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Antifungal Activity of Probiotic Lactobacilli Culture Supernatant against *Candida albicans*; the Possibility of Suppression Factor Other than Organic Acids

Yukako KOJIMA, Tomoko OHSHIMA*, Tomomi KAWAI*,
Chaminda Jayampath SENEVIRATNE** and Nobuko MAEDA*

Department of Oral Microbiology, Doctor of Philosophy in Dental Science,
Graduate School of Dental Medicine, Tsurumi University
(Chief: Prof. Nobuko MAEDA)
* Department of Oral Microbiology, School of Dental Medicine, Tsurumi University
(Chief: Prof. Nobuko MAEDA)
** Oral Sciences, Faculty of Dentistry, National University of Singapore, *Singapore*
(Chief: Chaminda Jayampath SENEVIRATNE)
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Abstract: Objectives: The frequent use of anti-fungal agents against various forms of *Candida* infections, including oral candidiasis has resulted in emergence of drug-resistant strains. Probiotic lactobacilli strains that exhibit potent antifungal activity have been proposed as an alternative therapy. However, some have raised concern over application of probiotics to the oral cavity due to the acidic nature of the products secreted by probiotic lactobacilli strains which may contribute to the tooth-decay or hypersensitivity of teeth. In this study, we examined the antifungal properties of lactobacilli culture supernatant (LCS) against *Candida albicans*, then compared to LCS-treated or lactic acid-treated *C. albicans* using transcriptomics to consider the action of antifungal substance.

Methods: The antifungal activities of LCS of five candidates, *Lactobacillus fermentum* 103 (LF103), *L. plantarum* 108 (LP108), *L. paracasei* 112 (LPa112), *Lactobacillus casei* 120 (LC120) and *L. plantarum* 122 (LP122), which were selected in our previous screening, were examined against yeast and hyphal forms of *C. albicans*. Acidity of culture supernatant of five strains and the type strain of *L. casei* (ATCC393) was measured as pH of LCS. The transcriptome analysis of *C. albicans* treated with LCS or 0.46% lactic acid (1/2 MIC) was carried out by RNA sequencing. Subsequently, quantitative real time PCR (qRT-PCR) was performed to confirm the variations in gene expression.

Results and discussion: Three *Lactobacillus* strains, LF103, LP108 and LP122, demonstrated highest antifungal activity then they were selected as the final candidates. Acidity of LCS of strains with higher antifungal activity as well as weak antifungal activity was approximately pH 4.0. When compared to the influence of LCS and lactic acid

Correspondence to: Tomoko OHSHIMA
Department of Oral Microbiology, School of Dental Medicine, Tsurumi University
2–1–3 Tsurumi, Tsurumi-ku, Yokohama-shi, Kanagawa 230–8501, Japan

for *C. albicans*, the former decreased not only the cell number but also the transition rate to hypha. The result of transcriptome analysis by RNA sequencing and subsequent qRT-PCR, indicated that the specific genes expression of *C. albicans* were down-regulated by LCS, those profiles were different from lactic acid-treated *C. albicans*.

Conclusions: All LCS of the three candidate probiotic strains showed antifungal effect against both yeast and hyphal forms of *C. albicans*. Comparative transcriptomic analysis of *C. albicans* treated with LCS revealed, the specific gene expression was different from those treated with lactic acid, implying the presence of other active ingredients. Further studies are required to demystify the mechanism of antifungal action exerted by these active ingredients in the LCS against *C. albicans*. If proven feasible, probiotic-based antifungal therapy can bring enormous benefits to the patients suffering from *Candida* infections, particularly at the mucosal surfaces such as oral candidiasis.

Key words：biogenics, *Candida albicans*, Lactobacilli, probiotics

1. Introduction

There has been a dramatic rise in the fungal infections over the past decade due to the increased number of elderly population and immnocompromised patients[1, 2]. *Candida albicans*, the major fungal pathogen of humans, which causes mucosal and systemic candidiasis has been a major concern among these compromised host populations[3, 4]. Moreover, frequent use of antifungal agents for candidiasis has increased the risks of emergence of drug-resistant strains[5].

Probiotics is well known for their beneficial effects in promoting the health of the human gastrointestinal tract[6]. The term "probiotics" was coined by W. Kollath in the 1950s and subsequently Lilly and Stillwell employed the term in 1965[7]. Recently, probiotics have been shown to bring benefits beyond promoting intestinal health, such as for bowel disease[8] and food allergy[9]. Therefore, Salminen et al. defined the term probiotic as "a viable microbial food supplement which beneficially influences the health of the host"[10]. Food and Agriculture Organization of the United Nations / World Health Organization define probiotics as "live microorganisms when administered in adequate amounts confer a health benefit on the host"[11].

The health benefits of oral probiotics have been investigated by several clinical trials[12, 13]. However, some have raised concern over application of probiotics to the oral cavity due the acidic nature of the products secreted by probiotic lactobacilli strains which may contribute to the tooth-decay or hypersensitivity of teeth[14]. Therefore, recent years attention has been focused on "biogenics" derived from lactic acid bacterial metabolites other than organic acids such as lactic and acetic acids[15]. Biogenics is defined as 'food ingredients which beneficially affect the host by directly immunostimulating or suppressing mutagenesiss, tumorigenesis, hypercholesterolemia or intestinel putrefyion'[16]. However, little is known about effective substance and the mechanism of biogenics action on oral health, although the activities of probiotics have been studied extensively[17]. In our previous study aiming at the application of probiotics to the treatment of oral diseases, we determined the antimicrobial activities against three oral diseases: dental caries (*Streptococcus mutans*), periodontal disease (*Porphyromonas gingivalis*), candidiasis (*Candida albicans*) by using 40 lactobacilli strains, and selected five strains among 40 strains as the candidate strains for the oral probiotic bacteria[15]. All of the strains were derived from human oral cavity. As the next step, it is imperative to determine the nature of the antifungal substance present in these oral probiotic bacteria. Hence, in the present study, we investigated the antifungal activity of cell-free lactobacilli culture supernatant (LCS) on yeast and hyphal forms of *C. albicans*. Moreover, we also aimed to examine the transcriptomics expression of *C. albicans* when treated with LCS as compared to lactic acid.

2. Material and Methods

2.1. Strains and culture conditions

C. albicans (ATCC18804) was stored at −80°C at the Department of Oral Microbiology, Tsurumi University

School of Dental Medicine until used. *C. albicans* was cultured on Sabouraud dextrose agar (Nissui, Tokyo, Japan) at 30°C for 2 days under aerobic conditions, then one inoculation loop-full of the colony was subcultured in Difco™ Lactobacilli MRS Broth (Becton Dickinson and company Sparks, MD, USA), and incubated under aerobic conditions at 30°C overnight (14–16 hours) with constant shaking to obtain a late logarithmic growth phase culture. The turbidity was 2.4 and the OD was measured at 620 nm (Ultrospec 4000 UV/Visible spectrophotometer, Pharmacia Biotech Inc., NJ, USA).

The candidates five strains of *Lactobacilli*, *L. fermentum* 103 (LF103), *L. plantarum* 108 (LP108), *L. paracasei* 112 (LPa112), *Lactobacillus casei* 120 (LC120), *L. plantarum* 122 (LP122), and the type strain of *Lactobacillus casei* (ATCC393), were stored at −80°C at the same Department[18]. They were cultured under anaerobic conditions (N_2: 80%, CO_2: 10%, H_2: 10%) at 37°C for two days and subcultured in MRS Broth at 37°C under anaerobic conditions for two days. Each culture suspension of lactobacilli was centrifuged (Avanti™ HP-20, Beckman Coulter, Inc., CA, USA) at 7000 rpm at 4°C for 20 min and subsequently filtered through a 0.22 μm pore size membrane (Millex®-GV Filter Unit, Low Protein Binding Durapore® (PVDF) Membrane, Co. Cork, IRELAND) to collect the cell-free supernatant of lactobacilli culture (LCS).

2.2. *C. albicans* growth inhibitory tests with LCS by CFU and turbidity assay

Ten microliters of culture suspension of the yeast form of *C. albicans* ($1\times10^4-10^5$ CFU/10 μl) was added to 100 μl of serial dilutions of lactic acid or each LCS and 100 μl of MRS as the control, and incubated under aerobic conditions in 96 well plates at 30°C overnight. MIC (minimum inhibitory concentration) of lactic acid (DL-Lactic Acid 92%, Wako Pure Chemical Industries, Osaka, Japan) was determined by turbidity with the naked eye using standard assay of 1/2 serial dilution method in MRS broth. Antifungal activity of LCS was determined by the Colony Forming Units (CFU) assay.

For hyphal inhibition assay, 10 μl of the yeast form of *C. albicans* ($1\times10^4-10^5$ CFU/10 μl) suspension was added to 100 μl of each LCS and 100 μl of double concentrated RPMI 1640 broth® (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (Gibco™, Invitrogen Corporation, Grand Island, N.Y., USA) to stimulate hyphal formation. *C. albicans* was incubated under aerobic conditions in 96 well plates at 37°C overnight with constant shaking. The initial stage of hypha formation (three hours) was observed with microscope and hyphal transition rate was calcurated. The growth amount of *C. albicans* was determined by measuring the turbidity (OD measured at 620 nm).

2.3. RNA-seq analysis of *Candida albicans* treated with LCS and lactic acid

One milliliter of *C. albicans* ($1\times10^5-10^6$ CFU/ml) suspension was added into each 5 ml of LCS or MRS with 0.46% lactic acid (as 1/2 MIC), and 5 ml of double concentrated RPMI 1640 broth® supplemented with 10% fetal bovine serum (total 11 ml). The cultures were incubated aerobically at 37°C for three hours with constant shaking, continually the *Candida* cell pellets were collected by centrifugation (3000 g for 5 min). A pretreatment of *C. albicans* was carried out with 0.1% β-mercapto ethanol and 25 U/ml lyticase (Sigma, St. Louis, USA) in 1 M sorbitol and 0.1 M EDTA (pH 7.0) at 30°C for 10–30 minutes. Total RNA was extracted using the RNeasy® Mini Kit from QIAGEN along with DNase digestion as described in the manufacturer's manual. Qubit® 3.0 Fluorometer and Qubit® assays kit (Life Technologies, Singapore) were used to determine the concentration of the isolated RNA.

For RNA-sequencing analysis, 2 μg of total RNA was used to isolate the polyA fraction (mRNA), followed by its fragmentation. Then double-stranded (ds) cDNA was reverse transcribed from fragmented mRNA. The ds cDNA fragments were processed for adaptor ligation, size selection (for 200 bp inserts) and amplification to generate strand-specific cDNA libraries. Prepared libraries were subjected to sequencing for the Illumina HiSeq platform® (Illumina, San Diego, CA, USA). Aligned data was calculated by Samtools® (ver. 0.1.19) for statistical results of mapping, normalized by edge® (ver. 3.16.1). The *C. albicans* samples treated with LCS and lactic acid-containing medium samples were compared to extract genes with large expression variation (P value < 0.01). Furthermore, genes of high scores were selected to prepare a heat map.

2.4. Quantitative real time PCR (qRT-PCR) for confirmation of gene expression

For each qRT-PCR sample, 5 μg of total RNA was

Table 1 qRT-PCR primer

Gene	Sequence (5'- > 3')	
	Forward primer Template	Reverse primer Template
PMA1 (House keeping)	TTGAAGATGACCACCCAATCC	GAAACCTCTGGAAGCAAATTCG
OP4	TGCCGAAGGTGAAGACTTGT	TGTTGGTAAGGGAGCTGGAA
C2_10070W_A	GTTATGAATTAATCACCACTGCT	AGCTGGGAAAAACGGAGTAG
C5_03770C_A	ACTCTGGTGGTAAACACGCTA	CAAAAGTGGATGTTGGGTAAGGA
AOX1	AGATGGGAAATGACCGAGGG	CCAGGAACACCAGCTATGGA
HSP31	GCTTGGGGTTTGAGTGAAGG	AAATGGTTGGCATGAGACCG
FDH1	TGGTTCTTTACGCTGGTGGT	CAGAAGTTGGTGCAGGTTCC
RBT1	GGGTTTGCTTTTGGGTTTGC	TGGAGATGGGGTGTCACATT
TPO3	TGTTGGTCCTTTGCTTTGGG	AAGGCACAGGGGATGTTGAA

Table 2 The antifungal activity of candidate strains and type strain -LCS against *C. albicans* under the condition of yeast type or hyphal type growth

	Yeast growth condition		Hyphal growth condition		Hyphal transition rate	
	log CFU mean	(SD)	OD (620 nm) mean	(SD)	mean (%)	(SD)
LF103	0.00	(0.00)	0.20	(0.09)	1.8	(1.50)
LP108	0.00	(0.00)	0.17	(0.10)	2.1	(2.03)
LPa112	1.67	(1.97)	0.22	(0.03)	ND	ND
LC120	0.27	(0.72)	0.26	(0.05)	ND	ND
LP122	0.00	(0.00)	0.25	(0.05)	1.8	(1.67)
L. casei (ATCC393)	1.98	(1.95)	ND	ND	ND	ND
Control (MRS)	4.00	(0.00)	0.69	(0.13)	15.7	(10.73)
Lactic acid 0.46%	ND	ND	0.74	(0.07)	30.5	(11.34)

ND: Not done

reverse transcribed into cDNA using GoTaq® 2-Step RT-PCR System (Promega, Singapore). Primers amplifying the 14 target genes and PMA1 (housekeeping gene) used for qRT-PCR are listed in the Table 1. They were constructed using the Primer-BLAST, and given dry checks about hairpin, self dimer and cross dimer by the NetPrimer (http://www.premirebiosoft.com/netprimer/index.html). Diluted cDNA, gene specific forward and reverse primers and qPCR master mix involved SYBR Green were mixed in reaction mixture and qPCR was performed using the CFX Connect Real-Time PCR System (BioRad, USA). This was done under adequate thermal cycling conditions (Holding stage 95°C for 2 min, Cycling stage 95°C for 15 s, 60°C for 1 min for 40 cycles and Melting curve stage 60°C to 95°C). The resultant C_t values of the target genes of interest were normalized to the C_t values of the respective PMA1 as previously described[19]. Results were analyzed using the $2^{-\Delta\Delta Ct}$ relative expression method to calculate the fold changes[20].

3. Results

3.1. *C. albicans* culture tests with LCS by turbidity and CFU assay

Five strains of probiotic candidates (LF103, LP108, LPa112, LC120, LP122) were tested for antifungal activities against yeast and hyphal forms of *C. albicans*. A clear antifungal effect was observed in all of five strains against hyphal growth of *C. albicans* (Table 2). However there was no significant difference among the strains. On the other hand, the antifungal activities against yeast were strongly exhibited as fungicidal effect in LF103, LP108 and LP122, because any grow colony

was observed (Table 2). Their inhibition activity against hyphal transition of *C. albicans* was confirmed (Table 2).

3.2. MIC of lactic acid against *C. albicans* and pH of LCS

MIC of lactic acid for yeast form of *C. albicans* was 0.92% according to the standard assay of 1/2 serial dilution method. LCS of all strains with or without RPMI1640 medium showed almost the same pH level around 4.0 (Table 3).

Table 3 pH of LCS

	LCS	LCS + RPMI1640
*L. fermentum*103	3.8	4.0
*L. plantarum*108	3.8	3.9
*L. plantarum*122	3.8	3.9
L. casei ATCC393	4.1	–

3.3. Comparative transcriptomic analysis of *C. albicans* treated with LCS and Lactic acid

C. albicans was cultured supplemented with LCS or 0.46% lactic acid (1/2 MIC) for 3 hours and was observed with a microscope to calculate the ratio of yeast and hypha type cells (Fig. 1 (a), (b)). The medium containing 0.46% lactic acid of 1/2 MIC was set as maximum concentration at which *C. albicans* was not killed. This concentration was not only for adjustment of the pH but also for the control of lactic acid contained in LCS. When compared the influence of LCS and lactic acid for *C. albicans*, the former decreased not only the cell number but also the transition rate to hypha (Fig. 1 (a)1-3, Table 2). The hyphal transition rate in lactic acid was almost eight fold higher than that in LCS. The transcriptomic profile of *C. albicans* was determined by RNA sequencing analysis, and the differential expression

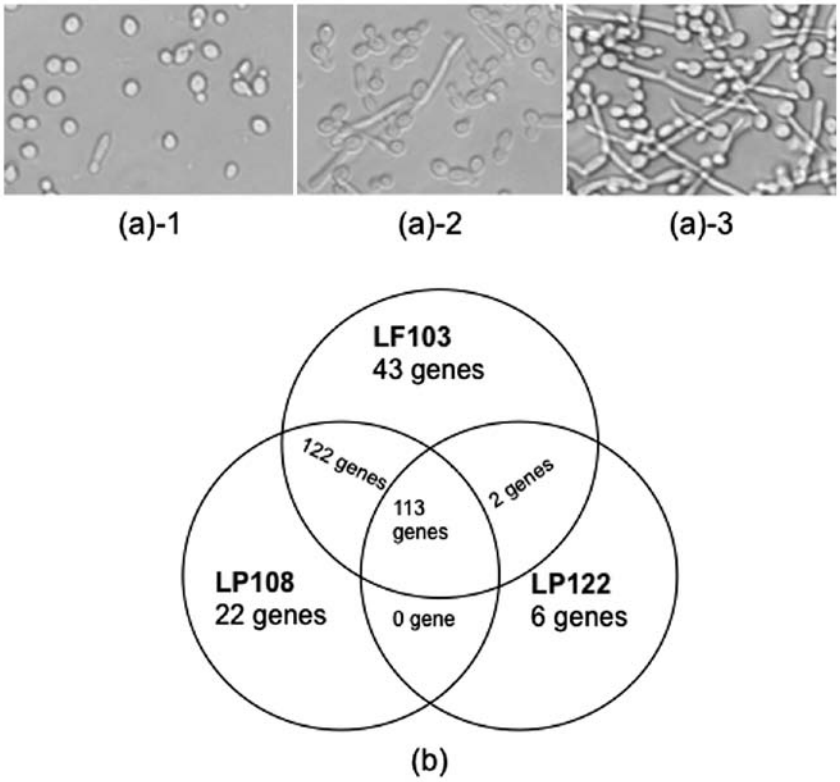

Fig. 1 Compared to variety genes in LCS by mRNA sequencing
(a) Microscopic observation after culturing for three hours with LCS or Lactic acid. The transition rate was showed in Table 1. (Magnification: 400×)
(a)-1; LP108, (a)-2; Control of Lactic acid 0.46%, (a)-3; Control of MRS (without LCS or Lactic acid)
(b) Down-regulate genes: 113 genes of *C. albicans* were commonly affected by LCS of three candidate strains (LF103, LP108, LP122).

with the presence of LCS of three probiotic candidates and 0.46% lactic acid (1/2 MIC) were extracted. A total of 113 common genes were down-regulated in all three lactobacilli strains compared to lactic acid. When considered LF103 and LP108, 122 genes were down-regulated in common (Fig. 1 (b), $p < 0.01$). On the other hand, the comparison of LP122 and LF103 or LP108 showed none or small number of down-regulated genes. The heat map shows the particularly large 13 down-regulated genes in *C. albicans* (Table 4) and no significant up-regulated gene was found.

3.4. Gene expression analysis by qRT-PCR

From the results of RNA sequencing, we selected particularly significant down-regulated genes in response to LCS whose functions were known or predicted, and primers for qRT-PCR were designed as shown in Table 1. The expression profile was confirmed by qRT-PCR for eight of down-regulated genes. Seven genes (C2_10070W_A, C5_03770C_A, AOX1, RBT1, HSP31, FDH1, OP4) among eight were also down-regulated by qRT-PCR (Fig. 2). Five genes (C2_10070W_A, C5_03770C_A, AOX1, RBT1, OP4) among seven were significantly down-regulated when compared with the samples treated with 0.46% lactic acid (CTL) (Fig. 2, $p < 0.05$).

4. Discussion

C. albicans is well known as a biphasic growth fungus that takes two different forms such as yeast and hypha[21]. *C. albicans* takes yeast form when exists on the mucous membrane or on skin surface. However, when invades into the tissues, *C. albicans* often takes hypha form which is more pathogenic than the yeast form[22]. *C. albicans* biofilm consists of a mixed state of yeast and hyphal forms[23]. In this study, we determined the antifungal activity of LCS against the yeast form as well as the hyphal forms of *C. albicans*.

We examined the antifungal activities of LCS produced by five probiotic candidate strains, which already were selected in our previous study[15]. The LCS of five strains showed almost equivalent antifungal activities against hypha production of *C. albicans*. However only LF103, LP108, and LP122 showed strong antifungal activities against yeast form (Table 2). Therefore, these three

Table 4 Comparison of *C. albicans* expression genes between LCS and pure Lactic acid treatment; down-regulated expression in *C. albicans*

gene name		LF103 logFC	LP108 logFC	LP122 logFC	Known or predicted gene function
C4_07020C_A	CAWG_03137	−12.74495242	−12.73107071	−11.95590228	Ortholog of *C. dubliniensis CD36*
OP4	CAWG_00140	−10.34048652	−10.09474851	−8.984861593	Ala- Leu- and Ser-rich protein
C2_10070W_A	CAWG_06078	−8.944462984	−8.87152917	−7.364475677	Predicted dehydrogenase
C1_04010C_A	CAWG_00992	−8.148080624	−8.179860884	−6.954783452	Protein with a NADP-dependent oxidoreductase domain
C5_03770C_A	CAWG_04749	−7.500265523	−7.311268516	−5.928199956	Protein similar to Candida boidinii formate dehydrogenase
C7_03560W_A	CAWG_05687	−6.537020372	−6.655579802	−5.411852635	Protein of unknown function
AOX1	CAWG_00513	−6.403088006	−6.44324353	−4.655450237	Alternative oxidase
HSP31	CAWG_01176	−6.385698247	−6.242183747	−6.043872094	Putative 30 kda heat shock protein
FDH1	CAWG_01849	−6.303691705	−6.108953026	−4.979999105	Formate dehydrogenase
RBT1	CAWG_03456	−5.680057175	−6.064578849	−5.527149542	Cell wall protein with similarity to Hwp1
TPO3	CAWG_00547	−5.589656238	−5.4705869	−5.082073851	Putative polyamine transporter
C2_02550C_A	CAWG_04023	−5.418422938	−5.38158395	−4.890709391	Protein of unknown function
C1_11320C_A	CAWG_00304	−5.298450577	−5.259007249	−3.940599087	Protein of unknown function

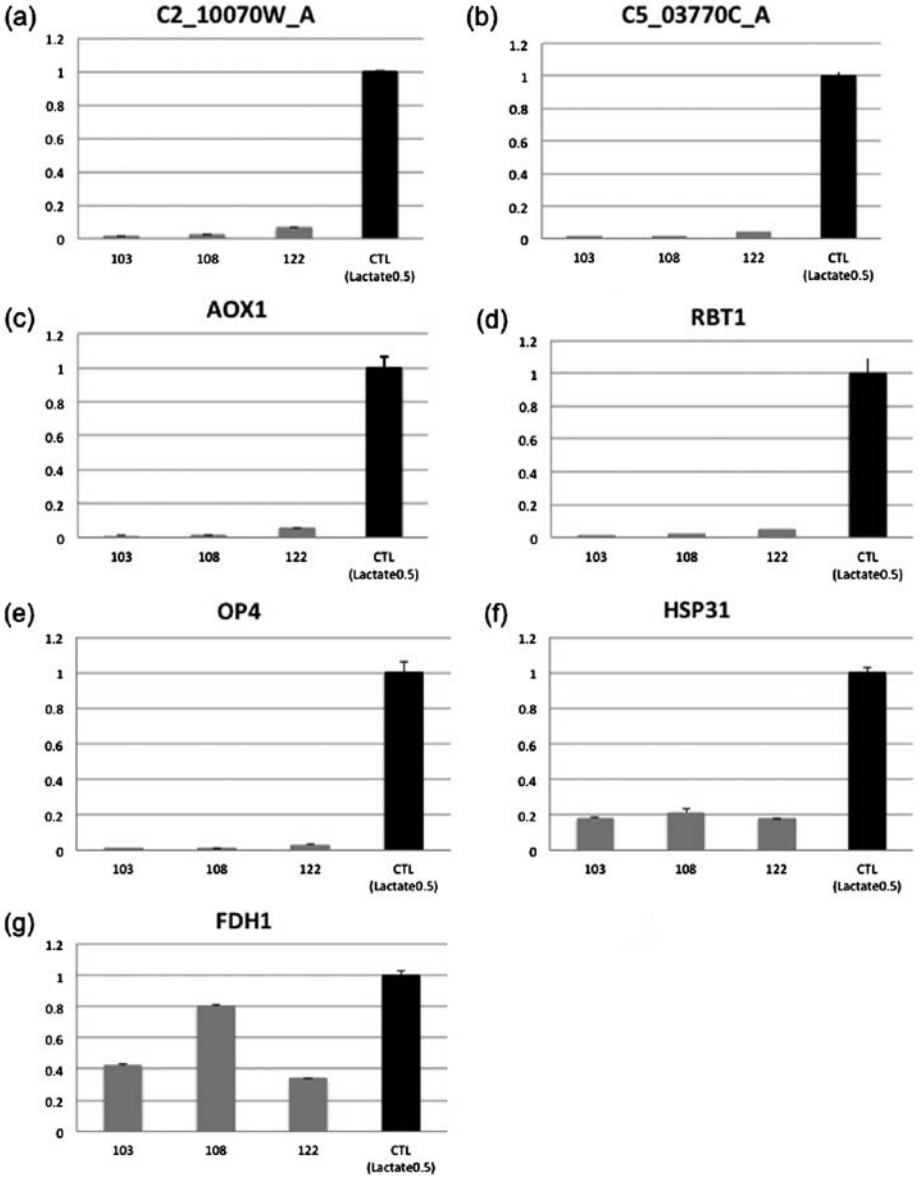

Fig. 2 Down-regulated expression of variety genes determined by qRT-PCR
CTL is treat with pure lactic acid. Down-regulation state of seven genes, C2_10070W_A (a), C5_3770C_A (b), AOX1 (c), RBT1 (d), OP4 (e), HSP31 (f) and FDH1 (g) were confirmed by qRT-PCR. The vertical axis of the graph shows $2^{-\Delta\Delta Ct}$ and the horizontal axis shows LCS or CTL. Significant decrease was shown in C2_10070W_A, C5_3770C_A, AOX1, RBT1 and OP4 (Kruskal-Wallis $p < 0.05$).

strains were regarded as the final candidate strains for probiotics or biogenics.

It is known that the pH of the culture influences hypha production of *C. albicans*. It has been shown that lower pH under 5.4 inhibits the transition from yeast to hyphae[24]. All tested LCS had a pH lower than pH 5.4 (Table 3), hence explains the yeast to hyphal inhibitory activity. However, products with low pH increased the risk of dental caries and hypersensitivity of tooth[25]. In order to find the antifungal activities other than acids (low pH), each LCS of aforementioned lactobacilli strains was fractionated by ethanol or acetone precipitation method, but the active fraction could not be separated from the organic acid (date not shown). Then we

performed the transcriptome analysis using RNA sequencing. The results showed the different fluctuating genes in the samples treated with three LCS when compared with the lactic acid treatment. There were eight significantly down-regulated genes although none was up-regulated. The qRT-PCR analysis corroborated the results of seven down-regulated genes. The functions of those seven genes were already suggested or predicted by previous studies. Both of C2_10070W_A and C5_03770C_A are related to the pathogenicity of *C. albicans*: the former was up-regulated in the infection model of oral candidiasis and the later was a protein similar to formate dehydrogenase which was required to express pathogenicity (*Candida* Genome Database; http://www.candidagenome.org/). RBT1 was similar to Hwp1, a cell wall protein gene, known as the pathogenic factor of *C. albicans*[26]. AOX1 is an alternative oxidase involved in the cyanide-resistant respiratory pathway in *C. albicans* to affect cell survival by inhibition[27]. Down-regulating OP4 could suppress the hyphal production of *C. albicans*[28]. HSP31 was a putative stress releasing protein[29], and FDH1 was format dehydrogenase[30]. Among seven genes, four genes of C2_10070W_A, C5_03770C_A, RBT1, AOX1 were significantly down-regulated (Fig. 2). The foregoing results suggest that LCS carry a suppression activity on the proliferation and survival capacity of *C. albicans* at gene expression level. Interestingly, the suppression of LCS on *C. albicans* is likely to be different from acidic mediated effect as response to lactic acid treatment is considerably different from the samples treated with LCS. Moreover, the active components that exert suppressive effect on *C. albicans* may not attribute to a single substance, but mixture of antifungal compounds present in LCS. Further studies are warranted to delineate the mechanism of antifungal action exerted against *C. albicans* by the active ingredients other than lactic acid contained in LCS.

Conclusions

All LCS of the three candidate probiotic strains showed antifungal effect against both yeast and hyphal forms of *C. albicans*. Comparative transcriptomic analysis of *C. albicans* treated with LCS revealed, the specific gene expression was different from those treated with lactic acid, implying the presence of other active ingredients. Further studies are required to demystify the mechanism of antifungal action exerted by these active ingredients in the LCS against *C. albicans*. If proven feasible, probiotic-based antifungal therapy can bring enormous benefits to the patients suffering from *Candida* infections, particularly at the mucosal surfaces such as oral candidiasis.

Disclosure

Ethical approval was not required for the present study.

The authors declare that have no conflicts of interest to disclose.

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Candida albicans に対するプロバイオティクス乳酸菌培養上清の抗真菌活性：有機酸とは異なる有効成分の可能性

小島由佳子　大島朋子*　河井智美*
Chaminda Jayampath SENEVIRATNE** 前田伸子*

鶴見大学大学院歯学研究科口腔微生物学講座
（主任：前田伸子教授）
*鶴見大学歯学部口腔微生物学講座
（主任：前田伸子教授）
**国立シンガポール大学歯学部口腔科学講座
（主任：Chaminda Jayampath SENEVIRATNE）

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目的：口腔カンジダ症を含むカンジダ感染に対する様々な抗真菌剤の汎用は，薬物耐性株の出現をもたらした．近年，抗真菌活性を示すプロバイオティクス Lactobacilli が代替療法として提案されている．しかし，特に口腔内においては Lactobacilli によって産出される乳酸などの有機酸により望まれない作用を引き起こす可能性が知られている．本研究では，*Candida albicans* に対する *Lactobacillus* 培養上清（LCS）の抗真菌性を調べ，トランスクリプトーム解析の結果を乳酸処理した場合と比較することで有機酸以外の有効成分による影響を検討した．

方法：プロバイオティクス候補菌 5 株の LCS の抗真菌活性を，酵母型および菌糸型の *C. albicans* に対して試験した．LCS で処理された *C. albicans* のトランスクリプトーム解析には RNA シークエンスを用い，さらに定量的リアルタイム PCR（qRT-PCR）を行い，遺伝子発現の変動を確認した．

結果：最も高い抗真菌活性を示した *Lactobacillus* 3 株を最終候補とした．高い抗真菌活性を示した菌株と活性の低かった菌株の LCS の酸性度はいずれも約 pH4.0 であった．トランスクリプトーム解析の結果，*C. albicans* の遺伝子発現は，高い抗真菌活性を有する 3 菌株全ての LCS によって down-regulate されたことが示された．また，LCS で処理した *C. albicans* の遺伝子発現プロファイルは，乳酸で処理したサンプルとは異なっていた．

結論：最終候補のプロバイオティック菌 3 株の LCS は，*C. albicans* の酵母および菌糸形態の両方に対して抗真菌効果を示した．また，LCS で処理された *C. albicans* の RNA シークエンスを用いたトランスクリプトーム解析結果より遺伝子発現は LCS 処理と 0.46％乳酸処理後で異なっており，乳酸以外の活性成分の存在が示唆された．LCS の活性成分によって発揮される抗真菌作用の機序の解明にはさらなる研究を要する．しかし，今後，プロバイオティクス菌産出物を用いた抗真菌療法は，カンジダ感染，特に口腔カンジダ症のような粘膜表面の疾患に対する治療に有効であると考えられる．