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Full Length Research Paper

Lack of correlation between H_2O_2 production and *in vitro* anti-staphyloccocal activity of vaginal *Lactobacillus* spp.

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Lactobacilli are considered to play important roles in human health as they are known to secrete inhibitory substances to prevent infection by pathogenic organisms. Previously we have isolated 77 strains of *Lactobacillus* spp. from human vaginas. In this study, using the plate diffusion method, strains showing *in vitro* antagonistic activity against pathogenic *Staphylococcus aureus* were screened. Because *Lactobacillus* spp. are known to produce hydrogen peroxide (H_2O_2) as an antimicrobial substance, we attempted to determine if there is a consistent link between *in vitro* anti-staphylococcal activity and H_2O_2 production by *Lactobacillus* spp. A simple quantitative analysis of H_2O_2 produced by *Lactobacillus* spp. was performed by a modified spectrophotometric method. A statistically significant correlation was not found between *in vitro* anti-staphylococcal activity and H_2O_2 production.

Key words: *Lactobacillus* spp., vaginal *Lactobacillus* spp., anti-staphylococcal activity, H_2O_2 production.

INTRODUCTION

In 1894, the German physician A. Doderlein found that *Lactobacillus* was the predominant bacterium in the vaginal microbial flora found in women of reproductive age (Redondo-Lopez et al., 1992). Lactobacilli are facultative anaerobes that colonize the moist surface of the vaginal epithelium, the intestinal tract, and oral cavity of humans and non-human animals (Sharpe, 1981; Redondo-Lopez et al., 1992). Lactobacilli play an important role in maintaining vaginal health by producing various inhibitory compounds, which can prevent the growth of anaerobic pathogenic bacteria (Vallor et al., 2001; Hillier et al., 1991). Lactobacilli metabolize glucose to a final end product of lactic acid, which contributes to the maintenance of a low vaginal pH (4.0 - 4.5) (Vallor et al., 2001; Sharpe, 1981). Many isolates of vaginal lactobacilli produce hydrogen peroxide, a compound having broad antimicrobial activity (Klebanoff, 1991; Hillier et al., 1991).

Lactobacilli, as well as other lactic-acid-producing bacteria, lack heme and thus do not utilize the cytochome system (which reduces oxygen to water) for terminal oxidation. Lactobacilli utilize flavoproteins, which generally convert oxygen to H_2O_2. This mechanism, together with the absence of the heme protein catalase, generally results in the formation of H_2O_2 in amounts in excess of the capacity of the organism to degrade it. The H_2O_2 formed may inhibit or kill other members of the microbiota (Dahiya and Speck, 1968), particularly those that lack or have low levels of H_2O_2-scavenging enzymes, such as catalase. More recently, it has been demonstrated that peroxidases combined with H_2O_2 and a halide have properties that are toxic against a variety of microorganisms (Klebanoff et al., 1991). Lactic acid producing bacteria, e.g., *Lactobacillus acidophilus*, release H_2O_2 required for this peroxidase-mediated antimicrobial system (Leher, 1969). What properties do these strains possess that make them effective probiotic agents? Although the answer is not fully known, some common denominators appear to exist, namely the ability to adhere to and colonize tissues and the pathogenesis of disease causing organisms (Figure 1) (Reid, 2001).

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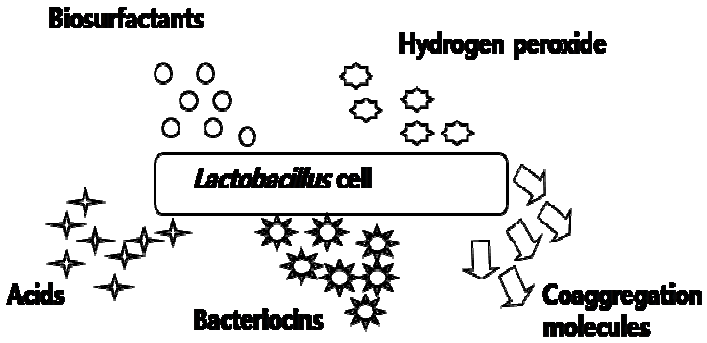

Figure 1. Byproducts of lactobacillus metabolism that have an antagonistic effect against urinary and vaginal pathogens. The biosurfactants inhibit adhesion; the acids, bacteriocins, and hydrogen.

Previously, we isolated 77 vaginal lactobacilli from healthy Korean women (KLB, Korean *Lactobacillus*, strains) (Chang et al., 2002). In this work, 77 KLB strains of *Lactobacillus* spp. were examined for detection of H_2O_2 and anti-microbial activity using *Staphylococcus aureus* as a target pathogen. The focus of this study was first to optimize the quantitative analysis of hydrogen peroxide in lactobacilli culture supernatant, and secondly, to determine the correlation between H_2O_2 production and *in vitro* anti-staphylococcal activity of *Lactobacillus* spp.

MATERIALS AND METHODS

Strains and media

In this study, 77 vaginal lactobacilli isolated from healthy Korean women were characterized in terms of their anti-microbial activity against *S. aureus* KTCC 25175 and hydrogen peroxide production. *Lactobacillus* spp. and *S. aureus* were cultured overnight anaerobically in 5 ml MRS broth and BHI broth, respectively. To measure H_2O_2 produced by *Lactobacillus* spp., SDM medium was used (Kimmel and Roberts, 1998) the medium contained 20 g of dextrose per liter, 1 ml of Tween 80 per liter, 2 g of ammonium citrate per liter, 5 g of sodium acetate per liter, 0.1 g of $MgSO_4 \cdot 7H_2O$ per liter, 0.05 g of $MgSO_4$ per liter, 2 g of K_2HPO_4 per liter, and 5 g of beef extract per liter.

Detection of anti-staphylococcal activity

Five □L of the overnight cultures of the *Lactobacillus* strains to be tested were spotted onto the surface of agar plates (MRS with 1.5% agar). Growth of the colonies was obtained after incubation at 37° for 5 h. *S. aureus* cells were transferred (1%, v/v) into 6 ml of soft BHI media (containing 0.7% agar) and poured over the plate on which the *Lactobacillus* strains were grown. The plate was incubated under anaerobic conditions at 37° for 12 h and examined for the capacity to inhibit the presence of an inhibition zone. Inhibition was scored positive if the width of the clear zone around the colonies of the *Lactobacillus* strain was 2.0 cm or larger (Corsetti et al., 1996; Otero and Nader-Macias, 2006).

Hydrogen peroxide quantification

The lactobacilli were inoculated (2% v/v) into 30 ml of SDM medium (100 ml Horex flasks) and then incubated with agitation at 250 rpm (SH-802F, HanSol, Korea) at 37℃. Samples were taken at specific time intervals of 6 h to determine H_2O_2 by measuring the optical density (OD) at 350 nm in 10 mm light path cuvettes (UV/Visible Spectrophotometer, PharmaciaBiotech, Ultropsec 2000), the pH with a pH meter (Metrohm, Swiss), and the colony-forming units (CFUs) per milliliter by the successive dilution method in a SDM agar plate. At appropriate intervals, samples were removed for measurement of biomass, cell growth by viable cell counts (CFU/ml), pH value, and H_2O_2. The hydrogen peroxide levels in lactobacilli spent supernatant fluid were detected by a spectrophotometric method (Chai et al., 2004) modified so as to be rapid and simple for quantitation of H_2O_2 produced by *Lactobacillus* spp. Briefly, a color agent, 2.4 mmol/L molybdate, was prepared by dissolving 0.10 g $(NH_4)Mo_7O_{24} \cdot 4H_2O$ in 250 ml of 0.5 mol/L H_2SO_4. A 3% hydrogen peroxide solution of analytical grade was used as the standard for calibration. Calibration was conducted by preparing a set of standard solutions, that is, by adding 2.5, 5, 10, 15, 20, and 25 µL of source hydrogen peroxide solution (3.01%) into 1 ml of the molybdate solution. Distilled water was used as the blank in the UV measurements. The sample size was 100 µL for the spent supernatant collected at appropriate intervals (usually depending on the estimated content of hydrogen peroxide in the supernatant), and the sample was transferred to a 1 ml molybdate reaction solution. Mixing of the solution was achieved by manual shaking. The UV absorption of each of the resulting solutions was measured at 350 nm. After 6 h of incubation at 37℃, supernatant fluids were separated by centrifugation, and H_2O_2 was quantified using an H_2O_2 standard curve. This modified procedure took at most 10 min.

Statistical analysis

A statistical analysis (analysis of variance [ANOVA]) of the correlation between anti-staphylococcal activity and hydrogen peroxide production of *Lactobacillus* culture was performed. A level of (≥ 0.05) was considered statistically significant EXCEL software was used (Mario, 2001).

RESULTS AND DISCUSSION

Screening of *Lactobacillus* spp. inhibiting *S. aureus*

In vitro anti-staphylococcal activities of *Lactobacillus* spp. screened in this study are presented in Table 1. Screening of all 77 strains of *Lactobacillus* revealed that they could be divided into 3 groups: high, moderate, and

Table 1. Anti-staphylococcal activity and H_2O_2 production of *Lactobacillus* spp.

Diameter of inhibition Zone (cm)	Strain number of Lactobacillus spp.	Hydrogen Peroxide (mmol/10^{-12} CFU)	Strain number of Lactobacillus spp. KLB
High≥2	212,224,225,239,249,260,261, 258,286,288,296,	High≥5	215,216,255,266,275,278, 282,290,301,
1<Moderate<2	201,202,204,205,206,209,211, 213,214,215,216,218,219,220, 221,227,232,236,237,242,243, 244,247,248,250,251,253,254, 255,258,264,265,266,267,268, 271,275,276,277,278,282,283 292,293,294,295,301,306	1<Moderate<5	201,202,203,204,205,206, 209,210,211,213,214,217, 219,220,224,225,227,232, 235,236,238,239,242,243, 244,247,248,249,251,253, 254,257,260,265,267,277, 285,286,288,292,293,295, 296,297,298,302,305
0≤No/weak≤1	203,207,208,210,217,233,235, 238,256,257,270,274,290,297, 300,302,305	No=0	207,208,212,218,221,233, 237,250,256,258,261,264, 268,270,271,274,276,283, 294,300,306

Table 2. Correlation between H_2O_2 production and anti-staphylococcal activity of *Lactobacillus* spp.

No. % of lactobacillus spp. with anti-staphylococcal activity				No. % of lactobacillus spp. with H_2O_2 production			
High(n=12)	Moderate(n=48)		No/weak(n=17)	High(n=9)	Moderate(n=47)		No/weak(n=21)
High H_2O_2 producers	0(0)	8(17)	1(16)	High anti-staphylococcal activity	0(0)	10(21)	2(10)
Moderate H_2O_2 producers	10(83)	28(58)	9(53)	Moderate anti-staphylococcal activity	8(89)	28(60)	12(57)
Non H_2O_2 producers	2(17)	12(25)	7(41)	No anti-staphylococcal activity	1(11)	9(19)	7(33)

no or weak anti-staphylococcal activity. The high inhibition group (inhibition zone size ≥ 2 cm) included 12 strains of *Lactobacillus* spp. (KLB 212, 224, 225, 239, 249, 260, 261, 285, 286, 288, 296, and 298). *Lactobacillus* sp. KLB 298 and 288 showed high anti-staphylococcal activity (size of inhibition zone, 2.1 and 2.2 cm respectively) whereas no activity was seen with KLB 270. Moderate anti-staphylococcal activity (1 < zone size < 2 cm) was found in 50 strains of *Lactobacillus*. No or weak inhibitory activity (zone size ≤ 1 cm) was observed with the following strains: KLB 203, 207, 208, 233, 257, 270, 274, 297, 300 and 305 (0 cm) and KLB 210, 217, 235, 238, 256, 290, and 302 (1 cm).

Quantitation of H_2O_2 produced by *Lactobacillus* spp.

Lactobacilli, principally the strains that are H_2O_2 producers, may have a protective effect against vaginal colonization by pathogenic species such as those that cause bacterial vaginosis (Hawes et al., 1996). Since *Lactobacillus* species were found to produce the highest amount of hydrogen peroxide during the exponential growth phase (data not shown) culture H_2O_2 supernatant by a modified method, as described in Materials and Methods.

We found that nine strains (KLB 215, 216, 255, 266, 275, 278, 282, 290 and 301) produced high amounts of hydrogen peroxide, from 5.0×10^{-12} to 2.1×10^{-11} mmol/CFU. Moderate hydrogen peroxide producers included 49 strains of *Lactobacillus*. However, the remaining 21 strains (KLB 207, 208, 212, 218, 221, 233, 237, 250, 256, 258, 261, 264, 268, 270, 271, 274, 276, 283, 294, 300 and 306) out of 79 strains did not produce H_2O_2 (Table 1).

Determination of correlation between anti-staphylococcal activity and hydrogen peroxide production by statistical analysis

As shown in Table 2, one (6%) out of 17 isolates showing no anti-staphylococcal activity (KLB 290) produced the highest amount of H_2O_2 while 9 (53%) isolates (KLB 203, 210, 217, 235, 238, 257, 302 and 305) produced a moderate amount of H_2O_2. The remaining 7 (41%) isolates (KLB 207, 208, 233, 256, 270, 274 and 300) produced no H_2O_2. Eight (17%) out of the 48 isolates showing moderate anti-staphylococcal activity (KLB 215, 216, 255, 266, 275, 278, 282 and 301) produced the highest amount of H_2O_2 while 28 (58%) isolates (KLB 201, 202, 204, 205, 206, 209, 211, 213, 214, 219, 220, 227, 232, 236, 242, 243, 244, 247, 248, 251, 253, 254,

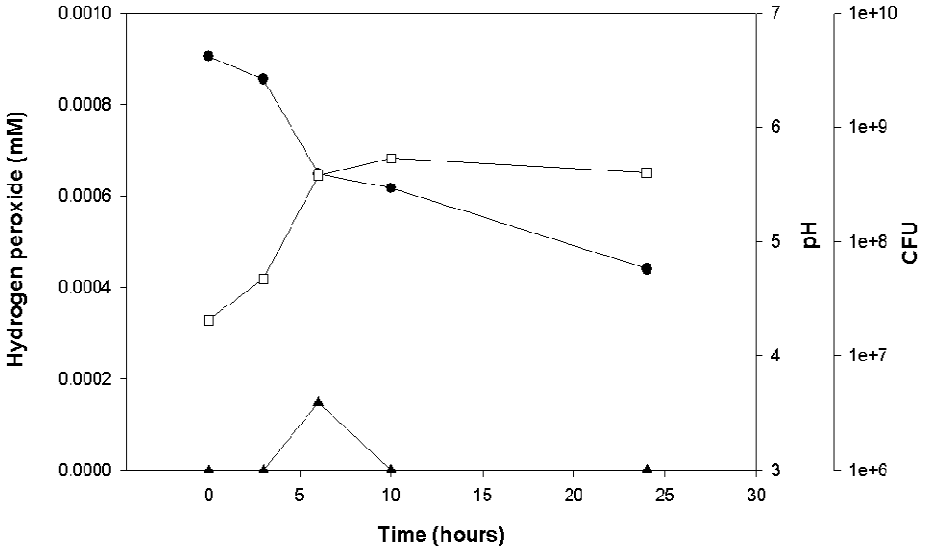

Figure 2. Correlation between quantitative of H_2O_2 production and antagonistic activity (2.3 cm) in *Lactobacillus* KLB 298; pH (●), Hydrogen peroxide (▲), CFU (■).

265, 268, 277, 292, 293 and 295) produced a moderate amount of H_2O_2. The remaining 12 (25%) isolates (KLB 218, 221, 237, 250, 258, 264, 268, 271, 276, 283, 294 and 306) produced no H_2O_2. No (0%) isolate producing high amount of H_2O_2 was included among the 12 isolates showing high anti-staphylococcal activity, but 10 (83%) isolates (KLB 224, 225, 239, 249, 260, 261, 285, 286, 288, 296 and 298) produced a moderate amount of H_2O_2. The remaining 2 (17%) isolates (KLB 212 and 261) produced no H_2O_2.

On the other hand, as shown in Table 2, two (10%) out of 21 isolates showing no H_2O_2 production KLB 212 and 261 were found to have high anti-staphylococcal activity while 12 (57%) isolates (KLB 218, 221, 237, 250, 258, 264, 268, 271, 276, 283, 294 and 306) were found to have moderate anti-staphylococcal activity. The remaining 7 (33%) isolates (KLB 207, 208, 233, 256, 270, 274 and 300) were found to have no anti-staphylococcal activity. 10 (21%) out of 47 isolates showing moderate H_2O_2 production (KLB 224, 225, 239, 249, 260, 285, 286, 288, 296 and 298) were found to have high anti-staphylococcal activity while 28 (60%) isolates (KLB 201, 202, 204, 205, 206, 209, 211, 213, 214, 219, 220, 227, 232, 236, 242, 243, 244, 247, 248, 251, 253, 254, 265, 267, 277, 292, 293 and 295) were found to have moderate anti-staphylococcal activity. The remaining 9 (19%) isolates (KLB 203, 210, 217, 235, 238, 257, 297, 302 and 305) were found to have no anti-staphylococcal activity. No (0%) isolate showing high anti-staphylococcal activity was included among the 9 isolates producing a high amount of H_2O_2, but 8 (89%) isolates KLB 215, 216, 255, 266, 275, 282 and 301 were found to have moderate anti-staphylococcal activity. The remaining 1 (11%) isolates (KLB 290) were found to have no anti-staphylococcal activity.

We have examined 83 KLB strains of *Lactobacillus* spp. for detection of H_2O_2 production, pH and anti-microbial activity at 6 h in a culture broth (Table 3). In quantitative measurement, KLB 298 (2.1 cm) with high anti-staphylococcal activity were found to produce H_2O_2 3.89×10^{-13} mM/CFU, viable cell counts 3.8×10^{8} CFU/ml, and to detect pH value 5.59 at 6 h of culture (Figure 2). Also, KLB 288 (2.2 cm) with high anti-staphylococcal activity were found to produce H_2O_2 3.60×10^{-14} mM/CFU, viable cell counts 3.73×10^{8} CFU/ml and to detect pH 5.57 at 6 h culture (Figure 3). KLB 271 (1.2 cm) with moderate anti-staphylococcal activity was found to produce H_2O_2 0 mM/CFU at 6 h culture (Figure 4). Also, KLB 227 (1.2 cm) with moderate anti-staphylococcal activity were found to produce H_2O_2 1.0×10^{-12} mM/CFU, viable cell counts 2.93×10^{8} CFU/ml and to detect pH 5.57 at 6 h culture (Figure 5).

A statistical analysis (ANOVA) was also applied to more precisely determine the correlation between hydrogen peroxide production and anti-staphylococcal activity. As a result of the statistical analysis though H_2O_2 quantitative measurement and inhibition zones of 83 KLB strains of *Lactobacillus* spp. after 6 h culture, there was no correlation between H_2O_2 production and *in vitro* anti-staphylococcal activity of *Lactobacillus* spp. No statistically significant correlation was found between anti-staphylococcal activity and hydrogen peroxide production, with a P value of 0.1565 and 0.1925 ($P > 0.05$). Previously *in vitro* inhibition of various microorganisms by H_2O_2-generating lactobacilli has been demonstrated by several authors. *S. aureus* was inhibited by *Lactobacillus gasseri* CRL1421, as demonstrated by morphological alterations and viability decreases (Mario, 2001). The

Table 3. Quantitative analysis of H_2O_2 production.

Lactobacillus spp.	H_2O_2	pH	CFU/ml
KLB 201	3.59×10^{-4}	5.74	1.80×10^{8}
KLB 202	1.67×10^{-4}	5.73	2.40×10^{8}
KLB 203	1.29×10^{-4}	5.58	1.53×10^{8}
KLB 204	1.29×10^{-4}	5.62	1.73×10^{8}
KLB 205	2.44×10^{-4}	5.68	2.20×10^{8}
KLB 206	1.29×10^{-4}	6.25	2.60×10^{7}
KLB 207	0.0	5.69	1.87×10^{8}
KLB 208	0.0	6.18	1.67×10^{8}
KLB 209	1.09×10^{-4}	5.59	4.07×10^{8}
KLB 210	2.63×10^{-4}	5.87	1.93×10^{8}
KLB 211	1.29×10^{-4}	5.69	1.47×10^{8}
KLB 212	0.0	6.00	1.87×10^{8}
KLB 213	1.34×10^{-5}	5.52	3.07×10^{8}
KLB 214	1.86×10^{-4}	6.14	2.27×10^{8}
KLB 215	3.60×10^{-4}	6.33	5.33×10^{7}
KLB 216	2.05×10^{-4}	6.29	4.00×10^{7}
KLB 217	1.09×10^{-4}	6.23	1.60×10^{8}
KLB 218	0.0	6.09	1.33×10^{8}
KLB 219	1.86×10^{-4}	6.39	2.47×10^{8}
KLB 220	3.21×10^{-4}	5.57	2.13×10^{8}
KLB 221	0.0	6.58	1.20×10^{8}
KLB 224	4.55×10^{-4}	6.16	1.80×10^{8}
KLB 225	2.82×10^{-4}	5.85	2.93×10^{8}
KLB 226	0.0	6.17	2.67×10^{7}
KLB 227	3.21×10^{-4}	5.80	2.93×10^{8}
KLB 230	5.70×10^{-4}	6.66	1.93×10^{8}
KLB 231	1.09×10^{-4}	5.42	4.20×10^{8}
KLB 232	9.02×10^{-4}	6.07	2.40×10^{8}
KLB 233	0.0	5.58	3.60×10^{8}
KLB 235	1.34×10^{-4}	6.19	9.33×10^{7}
KLB 236	9.02×10^{-5}	6.26	7.33×10^{7}
KLB 237	0.0	5.84	3.73×10^{8}
KLB 238	7.10×10^{-5}	6.12	1.33×10^{8}
KLB 239	7.10×10^{-5}	5.49	3.47×10^{8}
KLB 240	3.01×10^{-4}	5.33	8.00×10^{8}
KLB 242	3.01×10^{-4}	6.34	9.33×10^{7}
KLB 243	2.05×10^{-4}	5.65	4.13×10^{8}
KLB 244	2.25×10^{-4}	6.07	1.20×10^{8}
KLB 247	3.20×10^{-4}	6.33	9.33×10^{7}
KLB 248	2.05×10^{-4}	5.97	1.27×10^{9}
KLB 249	1.67×10^{-4}	6.00	2.93×10^{8}
KLB 250	0.0	6.38	7.33×10^{7}
KLB 251	1.09×10^{-4}	4.99	4.67×10^{8}
KLB 253	2.63×10^{-4}	6.22	6.87×10^{8}
KLB 254	9.02×10^{-4}	6.12	1.00×10^{8}
KLB 255	1.30×10^{-3}	5.74	2.60×10^{8}

Table 3. Contd.

KLB 256	0.0	6.35	4.00×10^{7}
KLB 257	3.26×10^{-5}	6.27	4.20×10^{7}
KLB 258	0.0	6.25	1.00×10^{8}
KLB 260	2.44×10^{-4}	6.28	5.20×10^{7}
KLB 261	0.0	5.74	1.67×10^{8}
KLB 263	2.44×10^{-4}	5.97	1.27×10^{8}
KLB 264	0.0	5.89	3.27×10^{7}
KLB 265	7.10×10^{-5}	4.92	1.40×10^{8}
KLB 266	5.70×10^{-4}	5.91	3.67×10^{7}
KLB 267	7.10×10^{-5}	6.19	3.07×10^{7}
KLB 268	0.0	6.46	1.27×10^{7}
KLB 270	0.0	6.46	1.93×10^{7}
KLB 271	0.0	6.49	2.47×10^{7}
KLB 274	0.0	5.57	2.53×10^{8}
KLB 275	1.15×10^{-3}	6.47	5.47×10^{7}
KLB 276	0.0	6.48	5.33×10^{6}
KLB 277	3.26×10^{-5}	5.49	2.13×10^{8}
KLB 278	3.59×10^{-4}	6.07	2.67×10^{7}
KLB 282	2.05×10^{-4}	6.6	1.13×10^{7}
KLB 283	0.0	5.52	5.40×10^{8}
KLB 285	2.44×10^{-4}	6.73	6.00×10^{7}
KLB 286	5.18×10^{-5}	5.75	5.13×10^{8}
KLB 288	1.34×10^{-5}	5.57	3.73×10^{8}
KLB 290	1.67×10^{-4}	6.27	3.13×10^{7}
KLB 292	3.26×10^{-5}	5.35	2.73×10^{8}
KLB 293	3.59×10^{-4}	5.96	2.53×10^{8}
KLB 294	0.0	5.79	3.80×10^{8}
KLB 295	3.26×10^{-5}	5.46	8.67×10^{7}
KLB 296	7.10×10^{-5}	6.15	3.93×10^{7}
KLB 297	2.05×10^{-4}	5.46	3.73×10^{8}
KLB 298	1.48×10^{-4}	5.59	3.80×10^{8}
KLB 299	0.0	6.45	5.33×10^{6}
KLB 300	0.0	6.48	6.67×10^{6}
KLB 301	1.09×10^{-4}	6.37	1.53×10^{7}
KLB 302	4.55×10^{-4}	6.1	1.40×10^{8}
KLB 305	2.25×10^{-4}	6.37	8.07×10^{7}
KLB 306	0.0	5.22	2.67×10^{8}

greatest inhibition was obtained under aerobic conditions, in which *L. gasseri* CRL1421produced the greatest amount of H_2O_2. Furthermore, when catalase was added to destroy the H_2O_2 produced by lactobacilli, the inhibitory effect was partially reversed. Therefore, the inhibitory effect on the growth of *S. aureus* was partially attributed to the H_2O_2 produced by *L. gasseri* CRL1421 (Mario, 2001). Haines and Hamon (1973) have reported that certain lactobacilli inhibited growth of *S. aureus* at H_2O_2 concentration of 0.18 mmol/L. At this concentration, H_2O_2 acted as bacteriostatic, and it became bactericidal at concentrations of 0.6 to 1.0 mmol/L. Collins and Aramaki (1980) have shown that some *L. acidophilus* of dairy origin were able to inhibit *Pseudomonas* species by producing 1.18 to 1.62 mmol/ L (40 to 55 µg / ml) H_2O_2 in agitated cultures. Wardle and Renninger (1975) observed significant bactericidal activity when 0.88 mol/L of H_2O_2 was applied to *Micrococcus* sp. or *Staphylococcus epidermidis*. As H_2O_2 produced by lactobacilli has been suggested to be an antimicrobial substance, we have

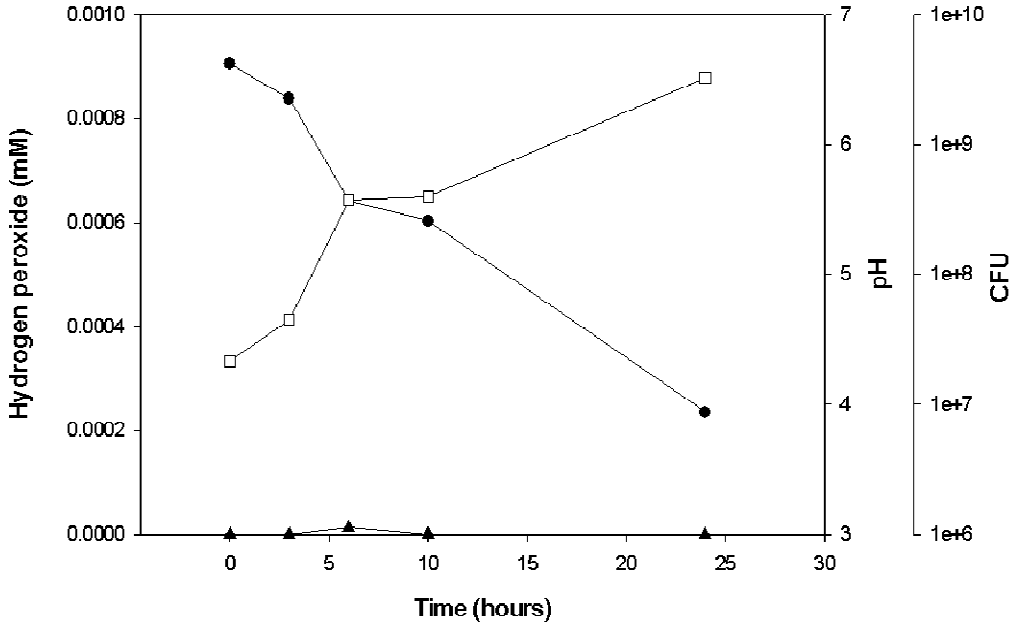

Figure 3. Correlation between quantitative of H_2O_2 production and antagonistic activity (2.2 cm) in *Lactobacillus* KLB 288; pH (●), Hydrogen peroxide (▲), CFU (■).

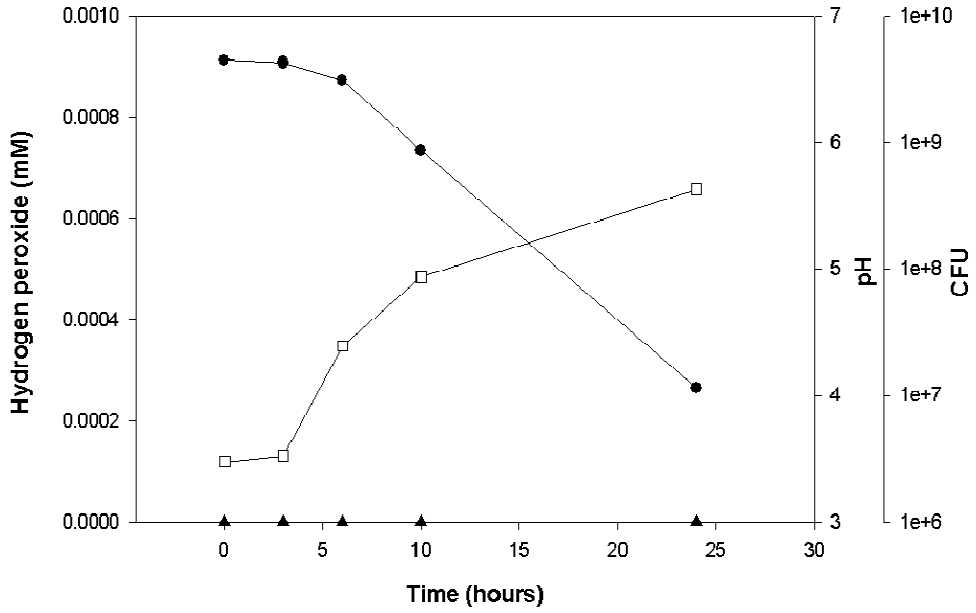

Figure 4. Correlation between quantitative of H_2O_2 production and antagonistic activity (1.2 cm) in *Lactobacillus* KLB 271; pH (●), Hydrogen peroxide (▲), CFU (■).

examined the H_2O_2 production and antagonistic activity *in vitro*. The results of the present study suggest that *Lactobacillus* spp. can inhibit *S. aureus*, but this *in vitro* experiment anti-staphylococcal activity was not necessarily associated with hydrogen peroxide production. Based on a statistical analysis though quantitative measurement, this report demonstrates for the first time that there is no correlation between H_2O_2 production and *in vitro* anti-staphylococcal activity of *Lactobacillus* spp. A recent paper describing a successful use of a probiotic, *Lactobacillus fermentum* RC-14 and its secreted biosurfactant to inhibit *S. aureus* is noteworthy (Gan et al., 2002).

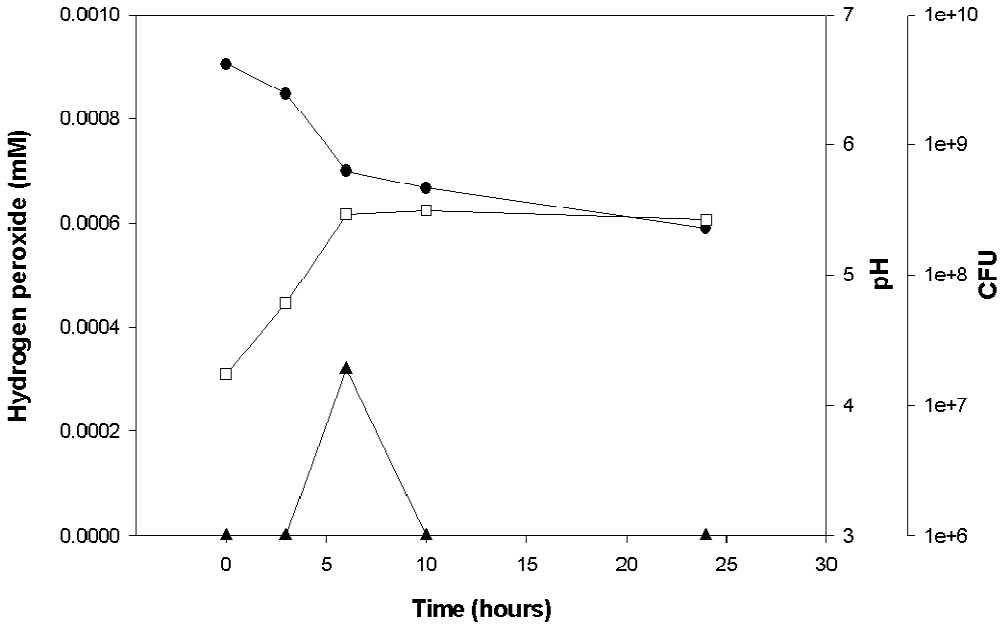

Figure 5. Correlation between quantitative of H_2O_2 production and antagonistic activity (1.2 cm) in *Lactobacillus* KLB 227; pH (●), Hydrogen peroxide (▲), CFU (■).

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