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Original Article

프로바이오틱 세균인 *Weissilla cibaria* CMU의 구취 억제 효과

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Inhibitory effect of the probiotic bacteria, *Weissella cibaria* CMU on halitosis: a randomized placebo-controlled study

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Objectives: Previous studies have suggested that the lactic acid bacterium, *Weissella cibaria* CMU has beneficial effects on halitosis, but its precise effects have not been evaluated in human subjects. We evaluated the efficacy and safety of *W. cibaria* CMU for reducing halitosis in adults (20-70 years old) whose exhibited volatile sulfur compound (VSC) concentrations exceeded 0.015 ng/mL and who scored ≥2 points in a halitosis sensory evaluation test.
Methods: A total of 60 participants were assigned to an experimental group (treated with *W. cibaria* CMU) and a control group (placebo). In total, 58 out of 60 participants (experimental group, 29; control group, 29) were ultimately included in gas chromatography (OralChroma) analyses of VSC concentrations and halitosis sensory evaluation tests.
Results: We found that the VSC concentration decreased by 0.030±0.062 ng/ml in the experimental group after 8 weeks (P=0.0138) and increased by 0.005±0.124 ng/ml in the control group (P=0.8198). However, the difference between groups was not statistically significant (P>0.05). In a sensory evaluation test, a significantly lower score was obtained for the experimental group than for the control group.
Conclusions: Overall, VSC concentrations and sensory evaluation scores were lower in the experimental group than in the control group, but only the latter was statistically significant. Thus, we conclude that *W. cibaria* CMU is involved in the reduction of halitosis.

Key Words: Halitosis, Probiotics, *Weissella cibaria*

Introduction

Halitosis, i.e., oral malodor, is a condition in which the odor of the breath upon expiration through the mouth and the nose causes displeasure in others[1]. In the modern world, characterized by intricate and diverse social networks, halitosis is becoming a common problem with a substantial influence on social life[2].

Halitosis may be caused by factors inside the oral cavity, such as dental plaque or a faulty prosthesis, or by factors outside the oral cavity, such as aging, fasting, drinking, or smoking. Intraoral factors cause approximately 90% of cases of halitosis[3]. Halitosis due to intraoral factors is mediated by bacterial decomposition and volatile sulfur compounds (VSCs)[4,5]. VSCs are produced by the decomposition of sulfur-containing amino acids, peptides, and proteins by anaerobic gram-negative bacte-

ria, including *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Fusobacterium* spp. (*F. nucleatum* and *F. polymorphum*). VSCs produced by these bacteria include hydrogen sulfide, methyl mercaptan, and dimethyl sulfide. These anaerobic gram-negative bacteria mainly reside on the tongue[6,7].

To treat halitosis, improved oral care to reduce the adherence of bacteria to the tooth and the tongue as well as the removal of tongue plaque should be performed. In addition, mouthwash containing antimicrobial agents that regulate the relevant microflora, antibiotics, and artificial saliva can be used[8]. However, antibiotics have various side effects and can lead to the emergence of resistant bacteria or superinfection. Despite the short-term effects of mouthwash, the powerful chemical components have the potential to remove harmless resident bacteria in the oral cavity in addition to pathogens, and therefore their long-term use has various side effects. As an alternative approach, natural substances, such as the main component of green tea extract epigallocatechin gallate, extracts of brown algae belonging to the family Laminaria or probiotics, have received recent attention[9-11].

Probiotics are defined as living microorganisms, principally bacteria, that are safe for human consumption and, when ingested in sufficient quantities, have beneficial effects on human health, beyond basic nutrition[12]. The most widely used and studied probiotics belong the genera *Lactobacillus* and *Bifidobacterium*[13]. The beneficial effects of these probiotics on various conditions, including diarrhea, enteritis, ulcerative colitis, reduced immunity, and hyperlipidemia have been established. Numerous recent studies have focused on the effects of probiotics on diseases of oral cavity, including caries, periodontal diseases, and halitosis[14].

Weissella are lactic acid bacteria previously assigned to the genus Lactobacillus[15]. *Weissella cibaria* is a gram-positive rod-shaped bacterium that does not form spores, is catalase-negative, and mediates nonmotile heterolactic fermentation. The strain is able to produce dextran from sucrose and is normally isolated from fermented foods, humans, and animals[16]. *W. cibaria*, without producing a strong acid, releases soluble glucan and hydrogen peroxide to lower the increase in VSCs by the halitosis-inducing bacteria *F. nucleatum* and *P. gingivalis*[17,18]. Previous studies have reported that *W. cibaria* CMU shows excellent adherence to epithelial cells and to bacteria, such as *F. nucleatum*, via various proteins on the bacterial surface, such as S-layer protein. The strain reduces 97% of the hydrogen sulfide and methyl mercaptan produced by *F. nucleatum*[18,19]. Doh et al.[20] confirmed that in healthy beagles, the oral application of *W. cibaria* CMU at 2×10^7, 2×10^8, and 2×10^9 CFU/g (CFU, colony forming unit) diluted in 2 ml of PBS improves halitosis within 6 weeks.

The inhibitory effects of *W. cibaria* CMU on halitosis have been evaluated by in vitro experiments and simple in vivo tests using animal models. In the present study, the effects of the oral administration of *W. cibaria* CMU on adults with halitosis were determined.

Materials and Methods

1. Subjects

Adult men and women between 20 and 70 years old who scored ≥2 points in an organoleptic test and whose VSCs concentration was ≥0.15 ng/ml were recruited. All subjects agreed to participate in this study and provided written consent. A total of 60 participants were recruited (30 in the experimental group and 30 in the control group). Excluding two subjects who dropped out, 58 participants were included in analyses.

The exclusion criteria were as follows: individuals currently receiving treatment or with a history of a systemic disease that may induce halitosis; individuals with a severe dental disease; individuals with diabetes treated with insulin or an oral hypoglycemic agent; individuals who had taken antibiotics within 2 weeks of the screening process; individuals who smoke; individuals who complained of gastrointestinal symptoms, such as heartburn or indigestion; individuals who had taken a probiotic-based functional food within a week of the screening process or who continuously (more than four times a week) consumed fermented milk; individuals who were pregnant, breastfeeding, or planning a pregnancy during the study period; individuals who were sensitive or allergic to the food used in the clinical study.

2. Food and method of ingestion

W. cibaria CMU was obtained in the powder form at 1.0×10^8 CFU/bag (Oradentics, Co., Ltd., Seoul, Korea). To eliminate the effects of maltodextrin used as a filler in powder production, the control group was administered maltodextrin alone. For ingestion, the powder was placed in the mouth immediately before going to bed and left until the powder got melted, with participants taking care not to swallow the powder. After ingestion of the powder, the participants were prohibited from drinking water or eating food.

3. Study design

The clinical study was conducted for 8 weeks (5 visits) based

on a randomized, double-blind study design. The study was approved by the IRB of D University (IRB: 2017-02-015-006). Prior to the study (Baseline; Visit 1), the study purpose and procedure were explained to the participants in detail, and written consent was obtained. The name (initial), gender, date of birth, and age of the participants were recorded, in addition to whether they drink alcohol and whether they have halitosis.

An organoleptic test and measurements of VSCs concentration were performed on Visits 1, 3, 4, and 5. The organoleptic test involved an identical investigator who noted the smell with her nose at a distance of 10 cm for the evaluation according to the following scoring system: 0, no halitosis; 1, ambiguous odor; 2, mild or moderate halitosis; 3, an intermediate level of halitosis; 4, a high level of halitosis; 5, severe halitosis. The VSCs concentration was recorded once per visit using a portable sulfide monitor (OralChroma, CHM-2, FIS Inc., Hyogo, Japan). The measurements were obtained prior to eating breakfast and without any brushing or rinsing. A self-evaluation of improvement was performed; each participant evaluated the improvement in the level of halitosis at Visits 3, 4, and 5 in comparison with the level prior to the ingestion (Visit 1). The evaluation was scored as an overall prominent improvement (1, far better); overall improvement in symptoms (2, better); no difference between before and after ingestion (3, no change); overall aggravation in symptoms (4, worse); overall substantial aggravation (5, far worse).

4. Statistical analysis

Statistical analyses were implemented in IBM SPSS Statistics 24.0 (IBM Inc., Armonk, NY, USA). For demographic characteristics, descriptive statistics was used. For VSCs concentrations and self-evaluated improvement, paired t-tests were used. To evaluate for intergroup differences at each visit, an analysis of covariance and two independent sample *t*-tests were used. However, for data that violated normality, the Wilcoxon rank sum test was utilized as the nonparametric test. The level of significance was set to 0.05 for the two-tailed test.

Results

1. Comparison of demographic factors and other pre-ingestion characteristics of study subjects

The experimental group included 8 men (27.6%) and 21 women (72.4%), while the control group included 10 men (34.5%) and 19 women (65.5%); the differences were not significant. The average age of individuals in the experimental group was 22.48 years, while that in the control group was 21.79 years. 'No' individual in either the experimental group or the control group smoked, and 'less than 1 bottle/week' was the most frequent response regarding alcohol consumption, reported in 12 individuals in the experimental group (41.4%) and 14 individuals in the control group (48.3%). Most individuals rated their oral malodor as 'a little,' including 23 individuals in the experimental group (79.3%) and 27 individuals in the control group (93.1%). Additionally, 17 individuals in the experimental group (58.6%) and 15 individuals in the control group (51.7%) sensed their oral malodor. 'No' was the most frequent response to the question 'Have you heard from someone that you have oral malodor?' (25 individuals in the experimental group (86.2%) and 27 individuals in the control group (93.1%)). 'No' was the most frequent response to 'Have you witnessed an act of someone that expresses you have oral malodor?' (28 individuals in the experimental group (96.6%) and 19 individuals in the control group (100.0%)) (Table 1).

2. Changes in volatile sulfur compound concentrations

After week 8 of ingestion, the VSCs concentration, as determined using a portable sulfide monitor, was reduced by 0.030

Table 1. Demographic information and pre-ingestion characteristics

		Experimental group (N=29)	Control group (N=29)
Gender, N (%)	Male	8 (27.6)	10 (34.5)
	Female	21 (72.4)	19 (65.5)
Age (years)	Mean±SD	22.48±2.08	21.79±1.082
	Min, Max	20.00, 30.00	20.00, 24.00
Smoking, N (%)	No	29 (100.00)	29 (100.00)
	Yes	0 (0.00)	0 (0.00)
Alcohol drinking, N (%)	No	6 (20.7)	5 (17.2)
	Quit	2 (6.9)	5 (17.2)
	<1 bottle/week	12 (41.4)	14 (48.3)
	1-3 bottles/week	9 (31.0)	3 (10.3)
	≥4 bottles/week	0 (0.00)	2 (6.9)
Halitosis awareness, N (%)	1. How would you rate your oral malodor?		
	None	5 (17.2)	2 (2.9)
	A little	23 (79.3)	27 (93.1)
	A high level	1 (3.40	0 (0.00)
	A very high level	0 (0.00)	0 (0.00)
	2. Do you sense your oral malodor?		
	Yes	17 (58.6)	15 (51.7)
	No	12 (41.4)	14 (48.3)
	3. Have you heard from someone that you have oral malodor?		
	Yes	4 (13.8)	2 (2.9)
	No	25 (86.2)	27 (93.1)
	4. Have you witnessed an act of someone that expresses you have oral malodor?		
	Yes	1 (3.4)	0 (0.00)
	No	28 (96.6)	29 (100.0)

ng/ml in the experimental group but increased by 0.005 ng/ml in the control group. The difference between groups was not statistically significant (Table 2).

3. Changes in the hydrogen sulfide concentration

After week 8 of ingestion, the hydrogen sulfide concentration decreased by 0.019 ng/ml in the experimental group and by 0.011 ng/ml in the control group, but the difference between groups was not statistically significant (Table 3).

4. Changes in methyl mercaptan concentrations

After week 8 of ingestion, the methyl mercaptan concentration increased by 0.006 ng/ml in the experimental group and by 0.029 ng/ml in the control group, although no statistically

Table 2. Changes in volatile sulfur compound concentrations

	Experimental group (N=29)			Control group (N=29)			*P*-value	*P*-value§
	VSCs*	*P*-value†	Change‡	VSCs*	*P*-value†	Change‡		
Baseline (visit 1)	0.171±0.014			0.176±0.022			0.5966‖	
Week 2 (visit 3)	0.210±0.142	0.1524	0.039±0.142	0.192±0.136	0.5146	0.016±0.130	0.4938‖	0.467
Week 4 (visit 4)	0.157±0.120	0.5212	−0.014±0.118	0.190±0.203	0.6955	0.014±0.191	0.8459‖	0.7202
Week 8 (visit 5)	0.141±0.068	0.0138	−0.030±0.062	0.181±0.132	0.8198	0.005±0.124	0.5809‖	0.2609

Value: Mean±SD, unit : ng/ml.
*VSC concentration.
†Compared within groups; *P*-value by Paired t-test.
‡Change from baseline.
§Compared between groups; *P*-value by ANCOVA adjusted by baseline.
‖Compared between groups; *P*-value by Wilcoxon rank sum test.

Table 3. Changes in hydrogen sulfide concentrations

	Experimental group (N=29)			Control group (N=29)			*P*-value	*P*-value§
	VSCs*	*P*-value†	Change‡	VSCs*	*P*-value†	Change‡		
Baseline (visit 1)	0.053±0.024			0.059±0.040			0.8825‖	
Week 2 (visit 3)	0.086±0.084	0.0666	0.033±0.092	0.055±0.045	0.6992	−0.004±0.057	0.2341‖	0.0955
Week 4 (visit 4)	0.051±0.051	0.8542	−0.002±0.049	0.069±0.081	0.5612	0.10±0.091	0.7617‖	0.3622
Week 8 (visit 5)	0.034±0.030	0.0109	−0.019±0.038	0.048±0.046	0.3172	−0.011±0.056	0.4909¶	0.1913

Value: Mean±SD, units: ng/ml.
*VSCs concentration.
†Comparison within groups; *P*-value obtained by the paired *t*-test.
‡Change from baseline.
§Comparison between groups; *P*-value obtained by ANCOVA adjusted by baseline.
‖Comparison between groups; *P*-value obtained by the Wilcoxon rank sum test.
¶Comparison between groups; *P*-value obtained by the two-sample *t*-test.

Table 4. Changes in methyl mercaptan concentrations

	Experimental group (N=29)			Control group (N=29)			*P*-value	*P*-value§
	VSCs*	*P*-value†	Change‡	VSCs*	*P*-value†	Change‡		
Baseline (visit 1)	0.032±0.023			0.041±0.043			0.6857‖	
Week 2 (visit 3)	0.058±0.046	0.0025	0.026±0.042	0.059±0.053	0.1014	0.018±0.057	0.3668‖	0.7955
Week 4 (visit 4)	0.041±0.042	0.2300	0.009±0.039	0.056±0.087	0.2374	0.015±0.066	1.0000‖	0.8021
Week 8 (visit 5)	0.038±0.039	0.4702	0.006±0.045	0.070±0.079	0.0376	0.029±0.070	0.2498‖	0.1045

Value: Mean±SD, units: ng/ml.
*VSCs concentration.
†Comparison within groups; *P*-value obtained by the paired *t*-test.
‡Change from baseline.
§Comparison between groups; *P*-value obtained by ANCOVA adjusted by baseline.
‖Comparison between groups; *P*-value obtained by the Wilcoxon rank sum test.

Table 5. Changes in dimethyl sulfide concentrations

	Experimental group (N=29)			Control group (N=29)			*P*-value	*P*-value§
	VSCs*	*P*-value†	Change‡	VSCs*	*P*-value†	Change‡		
Baseline (visit 1)	0.086±0.031			0.076±0.049			0.3284‖	
Week 2 (visit 3)	0.066±0.072	0.1570	−0.020±0.074	0.078±0.072	0.9091	0.002±0.098	0.2867¶	0.6337
Week 4 (visit 4)	0.065±0.072	0.1697	−0.021±0.082	0.065±0.078	0.5857	−0.011±0.104	0.6407¶	0.8239
Week 8 (visit 5)	0.069±0.052	0.1028	−0.017±0.055	0.063±0.074	0.4326	−0.013±0.085	0.6464¶	0.8017

Value: Mean±SD, units: ng/ml.
*VSCs concentration.
†Comparison within groups; *P*-value obtained by the paired *t*-test.
‡change from baseline.
§Comparison between groups; *P*-value obtained by ANCOVA adjusted by baseline.
‖Comparison between groups; *P*-value obtained by the two-sample *t*-test.
¶Comparison between groups; *P*-value obtained by the Wilcoxon rank sum test.

Table 6. Evaluation of improvement by the subjects

		Experimental group (N=29)	Control group (N=29)	*P*-value*
Self-evaluation of improvement	Visit 3	2.79±0.56	2.93±0.26	0.1889
	Visit 4	2.52±0.69	2.86±0.44	0.0301
	Visit 5	2.38±0.73	2.86±0.44	0.0038

Value: Mean±SD.
*Comparison between groups; *P*-value determined by the Wilcoxon rank sum test.

significant intergroup difference was observed (Table 4).

5. Changes in dimethyl sulfide concentrations

After week 8 of ingestion, the dimethyl sulfide concentration decreased by 0.017 ng/ml in the experimental group and by 0.013 ng/ml in the control group, although no statistically significant intergroup difference was observed (Table 5).

6. Self-evaluation of improvement

After week 2 of ingestion, the evaluation scores were 2.79 in the experimental group and 2.93 in the control group, with no statistically significant intergroup difference. After week 4 of ingestion, the evaluation scores were 2.52 in the experimental group and 2.86 in the control group, and the difference between groups was significant (*P*=0.0301). After week 8 of ingestion, the evaluation scores were 2.38 in the experimental group and 2.86 in the control group, and the difference between groups was significant (*P*=0.0038) (Table 6).

Discussion

Halitosis refers to the malodor released through the oral cavity. The condition arises in the oral cavity or nearby organs due to physiological, pathological, or psychological factors. Approximately 80-90% of halitosis originates in the oral cavity[21]. Its primary causes are bacterial byproducts and metabolic products, known as VSCs. Most halitosis is caused by microorganisms on the tongue, particularly the gram-negative bacteria *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *F. nucleatum*, and *F. polymorphum*[22,23].

According to Bosy (1997), the prevalence of halitosis in adults is 20-50%, and approximately 25% of cases are severe, affecting social life[24]. In addition, Yoon and Yoon (2008) reported that the frequency of self-awareness of halitosis is 79.3%, and 66.9% of patients respond positively to treatment, while 7.3% show high demand for treatment[25]. The most effective methods to reduce halitosis are aimed at growth inhibition or the removal of anaerobic gram-negative bacteria on the tongue. Training for brushing teeth or cleaning the tongue, the use of mouthwash containing antimicrobial agents, the administration of antibiotics, and the use of artificial saliva for reducing halitosis caused by xerostomia have all been applied[8]. However, antimicrobial agents are limited by side effects and recent studies are focused on the use of probiotics for reducing halitosis.

Suzuki et al.[26] showed that Enterococcus faecium could reduce halitosis by inhibiting *P. gingivalis* growth and neutralizing methyl mercaptan, and Lee and Baek[27] showed that Streptococcus thermophilus reduces halitosis by inhibiting *P. gingivalis* growth and neutralizing sulfur compounds directly or via metabolic products. Kim and Kim[28] confirmed that *L. salivarius* and *L. delbrueckii* subsp. lactis inhibit VSCs production by anaerobic bacteria via the release of hydrogen peroxide. Burton et al.[29] reported that *S. salivarius* prevents the colonization of bacteria that produce VSCs, thereby inhibiting VSCs production; however, the precise bacteria responsible for observed reductions in halitosis were not determined. *W. cibaria* CMU reduces the

production of VSCs by inhibiting the growth of *F. nucleatum* via the production of hydrogen peroxide, with excellent conglutination with *F. nucleatum* and adherence to epithelial cells[1,17,18].

We evaluated 58 healthy adult men and women who ingested *W. cibaria* for 8 weeks by analyses of the VSCs concentration, organoleptic properties, tongue plaque index, and a self-evaluation of improvement. The VSCs concentration decreased in the experimental group after week 4, although the difference between groups was not significant. In the organoleptic test, after week 2, both the experimental group and the control group displayed reductions in VSCs concentrations; while a greater reduction was detected in the experimental group than in the control group, the difference between groups was not significant. The tongue plaque index increased in both the experimental group and the control group; the increase was less substantial in the experimental group than in the control group, but there was no statistically significant difference between groups. The self-evaluation of improvement differed significantly between groups at week 4 and week 8 of ingestion.

We observed a reduction in halitosis in the experimental group, but the level was not significantly different from that in the control group. Nevertheless, this reduction in halitosis was supported by the self-evaluation by subjects, which showed a significant difference between groups.

This clinical study was conducted over an 8-week period. It was therefore difficult to maintain identical environmental conditions; an influence of such differences in conditions on the results cannot be ruled out. Moreover, the 8-week period may not have been sufficiently long for monitoring changes of halitosis. Further clinical studies with longer test periods and different food products and methods for ingestion with improved statistical power are needed.

This clinical study was designed to evaluate the efficacy and safety of W. civaria CMU ingestion for the reduction of halitosis in adult men and women. Our results supported the following major conclusions. After week 8, the VSCs concentration in the experimental group was reduced by 0.030±0.062 ng/ml and the VSCs concentration in the control group increased by 0.005 ±0.124 ng/ml, but the different between groups was not significant. The levels of hydrogen sulfide, methyl mercaptan, and dimethyl sulfide decreased in the experimental group but increased in the control group; however, the differences between groups were not statistically significant. A self-evaluation of improvement indicated that the level of halitosis was significantly lower in the experimental group than in the control group.

These results support for beneficial effects of *W. cibaria* CMU on halitosis, based on the improvement in self-evaluations, despite a lack of significance in measurements obtained using a device.

Conclusions

This anatomical application study involved adult males and females between the ages of 20 to 70 with an OLT (organoleptic test) score of 2 or above and a VSCs (volatile sulfur compounds) concentration in the oral cavity of 0.15 ng/ml or more. The study was conducted to evaluate the efficacy and safety of consumption of *W. cibaria* CMU in reducing halitosis compared to the control. A total of 60 subjects (30 in the experimental group and 30 in the control group) were randomly assigned to each group; then, 29 final subjects in the experimental group were given *W. cibaria* CMU to consume and 29 final subjects in the final control group were given the control food. After consumption, the following conclusions were obtained by measuring the mechanical halitosis concentration using a halitosis meter and evaluating the degree of improvement by the subjects themselves.

1. Analysis of the volatile sulfur compounds concentration through the halitosis meter showed that 8 weeks after consumption, the experimental group showed a decrease of 0.030±0.062 ng/ml and the control group showed an increase of 0.005± 0.124 ng/ml, yet there was no statistically significant difference between groups ($P>0.05$).

2. Hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$) were also shown to be decreased in the experimental group and increased in the control group, but there was no statistically significant difference between the groups ($P>0.05$).

3. In the evaluation of the degree of improvement by the subjects themselves, the experimental group was shown to be lower than the control group, and there was a statistically significant difference.

In this anatomical application study, the experimental group did show a decrease in halitosis, but there was no statistically significant difference in the measurements by the halitosis meter despite the statistically significant decrease in halitosis from self-evaluation by the subjects. Thus, the study was able to partially confirm the halitosis reduction effect of *W. cibaria* CMU.

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