysis. Values are expressed as the mean ± standard deviation (SD). The data were analyzed with the software IBM SPSS Statistic 19 (IBM,

Table 1. Rome III diagnostic criteria for functionally constipation.[1)]

Symptoms
Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis
1. Must include 2 or more of the following:
a. Straining[a]
b. Lumpy or hard stools[a]
c. Sensation of incomplete evacuation[a]
d. Sensation of anorectal obstruction/blockage[a]
e. Manual maneuvers to facilitate[a]
f. Fewer than 3 defecations per week
2. Loose stools are rarely present without the use of laxatives
3. There are insufficient criteria for irritable bowel syndrome

[a] ≥25% of defecations.

Table 2. Fecal properties and symptoms during defecation.

Fecal properties	Score
Color[a]	1. Blackish brown (No. 582), 2. Dark brown (No. 311), 3. Brown (No. 308), 4. Ochre (No. 240), 5. Orange (No. 205)
Shape[b]	1. Watery, no solid pieces
	2. Fluffy pieces with ragged edges, a mushy stool
	3. Soft blobs with clear cut edges
	4. Like a sausage or snake, smooth and soft
	5. Like a sausage or snake but with cracks on its surface
	6. Sausage-shaped, but lumpy
	7. Separate hard lumps, like nuts
Size	1. <1 unit, 2. 1 unit, 3. 2 units, 4. 3 units, 5. 4 units, 6. 5 units, 7. >5 units
Odor	1. Very strong, 2. Strong, 3. A little strong, 4. Odorless
Straining	1. Very difficult, 2. A little difficult, 3. Easy
Sensation of incomplete evacuation	1. Very unrefreshing, 2. A little unrefreshing, 3. Refreshing, 4. Very refreshing

Notes: Except for fecal shape, scores went from 'worse' to 'better,' and for fecal shape, scores were from 1 (softer) to 7 (harder) in incremental steps.
[a]According to a color sample book (Color Guide 19th ed., Dainippon Ink and Chemicals, Tokyo).
[b]According to the Bristol stool form scale.[12)]

Armonk, NY). Average per-day scores in the week before the test food/placebo intake began (Week 0) and the second week after intake (Week 2) were respectively calculated. Using these values, we examined the differences between before and after the ingestion with the Games–Howell test. Using a variation of these values, we examined the differences between the placebo and test groups with Mann–Whitney's *U*-test. Differences between means were considered significant at $p < 0.05$.

Results

Baseline data

This double-blind placebo-controlled randomized trial was conducted from September 2013 to December 2013. The study was completed by 268 subjects (59 males, 209 females, average age 50.6 years); 29 subjects dropped out due to their inadequate food intake (<80% of the study food) or inadequate questionnaire completion. Based on their responses to the questionnaire using the Rome III diagnostic criteria, we classified 137 subjects as functionally constipated subjects (51% of all subjects, 24 males, 113 females, average age 51.5 years) and the other 131 subjects were classified as non-functionally constipated subjects (49% of all subjects, 35 males, 96 females, average age 49.3 years).

Fecal size

The average per-day scores of fecal size in Week 0 and Week 2 were between 2 (1 unit) and 4 (3 units) in all subjects (Table 3). Compared to the average scores of before and after food/placebo ingestion, the fecal size increased significantly in all subjects (placebo, $p = 0.036$; test food, $p = 0.029$) and in the test food group of functionally constipated subjects ($p = 0.001$).

The changes in the average scores of fecal size are summarized in Table 5. Compared with each placebo group, the fecal size of the test food group was significantly increased in the functionally constipated subjects ($p = 0.034$, Fig. 2) and it tended to increase in the non-functionally constipated subjects ($p = 0.072$).

Fecal shape

Generally, the normal scores of fecal shape are between 3 (soft blobs with clear-cut edges) and 5 (similar to a sausage or snake but with cracks on its surface). In this study, the average per-day scores of fecal shape in all subjects were between 3 and 4 (similar to a sausage or snake, smooth and soft) although the subjects had a tendency for constipation (Table 3). Compared to the average scores from before and after ingestion, there were no significant differences among any of the groups.

The changes in the average scores of fecal shape are summarized in Table 5. Compared with each placebo group, the fecal shape reported by the test food subjects changed significantly in the group of functionally constipated subjects ($p = 0.039$, Fig. 2) and tended to change in all subjects ($p = 0.061$).

Table 3. Average per-day scores of fecal properties.

Group		Size		Shape		Color		Odor	
		Week 0	Week 2	Week 0	Week 2	Week 0	Week 2	Week 0	Week 2
All subjects (n = 268)	Placebo (Okara powder)	2.87 ± 0.13	3.30 ± 0.12*	3.56 ± 0.12	3.40 ± 0.09	2.34 ± 0.07	2.50 ± 0.07	2.27 ± 0.07	2.47 ± 0.06
	Test food (Lilac LAB)	2.80 ± 0.13	3.22 ± 0.10*	3.43 ± 0.12	3.60 ± 0.09	2.24 ± 0.08	2.45 ± 0.06	2.19 ± 0.08	2.49 ± 0.06**
Functionally constipated subjects (n = 137)	Placebo (Okara powder)	2.85 ± 0.17	3.18 ± 0.15	3.82 ± 0.17	3.58 ± 0.13	2.33 ± 0.09	2.48 ± 0.09	2.33 ± 0.11	2.49 ± 0.08
	Test food (Lilac LAB)	2.28 ± 0.16	3.10 ± 0.14**	3.40 ± 0.18	3.69 ± 0.14	1.97 ± 0.10	2.37 ± 0.07**	1.94 ± 0.10	2.46 ± 0.09**
Non-functionally constipated subjects (n = 131)	Placebo (Okara powder)	2.89 ± 0.18	3.42 ± 0.18	3.28 ± 0.16	3.20 ± 0.13	2.36 ± 0.11	2.52 ± 0.10	2.21 ± 0.10	2.46 ± 0.09
	Test food (Lilac LAB)	3.31 ± 0.18	3.34 ± 0.15	3.45 ± 0.16	3.51 ± 0.13	2.51 ± 0.11	2.53 ± 0.09	2.43 ± 0.12	2.52 ± 0.09

Notes: Scores refer to Table 2. Values are mean ± SD. The average scores in the week before intake (Week 0) and the second week after intake (Week 2) per day were respectively calculated. Using these values, we examined the differences between before and after the ingestion with the Games–Howell test.
*$p < 0.05$, significantly different from Week 0.
**$p < 0.01$, significantly different from Week 0.

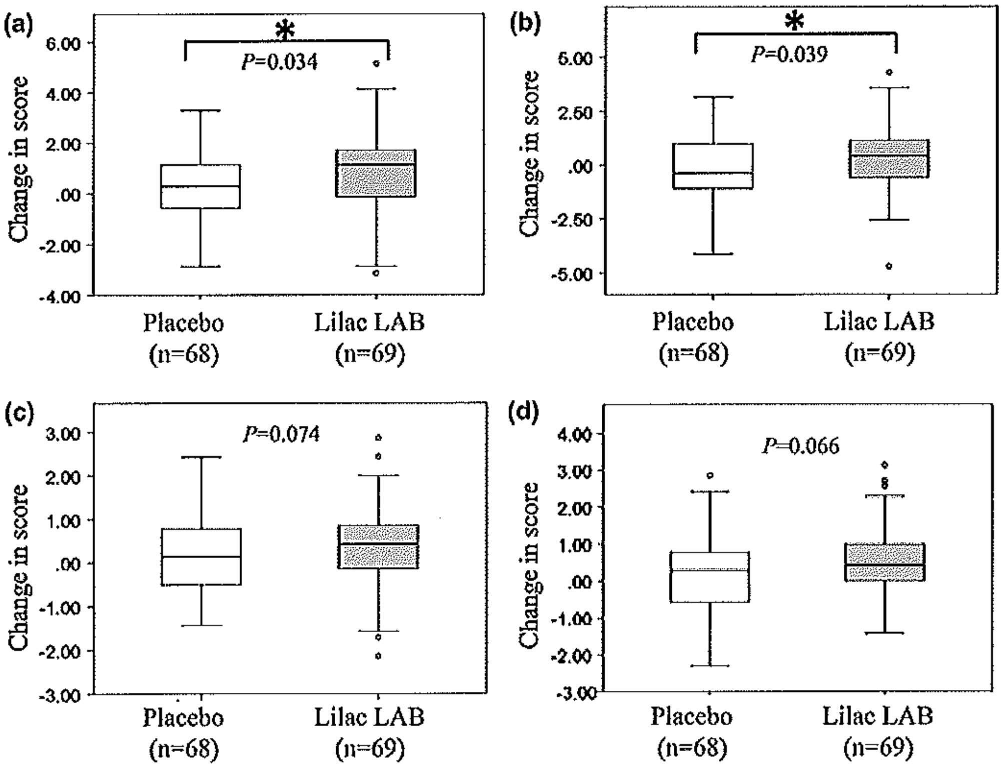

Fig. 2. Changes of average score in Week 0 and Week 2 of the 137 functionally constipated subjects.

Notes: (a) Fecal size, (b) fecal shape, (c) fecal color, and (d) odor. Box plots show 25th and 75th percentiles, median and range. The average per-day scores in the week before intake (Week 0) and the second week after intake (Week 2) were respectively calculated. Using a variation of these values, we examined the differences between the placebo and test groups with Mann–Whitney's *U*-test. $^{*}p < 0.05$ compared to the placebo group.

Table 4. Average per-day scores of defecation frequency and symptoms during defecation.

	Group	Straining		Sensation of incomplete evacuation		Defecation frequency	
		Week 0	Week 2	Week 0	Week 2	Week 0	Week 2
All subjects (n = 268)	Placebo (Okara powder)	1.65 ± 0.06	1.78 ± 0.05	1.88 ± 0.07	2.02 ± 0.06	0.99 ± 0.04	1.05 ± 0.04
	Test food (Lilac LAB)	1.64 ± 0.06	1.79 ± 0.05	1.82 ± 0.07	2.12 ± 0.06**	1.08 ± 0.06	1.20 ± 0.05
Functionally constipated subjects (n = 137)	Placebo (Okara powder)	1.58 ± 0.07	1.75 ± 0.07	1.80 ± 0.08	2.00 ± 0.08	0.97 ± 0.05	1.02 ± 0.05
	Test food (Lilac LAB)	1.39 ± 0.08	1.73 ± 0.07**	1.55 ± 0.08	2.09 ± 0.09**	0.91 ± 0.06	1.21 ± 0.08**
Non-functionally constipated subjects (n = 131)	Placebo (Okara powder)	1.72 ± 0.09	1.82 ± 0.08	1.97 ± 0.10	2.05 ± 0.09	1.02 ± 0.06	1.09 ± 0.06
	Test food (Lilac LAB)	1.90 ± 0.09	1.86 ± 0.07	2.08 ± 0.10	2.15 ± 0.09	1.24 ± 0.11	1.18 ± 0.06.

Notes: Scores refer to Table 2. Values are mean ± SD. The average scores in the week before intake (Week 0) and the second week after intake (Week 2) per day were respectively calculated. Using these values, we examined the differences between before and after the ingestion with the Gemes–Howell test.

$^{*}p < 0.05$, significantly different from Week 0.

$^{**}p < 0.01$, significantly different from Week 0.

Fecal color

The average per-day scores of fecal color were between 2 (dark brown) and 3 (brown) in all subjects (Table 3). Fecal color corresponds to fecal pH: Low pH turns feces yellow because of bilirubin. Compared to the average scores from before and after ingestion, the fecal color improved (turn to yellowish) significantly in the test food group of functionally constipated subjects (p = 0.004) and tended to improve in the test food group of all subjects (p = 0.075).

The changes in the average scores of fecal color are shown in Table 5. Compared to the values reported by each placebo group, the fecal color described by the test food groups tended to turn to yellowish among the functionally constipated subjects (p = 0.074, Fig. 2).

Table 5. Changes in average scores of fecal properties.

	Group	Size	Shape	Color	Odor
All subjects (n = 268)	Placebo (Okara powder)	0.43 ± 0.13	−0.16 ± 0.12	0.16 ± 0.08	0.20 ± 0.08
	Test food (Lilac LAB)	0.43 ± 0.14	0.17 ± 0.13	0.21 ± 0.09	0.31 ± 0.09
Functionally constipated subjects (n = 137)	Placebo (Okara powder)	0.33 ± 0.17	−0.24 ± 0.19	0.15 ± 0.11	0.16 ± 0.12
	Test food (Lilac LAB)	0.82 ± 0.20*	0.28 ± 0.18*	0.41 ± 0.11	0.52 ± 0.11
Non-functionally constipated subjects (n = 131)	Placebo (Okara powder)	0.53 ± 0.20	−0.07 ± 0.15	0.16 ± 0.13	0.24 ± 0.10
	Test food (Lilac LAB)	0.03 ± 0.19	0.06 ± 0.19	0.02 ± 0.14	0.09 ± 0.14

Note: Scores refer to Table 2. Values are mean ± SD. The average scores in the week before intake (Week 0) and the second week after intake (Week 2) per day were respectively calculated. Using a variation of these values, we examined the differences between the placebo and test groups with Mann–Whitney's U-test.
*$p < 0.05$, significantly different from the placebo group.

Table 6. Changes in average scores of defecation frequency and symptoms during defecation.

	Group	Straining	Sensation of incomplete evacuation	Defecation frequency
All subjects (n = 268)	Placebo (Okara powder)	0.13 ± 0.06	0.14 ± 0.07	0.06 ± 0.05
	Test food (Lilac LAB)	0.15 ± 0.07	0.30 ± 0.09	0.12 ± 0.07
Functionally constipated subjects (n = 137)	Placebo (Okara powder)	0.17 ± 0.09	0.20 ± 0.09	0.05 ± 0.06
	Test food (Lilac LAB)	0.34 ± 0.09	0.54 ± 0.11*	0.30 ± 0.09*
Non-functionally constipated subjects (n = 131)	Placebo (Okara powder)	0.09 ± 0.10	0.08 ± 0.10	0.07 ± 0.07
	Test food (Lilac LAB)	−0.04 ± 0.10	0.07 ± 0.13	−0.06 ± 0.09

Notes: Scores refer to Table 2. Values are mean ± SD. Average per-day scores in the week before intake (Week 0) and the second week after intake (Week 2) were respectively calculated. Using a variation of these values, we examined the differences between placebo and test groups with Mann–Whitney's U-test.
*$p < 0.05$ compared to the placebo group.

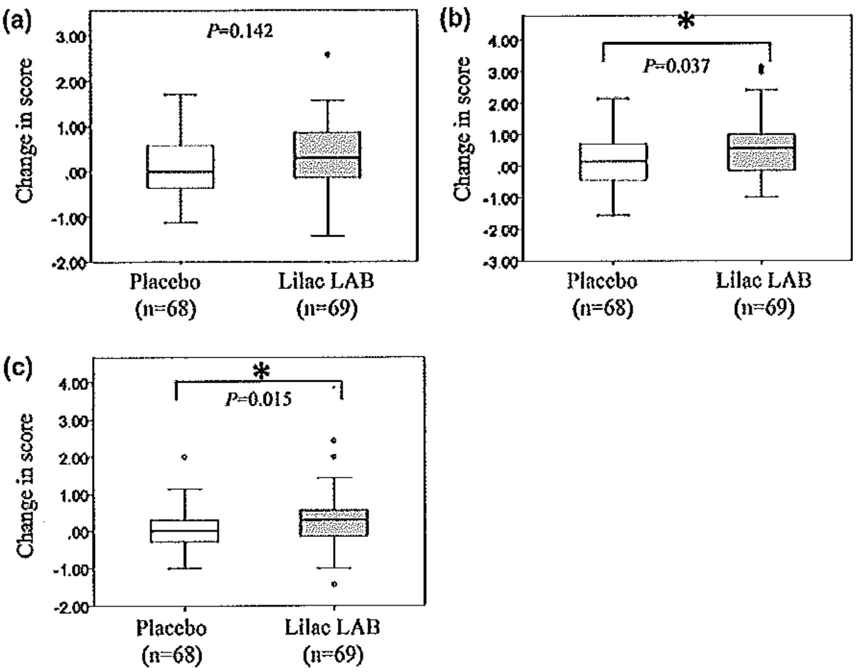

Fig. 3. Changes of average score in Week 0 and Week 2 of functionally constipated subjects.
Notes: (a) Straining, (b) sensation of incomplete evacuation, and (c) defecation frequency. Box plots show 25th and 75th percentiles, median and range. Average per-day scores in the week before intake (Week 0) and the second week after intake (Week 2) were respectively calculated. Using a variation of these values, we examined the differences between the placebo and test groups with Mann–Whitney's U-test. *$p < 0.05$ compared to the placebo group.

Odor

The average per-day scores of odor were between 2 (strong) and 3 (a little strong) in all subjects (Table 3). Compared to the average scores before and after ingestion, the odor decreased significantly in the test food groups of all subjects and the functionally constipated subjects ($p = 0.007$, $p < 0.001$, respectively).

The changes in average fecal odor scores are provided in Table 5. Compared with each placebo group, the odor score reported by the test food tended to decrease in the functionally constipated subjects ($p = 0.066$, Fig. 2).

Straining

The average per-day scores of straining were between 1 (very difficult) and 2 (a little difficult) in all subjects (Table 4). Compared to the average scores form before and after ingestion, straining improved significantly in the test food group of functionally constipated subjects ($p = 0.003$).

The changes in the average scores regarding straining are shown in Table 6. Compared with each placebo group, there were no significant differences in straining in the test food groups of all subjects, the functionally constipated subjects, and the non-functionally constipated subjects (Fig. 3).

Sensation of incomplete evacuation

The average per-day scores of sensation of incomplete evacuation were between 1 (very unrefreshing) and 3 (refreshing) in all subjects (Table 4). Compared to the average scores reported before and after ingestion, the sensation of incomplete evacuation improved significantly in the test food groups of all subjects and functionally constipated subjects ($p = 0.004, p < 0.001$, respectively).

The changes in the average scores of sensation of incomplete evacuation are given in Table 6. Compared with each placebo group, the sensation of incomplete evacuation reported by the test food subjects improved significantly among the functionally constipated subjects ($p = 0.037$, Fig. 3).

Defecation frequency

In all subjects, the average per-day scores of defecation frequency were around 1 time/d although the subjects had a tendency for constipation (Table 4). Compared to the average scores reported before and after ingestion, the defecation frequency increased significantly in the test food group of functionally constipated subjects ($p = 0.005$).

The changes in the average scores of defecation frequency are shown in Table 6. Compared with each placebo group, the defecation frequency of the subjects who ingested the test food increased significantly among the functionally constipated subjects ($p = 0.015$, Fig. 3).

Discussion

B. coagulans SANK 70258 (Lacbon®) has been used as an intestinal remedy since the 1960s in Japan. It has been shown to have highly effective therapeutic effects on intestinal catarrh (87%;[13] acute 86%, chronic 70%[14]), diarrhea (100%)[13], and acute colitis (83%)[14] at $0.75–6 \times 10^8$ CFU/d for 2–12 d[13] or 3×10^8–1.2×10^9 CFU/d for 3–24 d.[14] It has a low therapeutic effect on constipation (70%,[13] 29%[14]) at $3–7.5 \times 10^8$ CFU/d for 2–10 d[13] or $3–7.5 \times 10^8$ CFU/d for 2–24 d.[14] The effects of *B. coagulans* GBI-30, 6086 for irritable bowel syndrome (IBS) patients were examined in randomized, double-blind, placebo-controlled critical trials.[15,16] Dolin et al. reported that with of *B. coagulans* GBI-30, 6086 treatment, the number of bowel movements was significantly decreased, but other IBS symptoms were not improved ($n = 52$, 2×10^9 CFU/d, 8 weeks).[15] Hun reported that abdominal pain and bloating in the treatment group were significantly improved compared to baseline values in the treatment group ($n = 44$, 8×10^8 CFU/d, 8 weeks).[16]

This study and the study by Ara et al.[7] showed that *B. coagulans* lilac-01 and *B. coagulans* SANK 70258 significantly improved constipation symptoms in healthy subjects at a daily dose that was lower than that used for the treatment of diarrhea and constipation.

Relief from these symptoms of constipation is usually achieved by consuming probiotics (lactic acid bacteria). Lactic acid produced by lactic acid bacteria has the following effects: The fecal moisture content increases,[17] the stools become softer, defecation is easier, the defecation frequency increases, the sensation of incomplete evacuation reduces, and the symptoms of constipation decrease. In addition, due to the lowering of the fecal pH by lactic acid (the fecal color turns yellowish), the odor decreases because the number of "bad" bacteria producing an odor is reduced.

In the constipated subjects, Lilac LAB (10^8 CFU/d) improved the symptoms of constipation with a smaller viable number compared to those of general probiotics (10^9–10^{10} CFU/d).[18–20] Because spores of *B. coagulans* are resistant to acid, almost all of the spores reach the intestine. In human trials of *B. coagulans* SANK 70258 ($n = 20$, 1×10^8 CFU/d, 2 weeks), the fecal shape, color, odor, pH, and defecation frequency improved compared to the scores reported before ingestion. Moreover, the number of bifidobacteria increased significantly ($p < 0.05$), and the concentrations of humic substances (ammonia, indole, and *p*-cresol) in the feces were significantly decreased ($p < 0.05$).[7] In the present human trial, Lilac LAB ($n = 138$, 1×10^8 CFU/d, 2 weeks) improved the fecal size, odor, and sensation of incomplete evacuation significantly in the test food groups compared to the scores reported before ingestion ($p < 0.05$). In the functionally constipated subjects, the fecal size, color, odor, straining, sensation of incomplete evacuation, and defecation frequency all improved significantly ($p < 0.01$). Lilac LAB also increased the fecal size; the improvement of the fecal size in human trial of strain SANK 70258 was not described.[7] Lilac LAB contains spores of *B. coagulans* lilac-01 and okara powder. Okara has the effect of increasing fecal size, as shown in this study: The fecal size of the subjects after placebo (okara powder) ingestion increased significantly ($p < 0.05$). Because the increased bulk stimulates the intestines, the defecation frequency is also increased. Lilac LAB thus seems to efficiently reduce the symptoms of constipation due to the effects of its okara and spore-forming lactic acid bacteria (*B. coagulans*) components.

Additionally, compared with the placebo (okara) groups, Lilac LAB significantly improved the fecal size, sensation of incomplete evacuation, and defecation frequency in the test food groups of functionally constipated subjects ($p < 0.05$). Because Lilac LAB also contains okara, these effects seemed to be due to the dietary fiber with attached *B. coagulans* lilac-01.

In conclusion, the results of this study demonstrate that the test food (Lilac LAB) at 2 g/d effectively improves the bowel movements and fecal properties in subjects with functionally constipation (Rome III-defined) with a short-term ingestion (2 weeks).

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