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Effects of *Saccharomyces cerevisiae* NK-1 in Improving the Intestinal Environment and Quality of Sleep:
A Randomized, Double-blind, Crossover Study

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● **Abstract**

Background: *Saccharomyces cerevisiae* NK-1 is a simple, single-celled organism and is a yeast used for industrial and pharmaceutical purposes in the field of genetics and medicine. Yeast has intestinal regulating properties. Hence, the current study aimed to investigate whether *Saccharomyces cerevisiae* NK-1 can promote changes in the intestinal environment and sleep-related quality of life.
Methods: This was a randomized, double-blind, crossover study. Healthy men and women ingested a stick containing 10 billion *Saccharomyces cerevisiae* NK-1 or a placebo stick without *Saccharomyces cerevisiae* NK-1 three times a day for 4 weeks. Then, the proportion of intestinal flora was evaluated using the terminal restriction fragment length polymorphism methods, and quality of sleep was assessed with the Obstructive Sleep Apnea (OSA) questionnaire before and after ingestion. This study was registered in the UMIN Clinical Trials Registry prior to the study (UMIN-ID: UMIN000025190).
Results: The number of Bifidobacteria was significantly higher in the *Saccharomyces cerevisiae* NK-1 powder group than in the placebo group. Based on the OSA questionnaire findings, there were significant improvements in sleepiness and fatigue upon waking up in the *Saccharomyces cerevisiae* NK-1 powder group compared with the placebo group.
Conclusion: *Saccharomyces cerevisiae* NK-1 improved the intestinal environment by increasing the number of Bifidobacteria and sleepiness and fatigue upon waking up.

Key Words: *Saccharomyces cerevisiae* NK-1, Bifidobacterium, OSA Sleep Questionnaire, Randomized, Double-blind Study

1. BACKGROUND

The human intestine has different intestinal bacteria, which form a complex flora. The balance of microflora, or the intestinal environment, is significantly associated with overall health[1)-4)]. In human health, predominance of the Bifidobacterium genus should be maintained[5)]. Yeast has been utilized for the fermentation of foods since a long time ago and has several uses particularly in making bread, sake, and wine. It has a single cell with a simple biological structure; hence, it is applied for industrial and pharmaceutical purposes in the genetics and medical fields. *Saccharomyces cerevisiae* is a budding yeast. It is a well-known species and is popular due to its nutritional activity and nutrient and mineral content. Fiber-rich yeast is known to improve defecation.

Constipation is an abdominal symptom typically caused by a lack of fiber, which reduces peristalsis in the intestines, thereby causing difficulties in regular bowel movements. Prolonged constipation results in the retention of stool in the intestines, which causes loss of water and hardening of the stool. Extended retention of the stool in the intestine also increases the number of putrefactive bacteria in the intestinal microflora. A decrease in the proportion of lactobacilli leads the generation of indole, skatole, ammonia, and hydrogen sulfide in the intestine, thereby causing unpleasant symptoms such as inflammation of intestinal tissues and abdominal bloating.

In addition to abdominal symptoms, constipation can result in psychological symptoms such as decreased appetite and depression due to abdominal bloating and adverse effects on sleep. Thus, regular defecation is important in improving quality of life (QOL). We previously reported that the intake of *Saccharomyces*

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Table 1 Inclusion and exclusion criteria of the study

Inclusion criteria
Participants aged 20–64 years
Exclusion criteria
Participants with known allergy or sensitivity to any foods
Participants who use any medecines or supplements
Participants with a history of severe disease including intestinal diseases

Table 2 Composition of the experimental (*Saccharomyces cerevisiae* NK-1) and placebo food samples per day*

	Experimental food	Placebo
Saccharomyces cerevisiae NK-1	5.40 g (10×10^9 counts)	−
Lactose	−	5.38 g
Caramel color	−	0.02 g
Total	5.40 g	5.40 g

*3 sticks/day

cerevisiae NK-1 increased the frequency of defecation in constipated individuals with low defecation frequency. This result may be attributed to the effects of increased dietary fiber intake on the intestinal microbiota and the higher number of Bifidobacteria in the intestines, which may prevent the generation of putrefactive gas. Therefore, this randomized, double-blind study was conducted to compare the effect of *Saccharomyces cerevisiae* NK-1 and placebo on the intestinal environment and sleep.

2. METHODS

2.1. Study Design

This was a randomized, double-blind, crossover study. In total, 20 healthy adults who met the selection criteria, as depicted in **Table 1**, were included. All participants provided informed consent. The study details were discussed in advance by the JCCR Ethics Committee and permission was then obtained. Furthermore, this research was registered in the UMIN Clinical Trials Registry prior to the study (UMIN-ID: UMIN000025190).

2.2. Randomization

The participants were assigned to either one of two groups using the stratified blocked randomization method (stratification factors: gender and age). An independent assignee who was not involved in the trial developed a computer program using a random number table and classified the participants into two groups. Blinding of participants and the performer was maintained.

2.3. Experimental and Control Foods

The experimental product was a stick containing *Saccharomyces cerevisiae* NK-1. The participants received sticks containing 10 billion *Saccharomyces cerevisiae* NK-1 three times a day. Meanwhile, lactose, instead of *Saccharomyces cerevisiae* NK-1, here was no visual difference in terms of color, odor, or size between the two products. The components of the two products are shown in **Table 2**.

2.3. Intestinal Flora

Stool samples were collected from the participants before and 4 weeks after ingestion. Then, the occupancy of fecal microbiota was assessed using the terminal restriction fragment length polymorphism method at Techno Suruga Laboratory Co., Ltd.

2.4. Obstructive Sleep Apnea Questionnaire

Sleep status was assessed using the OSA-MA. The OSA-MA was administered at the participant's home soon after he or she woke up every day and on each of the previous 2 days. The participants were instructed to bring the questionnaires with them to the hospital for submission.

2. 5. Statistical Analysis

The student's paired *t*-test was used to assess each factor in the gut flora and the OSA questionnaire. The significance level was set at 5%. The Fisher's exact probability test was utilized to evaluate adverse events. Statistical analysis was conducted using SAS 9.4 at Kansai University of Social Welfare Science.

3. RESULT

3.1. Subjects

In total, 40 participants, 20 for each testing period, completed the study (**Table 3**).

3.2. Intestinal Flora

The results of the intestinal flora study are shown in **Table 4**. After the ingestion of *Saccharomyces cerevisiae* NK-1, a significant difference was observed in the percentage of Bifidobacterium in the feces between the *Saccharomyces cerevisiae* NK-1 powder and placebo groups. This finding indicated an increase in the percentage of Bifidobacterium in the feces. Moreover, the *Saccharomyces cerevisiae* NK-1 powder group had a higher percentage of Clostridium subcluster XIVa in the feces than the placebo group. This result indicated a decrease in the percentage of Clostridium subcluster XIVa.

3.3. OSA Questionnaire

The OSA questionnaire and OSA-MA findings of the *Saccharomyces cerevisiae* NK-1 powder and placebo groups are shown in **Table 5**.

The mean and standard deviation of the first factor (sleepiness upon waking up) from pre-intake to 4 weeks after ingestion ranged from 11.20 ± 1.57 to 12.14 ± 2.22 (difference: 0.94 ± 1.78) in the *Saccharomyces cerevisiae* NK-1 powder group and from 11.43 ± 1.98 to 11.43 ± 1.76 (difference: -0.23 ± 0.97) in the placebo group. The difference in score was significantly higher in the *Saccharomyces cerevisiae* NK-1 powder group than in the placebo group (20 ± 1.76 [difference: -0.23 ± 0.97 points], $p = 0.0139$).

The mean and standard deviation of the fourth factor (drowsiness and fatigue upon waking up after the ingestion of exhaustion) ranged from 12.00 ± 1.82 to 13.14 ± 2.24 (difference: 1.14 ± 1.91) in the *Saccharomyces cerevisiae* NK-1 powder group and from 11.46 ± 1.72 to 11.00 ± 1.85 in the placebo group (37 ± 1.85 [difference: -0.09 ± 1.75). The difference in score was significantly higher in the *Saccharomyces cerevisiae* NK-1 intake group than the placebo group at after 4 weeks of intake and change ($p < 0.001$ after 4 weeks of intake, change $p = 0.0403$).

3.4. Adverse Event

Based on the blood test results, both groups did not present with any medical problems. Mild adverse events including bloating occurred. However, there was no significant difference between the two groups.

4. DISCUSSION

The participants ingested *Saccharomyces cerevisiae* NK-1 powder (10 billion/pack) three times a day, and its

Table 3　Profile of the participants

Item	
Number of individuals	20
Sex ratio（male-to-female）	16/4
Age（mean ± SD）	43.59 ± 8.72

Table 4　Changes in fecal microbiota（% occupancy）

Microbiota	Foods	Before			4 weeks			Δ 4 weeks		
		N	Mean ± SD	*p*-value*	N	Mean ± SD	*p*-value*	N	Mean ± SD	*p*-value*
Bifidobacterium	NK-1	20	10.69 ± 8.72	0.2020	20	13.51 ± 10.21	0.1326	20	2.83 ± 4.37	0.0210
	Placebo	20	12.16 ± 9.30		20	10.91 ± 9.41		20	− 1.25 ± 5.67	
Lactobacillales	NK-1	20	2.45 ± 3.06	0.3568	20	3.24 ± 3.05	0.5521	20	0.80 ± 3.75	0.1687
	Placebo	20	3.41 ± 4.38		20	2.70 ± 2.85		20	− 0.71 ± 3.43	
Bacteroides	NK-1	20	50.96 ± 11.86	0.4176	20	45.64 ± 11.69	0.7717	20	− 5.33 ± 7.80	0.5024
	Placebo	20	48.56 ± 13.68		20	45.04 ± 11.33		20	− 3.52 ± 7.89	
Prevotella	NK-1	20	2.86 ± 8.18	0.3627	20	2.86 ± 8.11	0.7613	20	0.00 ± 6.72	0.4166
	Placebo	20	5.33 ± 12.82		20	3.25 ± 10.01		20	− 2.08 ± 8.29	
Clostridium cluster IV	NK-1	20	7.64 ± 4.72	0.6749	20	7.30 ± 4.84	0.4789	20	− 0.33 ± 5.23	0.5039
	Placebo	20	7.25 ± 4.10		20	7.74 ± 4.49		20	0.49 ± 3.70	
Clostridium subcluster XIVa	NK-1	20	13.41 ± 4.92	0.6056	20	12.97 ± 4.77	0.0450	20	− 0.43 ± 4.04	0.0192
	Placebo	20	12.76 ± 6.60		20	16.93 ± 8.30		20	4.17 ± 6.50	
Clostridium cluster IX	NK-1	20	4.46 ± 5.01	0.1818	20	4.92 ± 8.25	0.4691	20	0.46 ± 6.23	0.6884
	Placebo	20	3.01 ± 3.11		20	3.95 ± 6.48		20	0.94 ± 6.12	
Clostridium cluster XI	NK-1	20	0.61 ± 1.62	0.5643	20	0.55 ± 1.19	0.4804	20	− 0.06 ± 1.20	0.9528
	Placebo	20	0.43 ± 0.68		20	0.39 ± 0.82		20	− 0.04 ± 0.70	
Clostridium cluster XVIII	NK-1	20	1.68 ± 2.05	0.5785	20	1.34 ± 1.38	0.0253	20	− 0.34 ± 1.07	0.1905
	Placebo	20	1.91 ± 2.28		20	2.64 ± 3.52		20	0.73 ± 3.30	
Others	NK-1	20	5.25 ± 2.96	0.9207	20	7.66 ± 4.83	0.3734	20	2.41 ± 6.18	0.4995
	Placebo	20	5.18 ± 2.17		20	6.45 ± 2.96		20	1.27 ± 2.56	

*Between *Saccharomyces cerevisiae* NK-1 and placebo using the student's paired *t*-test

Table 5 Result of the OSA questionnaire

OSA factors	Food	Before			4 weeks			Δ 4 weeks		
		N	Mean ± SD	*p*-value*	N	Mean ± SD	*p*-value*	N	Mean ± SD	*p*-value*
First factor: sleepiness upon waking	NK-1	20	11.20 ± 1.57	NS	20	12.14 ± 2.22	NS	20	0.94 ± 1.78	0.0139
	Placebo	20	11.43 ± 1.98		20	11.20 ± 1.76		20	− 0.23 ± 0.97	
Second factor: onset and maintenance of sleep	NK-1	20	11.46 ± 2.30	NS	20	12.33 ± 2.05	NS	20	0.87 ± 1.72	0.0201
	Placebo	20	11.76 ± 2.71		20	11.35 ± 2.55		20	− 0.42 ± 1.63	
Third factor: frequent dreaming	NK-1	20	15.63 ± 3.37	NS	20	16.71 ± 3.91	NS	20	1.08 ± 4.08	NS
	Placebo	20	16.05 ± 3.33		20	16.01 ± 3.55		20	− 0.03 ± 3.38	
Fourth factor: feeling refreshed	NK-1	20	12.00 ± 1.82	NS	20	13.14 ± 2.24	< 0.001	20	1.14 ± 1.91	0.0403
	Placebo	20	11.46 ± 1.72		20	11.37 ± 1.85		20	− 0.09 ± 1.75	
Fifth factor: sleep duration	NK-1	20	12.43 ± 1.92	NS	20	11.78 ± 2.22	NS	20	− 0.65 ± 2.84	NS
	Placebo	20	11.54 ± 2.23		20	11.59 ± 2.33		20	0.05 ± 1.89	

*Between *Saccharomyces cerevisiae* NK-1 and placebo using the student's paired *t*-test

efficacy in improving defecation was assessed. Yeast is a non-motile, eukaryotic, unicellular microorganism with cell walls. It has no photosynthetic capacity, and its nutrients are derived from the decomposition and absorption of external organic matter. Morphologically, it has an almost featureless circular or oval shape, and it multiplies by budding and division. These features facilitate its production in the intestinal tract and expression of a higher number of good bacteria in the intestine. Moreover, since its a eukaryote, it has a fiber-like effect and has been a source of food for Bifidobacteria. There are various theories about how yeast improves bowel movement via its fiber-like action. In general, fiber is a substance that is not easily broken down in the wild, even if left their, nor is broken down by animal substances. Fibers that are not digested by human digestive enzymes are referred to as non-digestible fibers. This type of fiber has low nutrient contents and has not received much attention. However, they are now known as enzymes that are highly involved in human health functions and that have an important role in maintaining health. There are two types of fiber: insoluble and soluble. Insoluble and water-soluble dietary fibers have extremely different physiological effects. Insoluble fiber has good water retention, and it contributes to increased stool volume and promotes peristalsis in the intestines. Soluble fiber has physiological functions in the small intestine, including inhibiting the production of digestive enzymes involved in the breakdown of carbohydrates, such as alpha-glucosidase, and cholesterol by reducing the reabsorption of bile acids. In particular, water-soluble fiber can be broken down by the enzymes of intestinal bacteria, but not human digestive enzymes. Moreover, it can be a source of energy. Hence, it is believed to be a source for Bifidobacteria and lactobacilli, and it can reduce the percentage of putrefactive bacteria[8]. In the OSA questionnaire, sleepiness and fatigue upon waking up significantly improved in the *Saccharomyces cerevisiae* NK-1 powder group compared with the placebo group. There are different yeast strains. Among them, sake yeast was associated with improvement in sleep electroencephalogram (EEG) results. Improvement in sleep quality among individuals who consume sake yeast is attributed to the activation of adenosine A2A receptors[9] and increased secretion of growth hormone[9]. The secretion of growth hormones increases with greater delta wave power in sleep EEG[10)-12)], and an increase in delta wave power value promotes deep sleep[9]. These results showed that *Saccharomyces cerevisiae* NK-1 may have a similar effect. However, this is a subjective evaluation only. Thus, further research should be conducted to determine improvements in sleep EEG results.

5. CONCLUSION

A randomized, placebo-controlled, double-blind study was conducted using *Saccharomyces cerevisiae* NK-1 powder. Results showed that the percentage of Bifidobacteria in the intestinal flora was significantly higher in the *Saccharomyces cerevisiae* NK-1 powder group than in the placebo group. In addition, based on the OSA questionnaire, there was improvement in sleepiness and fatigue upon waking up. These results indicated that *Saccharomyces cerevisiae* NK-1 powder was effective in improving the intestinal environment and sleep-related QOL.

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