

Influence of Culture Conditions on Hydrogen Peroxide Production by *Lactobacillus jensenii*

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ABSTRACT

The purpose of this study was to determine how changes in certain culture conditions influence hydrogen peroxide production rate by the vaginal isolate *Lactobacillus jensenii*. Hydrogen peroxide production was detected for all variables, but the highest rates occurred at 13.8 mM glucose, pH 7, 40°C, and during early logarithmic growth stage when cell density was taken into account. Hydrogen peroxide was produced from *L. jensenii* regardless of the experimental variable; however, significant differences in production rate were detected within each variable. Since vaginal lactobacilli may play a crucial role in prevention of disease, it is important to know how *in vitro* environmental factors, which may emulate the vaginal environment or aid in the development of potential vaginal probiotic strains, influence production of hydrogen peroxide.

INTRODUCTION

Lactobacillus spp., which inhabit mucus membranes of humans and animals, are known to be dominant microorganisms in the reproductive tract of healthy women (Brown, 1978; Hill et al. 1984) and may provide protection from certain vaginal infections (Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Spurbeck and Arvidson, 2008). Disturbance or alteration of the *Lactobacillus*-dominated vaginal microbiota has been linked to an increased susceptibility to certain infections (Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Wilks et al. 2004). *Lactobacillus* spp. are thought to maintain or regulate the mucus-membrane microbial ecosystem by production of antimicrobial substances such as bacteriocins (Jack et al. 1995), lactic acid, (Boskey et al. 1999; Ogawa et al. 2001) and hydrogen peroxide (Eschenbach et al. 1989, Wilks et al. 2004). Hydrogen peroxide-producing lactobacilli seem to play a crucial role in regulating a healthy vaginal microbiota (Cherpes et al. 2008; Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Wilks et al. 2004). The absence of hydrogen peroxide-producing vaginal *Lactobacillus* spp. has been associated with an increased risk of bacterial vaginosis (Cherpes et al. 2008; Hawes et al. 1996), certain urinary tract infections (Gupta et al. 1998), and acquisition of human immunodeficiency virus (Martin et al. 1999). Wilks et al. (2004) and Cherpes et al. (2008) found that the presence of hydrogen peroxide-producing *Lactobacillus* spp. in the vagina of pregnant women was linked to a lower risk of bacte-

rial vaginosis and uterine infection. Among the numerous *Lactobacillus* species isolated from the human vagina (*L. casei*, *L. gasseri*, *L. iners*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. delbrueckii*, *L. vaginalis*, *L. salivarius*), *L. jensenii* is among the most abundant species (Antonio et al. 1999; Martin et al. 2008; Martin and Suarez, 2010; McGroarty et al. 1992; Vallor et al. 2001; Vásquez et al. 2002). In addition to abundance, nearly all of the vaginal *L. jensenii* strains reported in the literature make hydrogen peroxide (Antonio et al. 1999; Eschenbach et al. 1989; Martin et al. 2008; Martin and Suarez, 2010; McGroarty et al. 1992; Wilks et al. 2004) and at higher levels than most other vaginal *Lactobacillus* species (Martin and Suarez, 2010; Wilks et al. 2004). The level of hydrogen peroxide synthesis is an important characteristic of vaginal lactobacilli since there appears to be a relationship between its production and a reduced risk of genitourinary infections (Cherpes et al. 2008; Gupta et al. 1998; Hawes et al. 1996; Martin et al. 1999; Wilks et al. 2004). Therefore, hydrogen peroxide production is an important physiological characteristic for endogenous vaginal lactobacilli and for assessing the potential of a vaginal probiotic strain (Bolton et al. 2008; McLean and Rosenstein, 2000). Since vaginal lactobacilli may play a crucial role in prevention in disease, it is important to know which *in vitro* environmental factors will influence the rate of hydrogen peroxide production and to what extent. Very little information has been published about the influence of environmental conditions or growth phase on hydrogen peroxide production rate from important vaginal *Lactobacillus* species such as *L. jensenii*. A few other studies have also found that certain environmental or culture conditions such as aeration (Martin and Suarez, 2010; Otero and Nader-Macias, 2006; Tomas et al. 2003), incubation temperature, and culture pH (Tomas et al. 2003) can impact hydrogen peroxide production from vaginal *Lactobacillus* isolates. Recently, Martin and Suarez (2010) observed that vaginal *L. jensenii* cultures grown in media containing Fe^{3+} did not make hydrogen peroxide. The Fe^{3+} in the growth medium activated a peroxidase made by *L. jensenii* which degraded the hydrogen peroxide (Martin and Suarez, 2010). It has been previously reported that lactobacilli possess an atypical manganese-containing catalase/peroxidase system (Barynin et al. 2001; Yoder et al. 2000). Considering that lactobacilli possess a catalase/peroxidase and that complex growth media may contain Fe^{3+} , it is important to remove spent medium components from lactobacilli cells prior to measuring hydrogen peroxide synthesis. Most of the published literature dealing with vaginal lactobacilli, however, measure hydrogen peroxide production directly from spent growth media without using a wash step. In addition to a wash step, it is also important to measure the rate of hydrogen peroxide synthesis by lactobacilli rather than by end-point determination. Barnard and Stinson (1999) reported that rates of hydrogen peroxide were a more accurate assessment of the potential *in vivo* antagonistic ability of lactic acid bacteria rather than a simple end-point determination. As hydrogen peroxide diffuses away from the producing bacterial strain *in vivo*, the concentration is reduced and loses its killing effectiveness (Barnard and Stinson, 1999). Therefore, a vaginal lactobacillus isolate that can produce hydrogen peroxide at a high rate may be most effective for antagonism. Most of the published literature dealing with vaginal lactobacilli, however, measure hydrogen peroxide by using an end-point determination which is not a true kinetic assessment. *L. jensenii* was used for this study because it has been frequently isolated from the vagina of healthy women and most strains are strong producers of hydrogen peroxide (Antonio et al. 1999; Martin et al. 2008; Martin and Suarez, 2010; McGroarty et al. 1991; Wilks et al. 2004; Vallor et al. 2001; Vasquez et al. 2002). *L. jensenii* is not yet considered a probiotic strain and needs to be further evaluated based on the other characteristics

mentioned above. The purpose of this study was to determine how changes in glucose concentration, pH, temperature, and stages of growth influence hydrogen peroxide production rates by the important vaginal isolate and potential probiotic species *L. jensenii*.

MATERIALS AND METHODS

Hydrogen peroxide assay

The assay for hydrogen peroxide was adapted from Barnard and Stinson (1999). All assay reagents were prepared in sodium phosphate buffer (0.1 M, pH 7.8). The assay consisted of 20 μ l horseradish peroxidase (2 U, HRP, Sigma, St. Louis), 900 μ l of 2,2'-azino-bis-(3-ethylbenzthiazoline)-6-sulfonic acid (90 μ g, ABTS, Sigma), and 50 μ l of cell supernatant. The assay reagents were immediately mixed by inversion in a one ml cuvette and the absorbance (A_{414nm}) was measured using a spectrophotometer (Genesys 20, Thermospectronic, Madison, WI). The spectrophotometer was adjusted to zero absorbance with a blank which consisted of 920 μ l buffer and 50 μ l supernatant. Two controls were used for each reading to adjust for background from the enzyme or substrate. The enzyme control consisted of all components of the assay with the exception of ABTS. The substrate control consisted of all components of the assay with the exception of HRP. An additional control (reagent control) was tested with all of the components of the assay except the cell supernatant to insure that the assay was only detecting hydrogen peroxide production, and not background from the buffer. Cells from each trial were frozen and later counted with a Petroff-Hausser Chamber (data not shown). A standard curve of A vs hydrogen peroxide concentration was prepared using Microsoft Excel. The equation of the regression line for the standard curve was $y (A_{414nm}) = 0.094x (H_2O_2 \text{ mM}) - 0.0959$ ($r^2 = 0.998$) and was used to calculate hydrogen peroxide concentrations from each sample for all experiments. Initial rates of hydrogen peroxide production were used in this study because it is a more accurate assessment of the ability of certain lactic acid bacteria to control competing bacteria (Barnard and Stinson, 1999).

Data analysis

Four main experiments were performed in this study and included determination of the influence of glucose concentration, pH, temperature, and growth stage on hydrogen peroxide formation in *L. jensenii*. For each experimental variable, a plot of hydrogen peroxide concentration (mM) vs time (min) was prepared and a linear regression line was determined using Microsoft Excel. The slope of this linear regression line was used to determine units of activity for each experimental variable. One unit (U) of activity was defined as one mM of H_2O_2 produced per min under the assay conditions. Each variable was performed in triplicate and activity was reported as the mean $U \pm$ standard deviation (SD). ANOVA and Tukey multiple comparison tests were used to determine if differences existed among activity means within each experiment and were reported as P values. All statistical analyses were performed with Systat 8.0 software (SPSS Inc., Chicago, IL).

Bacterial strain, growth conditions, and cell preparation

Lactobacillus jensenii NRRL B-4550 was obtained from the United States Department of Agriculture, Agriculture Research Service Culture Collection in Peoria, IL. The *L. jensenii* strain used in this study (B-4550) was originally isolated from human vaginal dis-

charge as reported by Glasser et al. (1970) and is the *L. jensenii* type strain (Skerman et al. 1980). The strain has been used extensively in research related to the topic of vaginal lactobacilli and hydrogen peroxide (Antonio et al. 1999; Rabe and Hillier, 2003; Song et al. 1999; Spurbeck and Arvison, 2008; Zhong et al. 1998;). For preparation of stock cultures, isolated colonies were inoculated into Difco™ MRS Broth (10 ml) (Becton, Dickinson, & Co., Sparks, MD) and incubated overnight at 37°C. Cells from the overnight cultures were collected by centrifugation, resuspended in 1 ml fresh MRS Broth containing 20% (v/v) glycerol, and were stored frozen at -80°C until needed. For each experiment, a single vial of frozen stock cells was thawed, gently mixed, and 50- μ l was inoculated into the 10 ml MRS broth. Each spent frozen stock culture was not re-frozen but instead destroyed. A new vial of frozen stock culture was used for each experiment. This approach provided for very consistent inocula, much more consistent than performing multiple cultivations (two or three cultivations or transfers) prior to the assay. The inoculum provided consistent results for lag, log, and stationary phases and for enumerating cell numbers by microscopic cell count using Petroff-Hausser Chamber before each hydrogen peroxide assay was performed. The growth curve showed the expected lag, log, and stationary phases (Fig 1). The inoculated test tube culture (10 ml MRS broth) was subsequently incubated overnight at 37°C. Growth of the culture was measured by $A_{600\text{nm}}$ using a spectrophotometer (Spectronic 21, Bausch and Lomb). Cells were collected by centrifugation to a pellet (Sorvall angle centrifuge, Sorvall L.L.C., New Castle, DE) and washed three times with sodium phosphate buffer (0.1 M, pH 7.8, Breznak and Costilow, 1994) to remove medium components and glucose. The cells were resuspended in fresh phosphate buffer and adjusted to a A_{600} value of 1.0. This prepared culture was then used to determine the influence of environmental factors (glucose, pH, and temperature) on hydrogen peroxide production.

Influence of glucose on hydrogen peroxide production by *L. jensenii*

Samples (200 μ l) from a prepared culture were distributed into each of five pre-warmed (37°C water bath) microcentrifuge tubes containing 200 μ l of 0.1 M sodium phosphate buffer (pH 7.8) and varying concentrations of glucose (mM). The lids were closed and the tubes were incubated for up to 40 min at 37°C. One tube was removed from the water bath at each time interval (0, 10, 20, 30, 40 min). At each time interval, the tube was centrifuged for three min at 14,000 \times g (Eppendorf 5415C, Westbury, NY) to prepare a clarified supernatant, which was assayed for hydrogen peroxide.

Influence of pH on hydrogen peroxide production by *L. jensenii*

Cell preparation and the assay conditions were the same as previously described except that glucose concentration was maintained at 13.8 mM and the pH of the test buffers varied. The buffers used to test the effects of pH included citrate-phosphate buffer (pH 7 and pH 6) and acetate buffer (pH 5 and pH 4).

Influence of temperature on hydrogen peroxide production by *L. jensenii*

Cell preparation and assay conditions were the same as previously described except that glucose concentration was maintained at 13.8 mM, pH was maintained at 7.0, and the incubation temperatures were 30°C, 35°C, 40°C, and 45°C.

Influence of *L. jensenii* growth stage on hydrogen peroxide production.

A growth curve was prepared (data not shown) by inoculation of a *L. jensenii* thawed stock culture (65 μ l) into a 16-mm screw-cap test tube containing 13 ml of MRS broth which was incubated at 37°C in a water bath. Growth (A_{600nm}) was measured using a spectrophotometer (Spectronic 21) at three-h intervals for 15 h, and again at 20 h. Hydrogen peroxide formation was immediately detected from each sample collected during the growth assessment as described previously. The cells were washed and resuspended in fresh phosphate buffer using optimum conditions (13.8 mM, glucose, pH 7). Samples were assayed during early logarithmic (3 h), late logarithmic (6 h), early stationary (9 h), and late stationary phase (15 h) of growth.

RESULTS

Hydrogen peroxide was produced regardless of the glucose concentrations tested (Figure 2). The highest rate of hydrogen peroxide production occurred at 13.8 mM glucose (0.27 U \pm 0.08) and the lowest rate was at 55.5 mM glucose with an activity of 0.06 U \pm 0.01 (Figure 2). Hydrogen peroxide production at 55.5 mM glucose and when no glucose was added to the assay was significantly different than at 13.8 mM glucose ($P = 0.031$, $P = 0.003$, respectively) (Figure 2). In addition, hydrogen peroxide production at 55.5 mM glucose was significantly different from 27.7 mM glucose and 41.6 mM glucose ($P = 0.028$, $P = 0.025$) (Figure 2).

Hydrogen peroxide was produced regardless of pH conditions tested (Figure 3). The highest rate of hydrogen peroxide production was detected at pH 7, with an activity of 0.31U \pm 0.09 and the lowest rate of production was at pH 5 (0.07 U \pm 0.00) (Figure 3). There was a significant difference in hydrogen peroxide production between pH 7 and pH 5 ($P = 0.004$), pH 7 and pH 4 ($P = 0.021$), and pH 5 and pH 6 ($P = 0.020$) (Figure 3).

Hydrogen peroxide was produced regardless of the assay temperatures tested (Figure 4). The highest rate of hydrogen peroxide production occurred at 40°C (0.19 U \pm 0.01) and the lowest rate of production occurred at 30°C (0.05 U \pm 0.01) (Figure 4). A significantly lower rate of hydrogen peroxide production occurred at 30°C when compared to the other temperatures tested (35°C, $P = 0.003$; 40°C, $P = 0.000$, and 45°C, $P = 0.000$) (Figure 4). A significant difference in hydrogen peroxide production also occurred between 40°C and 35°C ($P = 0.003$) (Figure 4).

The relationship between growth stage and hydrogen peroxide production by *L. jensenii* is shown in figures 5 and 6. Hydrogen peroxide production was calculated as mM L⁻¹ min⁻¹ (U) in figure 5 and as mM L⁻¹ min⁻¹ / A in figure 6. Hydrogen peroxide assays calculated as mM L⁻¹ min⁻¹ / A value were determined to account for differences in hydrogen peroxide production due to cell density (Figure 6). Hydrogen peroxide was produced regardless of the growth stages tested (three h, mid log; six h, late log; nine h, early stationary; and 15 h, late stationary, Figures 5 and 6). When calculated as mM L⁻¹ min⁻¹, the highest rate of hydrogen peroxide production occurred after six h growth (late log phase, 0.24 U \pm 0.05) and the lowest rate occurred after three h growth (mid log phase, 0.07 U \pm 0.02) (Figure 5). A significant difference in hydrogen peroxide production occurred between three h and six h growth ($P = 0.0002$), six h and nine h growth ($P = 0.024$), and three h and 15 h growth ($P = 0.007$) (Figure 5). When cell density was

taken into account as $\text{mM L}^{-1} \text{min}^{-1} / A$, the highest hydrogen peroxide production occurred at three h growth (mid log phase, 1.11 ± 0.35) and the lowest activity occurred at nine h growth (early stationary phase, 0.10 ± 0.01) (Figure 6). A significant difference in hydrogen peroxide production occurred between three h and six h growth ($P = 0.006$), three h and nine h growth ($P = 0.002$), and three h and 15 h growth ($P = 0.002$) (Figure 6).

DISCUSSION

The purpose of this study was to determine how certain *in vitro* culture conditions influence the rate of hydrogen peroxide production by *L. jensenii*. It was apparent from this study that many factors can influence *in vitro* hydrogen peroxide production by *L. jensenii*. For example, hydrogen peroxide production by *L. jensenii* was lowest at the highest glucose concentration (55.5 mM) and at the lowest glucose concentration (0 mM) tested. These data were similar to findings by Barnard and Stinson (1999) who studied hydrogen peroxide synthesis in *Streptococcus gordonii*. *S. gordonii* is a lactic acid bacterial species associated with oral health. Barnard and Stinson (1999) showed that there was essentially a bell shaped curve associated with hydrogen peroxide production as it related to glucose concentration. In their study, hydrogen peroxide synthesis was lowest at the lowest (0.01 mM) and highest (1000 mM) glucose concentrations tested and was higher at the intermediate glucose concentrations (0.1mM, 1.0 mM, and 10 mM). The fact that both *L. jensenii* (in this study) and *S. gordonii* (Barnard and Stinson (1999)) displayed lower hydrogen peroxide synthesis at the highest glucose concentrations tested in each study may reflect a common mechanism used by lactic acid bacteria to efficiently regulate bacterial competition in a vaginal or oral environment. Competition for nutrients among various microbial groups in the oral or vaginal environment would be less important when carbohydrate concentration is abundant. Consequently, a high rate of hydrogen peroxide synthesis by lactic acid bacteria is not needed to suppress competing bacteria and the cells can devote resources to more important metabolic processes. *L. jensenii* and *S. gordonii* (Barnard and Stinson 1999) both produced hydrogen peroxide when glucose was not added to the assay conditions. The energy needed for hydrogen peroxide synthesis by lactic acid bacteria in the absence of extraneous glucose may have come from metabolism of intracellular polysaccharide stores (Barnard and Stinson, 1999; Minah and Loesche, 1977). Minah and Loesche (1977) showed that up to 14% of the carbohydrate consumed by hydrogen peroxide-producing oral streptococci is converted into intracellular carbohydrate. The ability of *L. jensenii* to produce an intracellular polysaccharide reserve, however, was not determined in this study. Although not statistically significant, *L. jensenii* made twice as much hydrogen peroxide with no exogenous glucose (0 mM) than with the highest glucose concentration tested (55.5 mM). The ability to make hydrogen peroxide without exogenous carbohydrate present may reflect the need for certain lactic acid bacteria (*L. jensenii* or *S. gordonii*, Barnard and Stinson, 1999) to inhibit competing bacteria in their particular environment (vaginal or oral) under low carbohydrate conditions when competition for nutrients would be more intense (Barnard and Stinson, 1999). Vaginal glucose in the form of glycogen (Gregoire et al. 1971; Paavonen, 1983) is used by lactic acid bacteria as an energy source for hydrogen peroxide synthesis (Barnard and Stinson, 1999; Minah and Loesche, 1977) and is also metabolized to form lactic acid (Boskey et al. 2001). Glycogen content will vary based on the cycle stage and

vaginal location but has been determined to be 1122 μg to 1667 μg of glycogen per 100 mg of tissue wet weight epithelial tissue (Gregoire et al. 1971).

Vaginal glycogen levels are regulated by estrogen (Gregoire et al. 1971; Paavonen, 1983). Consequently, fluctuations in estrogen such as menarche, menopause, oral contraception, and hormone replacement therapy could conceivably alter the vaginal biota including hydrogen peroxide-producing lactobacillus species (Cauci et al. 2002). Increased estrogen production can cause higher amounts of glycogen to be deposited into the vagina (Boskey et al. 2001; Gregoire et al. 1971; Paavonen, 1983). It is hypothesized that decreased vaginal glycogen levels may increase the antimicrobial competitive activities (lactic acid and hydrogen peroxide production) of vaginal lactobacilli.

Production of hydrogen peroxide by *L. jensenii* in this study was also influenced by pH. The higher production rates of hydrogen peroxide detected in this study at pH 6 and pH 7 than at pH 4 and 5 may be a response by *L. jensenii* to inhibit microbial competition in the vaginal environment as the pH increases. The healthy vagina should have a pH range of 4.0-4.7 (Gardner and Dukes 1955) and an abundance of the lactobacilli morphotype (large gram-positive rod) (Nugent et al. 1991; Spiegel, 1991). An increase in vaginal pH above 4.5, a decrease in lactobacilli, and the appearance of non lactobacilli bacterial morphotypes (gram-negative rods, gram-variable rods and curved rods, gram positive cocci) are among the clinical signs associated with bacterial vaginosis (Amsel et al. 1983; Nugent et al. 1991; Spiegel, 1991). The lowest rates of hydrogen peroxide synthesis obtained in this study were detected at pH 4 and pH 5. This response to pH may be a mechanism for *L. jensenii* to conserve energy during times of lower pH because the normal acidic vaginal environment (pH 4.0-4.7) itself would help inhibit the growth of pathogenic organisms (Ogawa et al. 2001; Stamey and Timothy, 1975).

Temperature was also an important factor in the production of hydrogen peroxide by *L. jensenii*. The peak rates of production ($0.19 \text{ mM L}^{-1} \text{ min}^{-1}$ and $0.16 \text{ mM L}^{-1} \text{ min}^{-1}$) occurred at the two highest temperatures tested (40°C and 45°C), indicating that increased temperature enhances the production of hydrogen peroxide in *L. jensenii in vitro*. Although the difference in rates between the two highest temperatures was not significant, cells assayed for hydrogen peroxide production at 45°C may have been slightly temperature stressed resulting in the lower rate. In general, higher hydrogen peroxide synthesis rate coordinated with optimum growth temperatures (37°C - 44°C) associated with *Lactobacillus* species isolated from the vagina (McLean and Rosenstein, 2000; Tomás et al. 2003). The coordination of high hydrogen peroxide synthesis with optimum growth temperatures that mimic the human vagina (37°C) is an important physiological characteristic for endogenous vaginal lactobacilli and for assessing the potential of a vaginal probiotic strain (McLean and Rosenstein, 2000).

In addition to the other environmental factors mentioned in this study, hydrogen peroxide production by *L. jensenii* coordinated with growth stage (Fig 6) rather than by cell numbers alone (Fig 5). That is, actively growing cells of *L. jensenii* and not necessarily high cell numbers produced the highest rates of hydrogen peroxide. For example, when measured as $\text{mM L}^{-1} \text{ min}^{-1}$, hydrogen peroxide synthesis was assessed by cell numbers during the growth cycle (Fig 5). For this cell number assessment, hydrogen peroxide synthesis was lowest during mid-log ($0.07 \text{ mM L}^{-1} \text{ min}^{-1}$) but peaked during late-log growth phase

(0.24 mM L⁻¹ min⁻¹). When calculated as mM L⁻¹ min⁻¹ / A value, hydrogen peroxide synthesis peaked during mid-log growth (1.11 mM L⁻¹ min⁻¹ / A) followed by a sharp reduction in activity as the growth cycle proceeded into late log (0.29 mM L⁻¹ min⁻¹ / A) and stationary phase (0.10 mM L⁻¹ min⁻¹ / A; 0.13 mM L⁻¹ min⁻¹ / A) (Fig 6). This activity assessment (mM L⁻¹ min⁻¹ / A value) is an indication of hydrogen peroxide synthesis efficiency by *L. jensenii*. This value may be a more accurate assessment of the hydrogen peroxide capability of a probiotic culture since lactobacilli cell numbers in the healthy vagina (Boskey et al. 1999) would not reach the concentration or density as is typically obtained *in vitro* (Tomás et al. 2003). In addition, these data may also indicate that hydrogen peroxide production in *L. jensenii* coordinates with the synthesis of cell enzymes needed for primary metabolism such as NADH oxidases (Marty-Tysset et al. 2000; Talwalkar and Kailasapathy, 2003).

This *in vitro* study provides the first evidence that hydrogen peroxide synthesis rate by the vaginal isolate *L. jensenii* is not static but is highly influenced by environmental factors. *L. jensenii* can adjust hydrogen peroxide synthesis rate in response to changes in glucose concentration, pH, temperature, and growth phase. Parameters such as glucose concentration and pH are important environmental conditions that can influence composition of the vaginal microbiota. It is not ideal to correlate results of an *in vitro* study to an *in vivo* environment such as the vagina. The response in hydrogen peroxide synthesis by *L. jensenii* to glucose and pH fluctuations, however, may reflect a common mechanism used by lactic acid bacteria to efficiently regulate bacterial competition in the vaginal environment. This study may help to establish a scientific basis for the selection of vaginal probiotic bacterial strains and also may be used as a guideline for studies focused on differential gene expression in response to changes in environmental conditions.

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Figure 1. Growth curve of *L. jensenii* after inoculation into a 10 ml MRS broth culture. The 10-ml MRS culture was started from a frozen stock culture (50 μ l) of *L. jensenii*. Each time point represents data from three growth trials. Absorbance values were exactly the same for each time point.

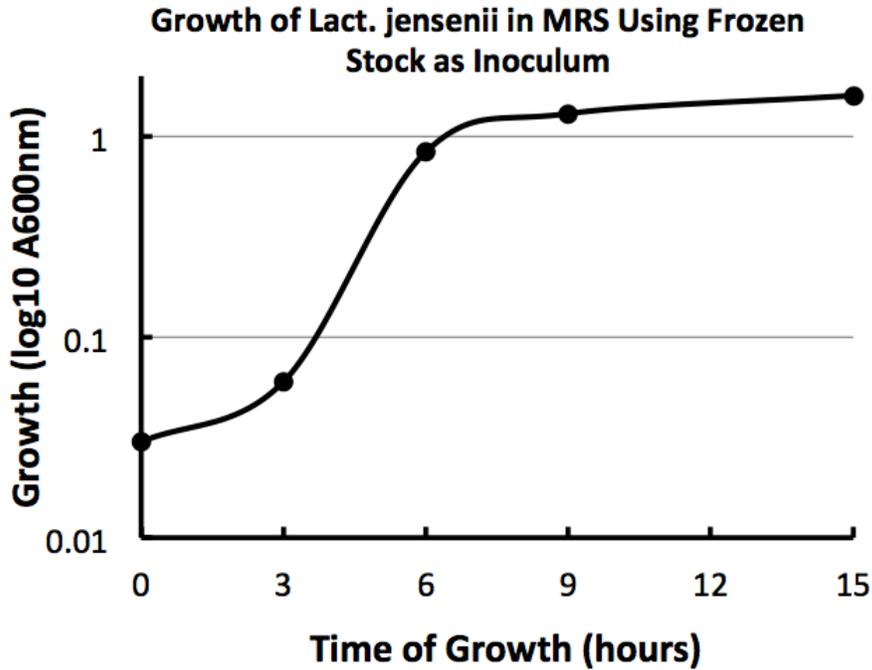


Figure 2. Influence of glucose concentration on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate ($\text{mM L}^{-1} \text{min}^{-1}$) was determined from three trials for each glucose concentration and reported as mean \pm SD.

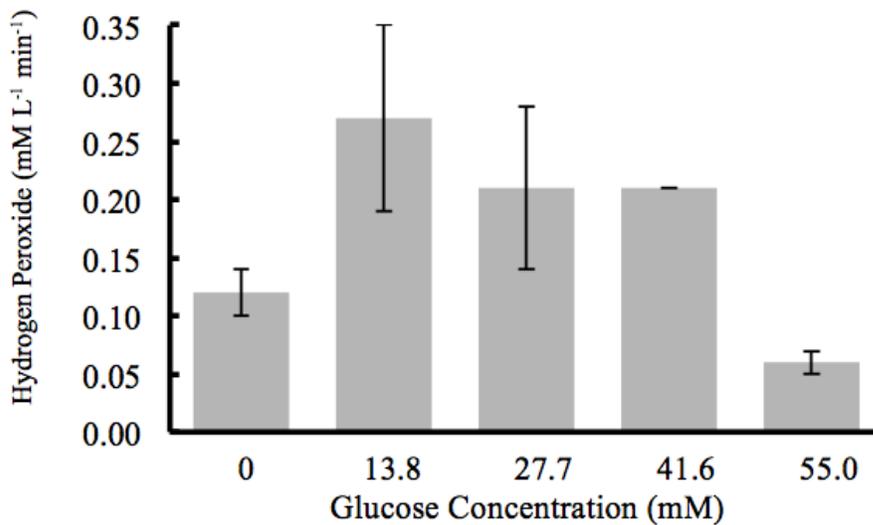


Figure 3. Influence of pH on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate ($\text{mM L}^{-1} \text{min}^{-1}$) was determined from three trials for each pH and reported as mean \pm SD.

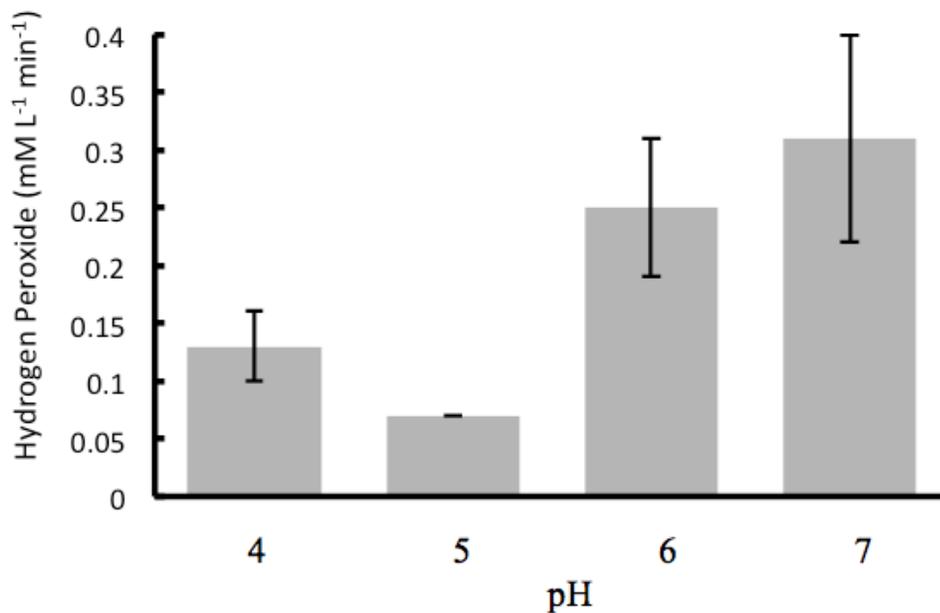


Figure 4. Influence of temperature on hydrogen peroxide production by *L. jensenii*. Hydrogen peroxide production rate ($\text{mM L}^{-1} \text{min}^{-1}$) was determined from three trials for each temperature and reported as mean \pm SD.

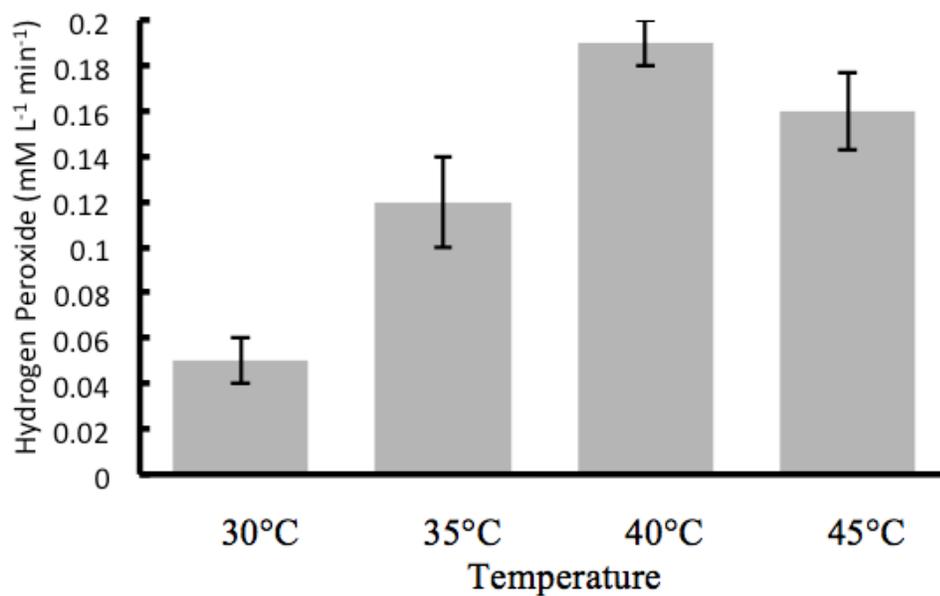


Figure 5. Influence of *L. jensenii* growth stage on hydrogen peroxide production measured as $\text{mM L}^{-1} \text{min}^{-1}$. Hydrogen peroxide production rate ($\text{mM L}^{-1} \text{min}^{-1}$) was determined in triplicate for each growth stage and reported as mean \pm SD.

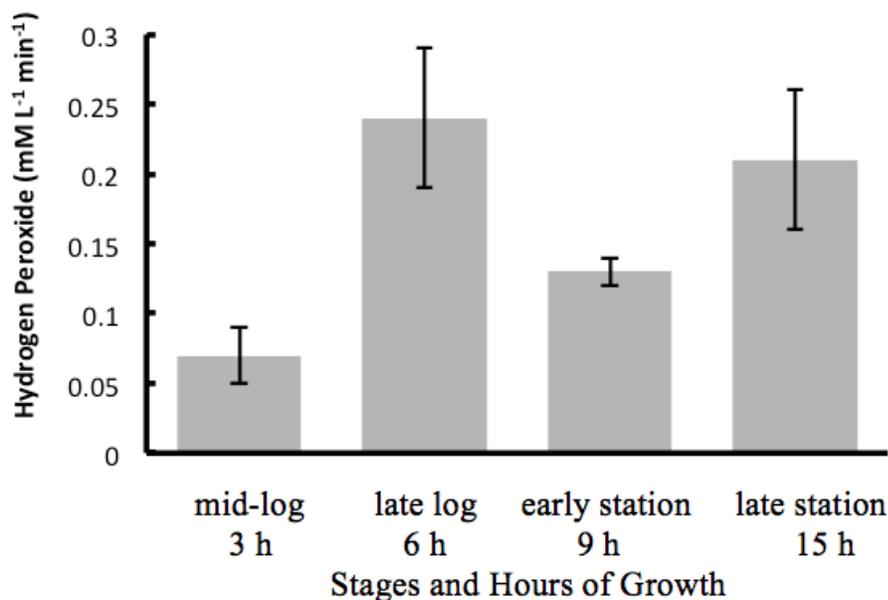


Figure 6. Influence of *L. jensenii* growth stage on hydrogen peroxide production measured as ($\text{mM L}^{-1} \text{min}^{-1} / A$). Hydrogen peroxide production rate ($\text{mM L}^{-1} \text{min}^{-1} / A$) was determined from three trials for each growth stage and reported as mean \pm SD.

