

Stress Resistance of Biofilm and Planktonic *Lactobacillus plantarum* subsp. *plantarum*
JCM1149

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1 **ABSTRACT**

2 The purpose of this study was to investigate the change in resistance of biofilm and
3 planktonic food spoilage lactic acid bacteria (LAB) to environmental stresses, which
4 strongly inhibit bacterial growth and are important in food preservation or in disinfection.
5 The stress responses of biofilm and planktonic cells of *Lactobacillus plantarum* subsp.
6 *plantarum* JCM1149, which was used as a model spoilage bacterium, in various organic
7 acids (namely, acetic acid, citric acid, lactic acid, and malic acid), ethanol, and sodium
8 hypochlorite, were investigated using survival tests. The bacterial cells in biofilms showed
9 greater resistance to all treatments than the planktonic bacterial cells in either the stationary
10 or logarithmic phase. The planktonic bacterial cells showed reduced resistance to acetic
11 acid after the cell suspension was diluted; however, intriguingly, the bacterial cells in
12 biofilms maintained their resistance to acetic acid even after they were suspended or the
13 cell suspension was diluted. These findings suggested the risk for food spoilage due to LAB
14 derived from biofilms and suspended or diluted in foods, and demonstrated the importance
15 of controlling biofilms of LAB in the food industry.

16

17 **Key words**

18 acid resistance, ethanol resistance, sodium hypochlorite, biofilm, *Lactobacillus plantarum*

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1 **1. Introduction**

2

3 Lactic acid bacteria (LAB) are noteworthy spoilage bacteria of foods and pose a
4 constant concern for the food industry, while they are also well known as beneficial
5 bacteria, which include probiotic bacteria that help to maintain a good enteral environment
6 and produce biopreservatives. They cause contamination or deterioration of many types of
7 food such as vinegar (Dakin and Radwell, 1971; Entani *et al.*, 1986), meat products (Niven
8 *et al.*, 1949; Björkroth *et al.*, 1997; Aymerich *et al.*, 2003), pickles (Sheneman and
9 Costilow, 1955), mayonnaise (Kurtzman *et al.*, 1971), salad dressing (Kurtzman *et al.*,
10 1971), marinated herring (Lyhs *et al.*, 2001), cheese (Somers *et al.*, 2001), *miso* (fermented
11 soybean paste) (Nikkuni *et al.*, 1996), and beer (Behr *et al.*, 2006). In most cases, the
12 spoilage LAB originate from the production line or raw materials and are resistant to
13 environmental stresses. *Lactobacillus fructivorans* was isolated from spoiled mayonnaises
14 of pH 3.7-3.8 (Kurtzman *et al.*, 1971). *L. fructivorans* also spoiled *miso*, which contains salt
15 (Nikkuni *et al.*, 1996). *Lactobacillus acetotolerans* responsible for the spoilage of vinegar
16 could survive in over 4% acetic acid (Entani *et al.*, 1986). The beer spoilage bacterium
17 *Lactobacillus brevis* that lacks the hop transport ability showed resistance to both acid and
18 ethanol (Behr *et al.*, 2006).

19 The stress responses and survival strategies of LAB in stressful environments have been
20 reviewed (Cotter and Hill 2003; Van de Guchte *et al.*, 2002). One of them is biofilm
21 formation. Biofilm formation by *Lactobacillus* species has also been reported (Kawarai *et*
22 *al.*, 2007; Kubota *et al.* 2008; Lebber *et al.*, 2007; Millsap *et al.* 1997; Somers *et al.*, 2001;

1 Sturme *et al.*, 2005; Tannock *et al.*, 2005) and some reports have described the genes
2 responsible for quorum sensing, adhesion or biofilm formation (Fujii *et al.*, 2008; Lebber *et*
3 *al.*, 2007; Sturme *et al.*, 2005; Tannock *et al.*, 2005). Sturme *et al.* (2007) reviewed quorum
4 sensing focusing on *Lactobacillus plantarum* WCFS1. However, the stress responses of
5 LAB biofilms associated with food production and raw materials have not been studied in
6 detail. The response of LAB biofilms to low pH was reported for the oral bacterium,
7 *Streptococcus mutans* (Li *et al.*, 2001). The two-component regulatory system in *S. mutans*
8 is involved in both biofilm formation and acid resistance (Li *et al.*, 2002). We have
9 embarked on the study of LAB biofilms, particularly on biofilm formation by the LAB
10 isolated from raw materials and on their changes in stress resistance (Kubota *et al.*, 2008).
11 We demonstrated that three *Lactobacillus* type strains and 43 LAB isolated from onions
12 formed biofilms. We also showed that the bacterial cells in biofilms of *L. plantarum* either
13 from culture collection or the isolates had greater resistance than planktonic bacterial cells
14 to acetic acid and ethanol, which are strong growth inhibitors for bacteria and important in
15 food preservation. The enhanced resistance to acid and ethanol in *Lactobacillus* biofilms
16 was the first finding. In this study, we tested *Lactobacillus plantarum* subsp. *plantarum*
17 JCM 1149 as a typical spoilage model species because it was isolated from acidic food,
18 namely, pickled cabbage (Dellaglio *et al.*, 1975), formed biofilms well and showed greater
19 acid and ethanol resistance in biofilms (Kubota *et al.*, 2008). To elucidate the difference in
20 resistance between planktonic and bacterial cells in biofilms more closely, we have
21 examined in detail the difference in resistance among bacterial cells in biofilms and
22 planktonic bacterial cells in two different representative growth phases to various organic

1 acids, ethanol and sodium hypochlorite, which are used as typical food acidulants,
2 preservatives, disinfectants, or known as fermentation products of LAB. In addition, we
3 focused our attention on the change in resistance of the cells derived from biofilms and
4 suspended or diluted in foods.

5

6 **2. Materials and Methods**

7

8 2.1 Bacterial strains and growth condition

9

10 *Lactobacillus plantarum* subsp. *plantarum* JCM 1149 obtained from the Japan
11 Collection of Microorganisms (JCM) was used in the study. The strain was grown in de
12 Man, Rogosa, Sharpe (MRS) broth (Oxoid Ltd., Cambridge, UK) and on MRS agar (Oxoid
13 Ltd.). Inoculated plates were incubated for 1 to 3 days at 30 °C under anaerobic conditions
14 created in an AnaeroPack Rectangular jar with an AnaeroPack-Anaero sachet (Mitsubishi
15 Gas Chemical Company, Inc., Tokyo, Japan). Inoculated broths in glass tubes (Iwaki 18 ø ×
16 150 mm, Asahi Techno Glass Co., Ltd., Tokyo, Japan) were incubated statically under
17 aerobic conditions for 24 h at 35 °C.

18

19 2.2 Preparation of planktonic and biofilm cells for assay

20

21 To prepare planktonic cells, *L. plantarum* subsp. *plantarum* JCM 1149 cells were
22 incubated statically in glass tubes containing 10 mL of MRS broth under aerobic conditions

1 for 24 h at 35 °C; the broths were centrifuged for 15 min at $2900 \times g$ and the cells were
2 resuspended in 0.85% saline to yield an initial concentration of 10^9 – 10^{10} CFU/mL. To
3 prepare biofilms, on the basis of the method of Sturme *et al.* (2005), the biofilms were
4 grown and formed on glass cover slips (12 mm ϕ , Thermo Fisher Scientific, Waltham, MA,
5 USA) in the wells of static 24-well polystyrene microtiter dishes (Iwaki 1820-042N, Asahi
6 Techno Glass Co., Ltd.) containing 1 mL of MRS broth under aerobic conditions for 24 h at
7 35 °C. The broth was then removed, and the bacteria on the glass cover slips were carefully
8 rinsed with 1 mL of 0.85% saline and the remaining materials were defined as biofilms. To
9 prepare a dilute suspension of bacterial cells in biofilms, the biofilms on glass cover slips in
10 1 mL of 0.85% saline were dispersed by pipetting up and down over ten times and the cell
11 suspension was diluted with 0.85% saline.

12

13 2.3 Assay for stress resistance to acids, ethanol, and sodium hypochlorite

14

15 To prepare organic acid solutions, acetic acid (Wako, Tokyo, Japan) was dissolved in
16 50 mM sodium acetate solution or ion-exchanged water. Citric acid (Wako), malic acid
17 (Wako), and lactic acid (Wako) were dissolved in ion-exchanged water. Biofilms formed on
18 glass cover slips and planktonic bacterial cells (10^8 – 10^9 CFU/mL) were placed in 1.1 mL of
19 the acid solutions in 24-well polystyrene microtiter dishes (Iwaki 1820-042N, Asahi Techno
20 Glass Co., Ltd.) for 30 min at room temperature; 0.85% (w/v) saline was used as control
21 instead of the test solutions. The bacterial cells in biofilms placed in 1.1 mL of the acid
22 solutions were suspended by pipetting up and down over ten times after the tests. The

1 suspensions were confirmed to show no large aggregations, and no bacterial cells
2 remaining on the glass cover slips were observed by microscopy (data not shown). Aliquots
3 (1 mL) were suspended in 9 mL of saline and aliquots (100 μ L) of the suspension or diluted
4 solution were plated onto MRS agar plates. The plates were incubated for 72 h at 30 $^{\circ}$ C
5 under anaerobic condition and the number of viable cells was determined. To test survival
6 in ethanol, biofilms on glass cover slips and planktonic bacterial cells (10^8 - 10^9 CFU/mL)
7 were placed in 1.1 mL of 18-45% ethanol in 24-well polystyrene microtiter dishes (Iwaki)
8 for 60 min at room temperature. The subsequent procedures were the same as in the test
9 using acids. To test survival in sodium hypochlorite, biofilms and planktonic bacterial cells
10 (10^8 - 10^9 CFU/mL) were placed in 1.1 mL of 10-275 ppm (effective chlorite concentration)
11 sodium hypochlorite in 24-well polystyrene microtiter dishes (Iwaki) for 30 min at room
12 temperature. Aliquots (1 mL) were suspended in 1 mL of equimolar sodium thiosulfate
13 solution to inactivate sodium hypochlorite and aliquots (1 mL) of the suspension were
14 suspended in 9 mL of saline. The subsequent procedures were the same as in the test using
15 acids.

16

17 2.4 Data analysis

18

19 Viable cell counts were converted to colony forming units (CFU) per milliliter on the
20 basis of 1.1 mL of the treatment solutions to compare the number of viable cells between
21 biofilm and planktonic bacteria. The data presented are means \pm standard deviations
22 obtained from three independent experiments. The detection limit of all the stress resistance

1 assays except for those in sodium hypochlorite was 100 CFU per milliliter of 1.1 mL of the
2 treatment solutions. The detection limit of the assays in sodium hypochlorite was 200 CFU
3 per milliliter of 1.1 mL of the treatment solutions. The bacteria populations were converted
4 to log CFU per milliliter on the basis of the treatment solutions. The converted detection
5 limit was 2.0 log CFU/ml (100 CFU/ml) or 2.3 log CFU/ml (200 CFU/ml) on the basis of
6 the solutions. In some cases, one or two of three replications had populations below the
7 detection limit; in this case, the value “2.0” (except for the assay in sodium hypochlorite) or
8 “2.3” (for the assay in sodium hypochlorite) was entered in the calculation.

9

10 **3. Results**

11

12 3.1 Survival of biofilm and planktonic *L. plantarum* subsp. *plantarum* JCM 1149 in acetic
13 acid

14

15 Figure 1 shows percent survivals of biofilm and planktonic *L. plantarum* subsp.
16 *plantarum* JCM 1149 in 8.2% acetic acid dissolved in 50 mM sodium acetate (pH 3.0). The
17 bacterial cells in biofilms grown for 24 h and the planktonic bacterial cells that were
18 incubated for 24 h corresponding to the stationary phase showed greater resistance to acetic
19 acid (pH 3.1) than the planktonic cells incubated for 4.5-14 h corresponding to the
20 logarithmic phase.

21 The percent survivals of planktonic and bacterial cells in biofilms in 0.85% saline
22 (control) and 5.5-11.0% (v/v) acetic acid dissolved in 50 mM sodium acetate (pHs 7.4

1 (saline) and 3.3-2.9 (acetic acid)) are shown in Fig. 2. Both the biofilm and planktonic cells
2 in the stationary phase were more resistant to 5.5-9.0% acetic acid than the planktonic cells
3 in the logarithmic phase. The bacterial cells in biofilms showed greater resistance even at
4 higher concentrations (9.0-11.0%).

5

6 3.2 Survival of diluted suspensions of biofilm and planktonic *L. plantarum* subsp.
7 *plantarum* JCM 1149 in acetic acid

8

9 The suspensions of the biofilm cells were confirmed to show no large aggregations by
10 microscopy observation, which is similar to the case of planktonic cells (data not shown).
11 The biofilms (10^9 CFU/ml), the suspended cells (10^9 CFU/ml) from the biofilms, and the
12 planktonic bacterial cells (10^9 CFU/ml) that were grown for 24 h, the state of which
13 corresponds to the stationary phase, showed resistance to 8.2% acetic acid in 50 mM
14 sodium acetate (pH 3.0) (Fig 3). The biofilm cells maintained their resistance to acetic acid
15 even after they were suspended (10^9 CFU/ml) or the cell suspension was diluted (10^8 and
16 10^7 CFU/ml). In contrast, the planktonic bacterial cells showed reduced resistance to acetic
17 acid after the cell suspension was diluted (10^8 and 10^7 CFU/ml).

18

19 3.3 Survival of biofilm and planktonic *L. plantarum* subsp. *plantarum* JCM 1149 in various
20 organic acids

21

22 Figure 4 shows the results of the survival tests using biofilm and planktonic *L.*

1 *plantarum* subsp. *plantarum* JCM 1149 in organic acids dissolved in ion-exchanged water:
2 2.7-27.0% (v/v) acetic acid (pHs 2.5-1.7) (Fig. 4a), 4.6-27.0% (w/v) citric acid (pHs
3 2.0-1.4) (Fig. 4b), 0.9-20.0% (v/v) lactic acid (pHs 2.3-1.5) (Fig. 4c), and 2.7-18.0% (w/v)
4 malic acid (pHs 2.1-1.5) (Fig. 4d). The planktonic cells in the logarithmic phase were less
5 resistant to these organic acid solutions than those in the stationary phase. The bacterial
6 cells in biofilms showed higher resistances to all the organic acids tested than the
7 planktonic bacterial cells.

8

9 3.4 Survival of biofilm and planktonic *L. plantarum* subsp. *plantarum* JCM 1149 in ethanol
10 and sodium hypochlorite

11

12 The percent survivals of *L. plantarum* subsp. *plantarum* JCM 1149 in 18.0-45.0%
13 ethanol are shown in Fig. 5a. The bacterial cells in biofilms showed greater resistance to
14 23.0-45.0% ethanol than the planktonic bacterial cells in both the logarithmic and stationary
15 phases. The bacterial cells in biofilms could still survive in 45.0% ethanol, while the
16 number of surviving planktonic cells was below the detection limit.

17 The percent survivals of *L. plantarum* subsp. *plantarum* JCM 1149 in 10-275 ppm
18 (effective chlorite concentration) sodium hypochlorite are shown in Fig. 5b. The bacterial
19 cells in biofilms showed greater resistance at all the concentrations tested than the
20 planktonic bacterial cells in either the logarithmic or stationary phases.

21

22 **4. Discussion**

23

1 First, to determine the percent survivals of biofilm and planktonic cells, acetic acid
2 solutions were adjusted to approximately pH 3.0 because the usual pH of acidic foods is
3 over 3. The test concentration of acetic acid was decided as 8.2% on the basis of the
4 following two reasons: 1) to compare percent survivals, the survival tests should be
5 performed at a concentration lower than the minimum bactericidal concentration for a 30
6 min treatment of planktonic *L. plantarum*, which was approximately 10% at pH 3
7 determined in our preparatory test, and 2) for applications in food preservation, the data
8 were obtained in the higher concentration range (7% - 9%) of vinegar that prevails on the
9 market as food materials (3% - 10%).

10 The cells in a model food spoilage LAB, *L. plantarum* subsp. *plantarum* JCM 1149,
11 which were grown for 24 h (corresponding to the stationary phase) by the static culture
12 method and grown in the biofilms for the same period, showed resistance in 8.2% acetic
13 acid solutions, and the cells in the biofilms showed greater stress resistance in more than
14 10% acetic acid solutions at the usual pH of acidic foods than the planktonic cells
15 regardless of the growth phase. Moreover, we found that the biofilm cells (10^9 CFU/ml)
16 maintained their resistance to acetic acid even after they were suspended (10^9 CFU/ml) or
17 the cell suspension was diluted (10^8 and 10^7 CFU/ml). These results suggest the risk for
18 food spoilage due to LAB derived from biofilm cells and suspended or diluted in foods. It
19 provides instructive information for controlling *Lactobacillus* spoilage in food production.

20 There are two aspects of controlling spoilage bacteria in biofilms. One is to prevent
21 biofilm formation, and the other is to effectively control the already formed biofilms. To
22 prevent biofilm formation, it is important not to introduce spoilage bacteria, which have the

1 potential of forming biofilms on raw materials and in production environment, into food
2 production. The genes responsible for quorum sensing, adhesion or biofilm formation of
3 *Lactobacillus* species have already been reported (Fujii *et al.*, 2008; Lebbet *et al.*, 2007;
4 Sturme *et al.*, 2005; Tannock *et al.*, 2005). It is considered that control of these specific
5 genes or other genes related to the formation of biofilms might control biofilm formation
6 during food production process or fermentation in the future.

7 To effectively control the already formed biofilms, on the other hand, it is needed to
8 understand the potentials of resistance to various stresses and elucidate the mechanism
9 underlying stress responses. Understanding the potentials of acid resistance, the survivals at
10 various concentrations of four organic acids (no pH adjustment, pHs below 2.5), which are
11 general food acidulants, were examined. The bacterial cells in biofilms showed higher
12 resistances to all the organic acids tested, notably to acetic acid and lactic acid, than the
13 planktonic bacterial cells. To the best of our knowledge, this is the first report of such
14 findings. Few reports show the results of survival tests on *L. plantarum* strains as we
15 examined but some show the resistance of cells under growing condition. Twenty *L.*
16 *plantarum* strains isolated from red wines showed high pH resistance and proliferated in the
17 pH range from 3.2 to 3.6 under growing condition (G-Alegria *et al.*, 2004). The *L.*
18 *plantarum* strain used in this study was also grown in this pH range and survived even
19 below pH 2.5, and survived in the lower pH range by forming biofilms. The bacterial cells
20 in biofilms also showed greater resistance at high concentrations of ethanol and sodium
21 hypochlorite than the planktonic bacterial cells in both the logarithmic and stationary
22 phases, suggesting the high risk for spoilage associated with LAB in biofilms during food

1 production. These results demonstrated the significance of focusing attention to biofilms
2 formed by spoilage lactobacilli on the raw materials treated even with acids at low pH,
3 ethanol and peroxidation stresses. We need to obtain in-depth data on the stress resistances
4 of spoilage lactobacilli that form biofilms and elucidate the stress resistance mechanisms to
5 determine the appropriate types and amounts of preservatives to ensure suitable tastes and
6 flavors of food, while maintaining a balance with antibacterial effects.

7 In general, aside from the low pH stress, the antibacterial effectiveness of organic acids
8 depends on the concentrations of their undissociated molecules, which are hydrophobic and
9 can affect the hydrophobic membrane of cells thus providing a major antibacterial
10 mechanism (Brown and Booth, 1991; Brul and Coote, 1999). Although the pH ranges of
11 treatment acid solutions are from 2.5 to 1.4, the difference in resistance between biofilm
12 and planktonic cells is pronounced in the case of acetic acid and lactic acid, whose
13 undissociated molecules are more hydrophobic than the other two organic acids (Fig. 4).
14 This difference in resistance could be caused by the biofilms gaining resistance against
15 hydrophobic antibacterial materials. The result that the biofilm cells showed great ethanol
16 resistance also indicated that the biofilms show enhanced resistance to hydrophobic
17 materials. We also examined the effect of pH stress on the survival of the bacterium treated
18 with 50 mM acetic acid-HCl buffer (pHs 3.5-1.0). The biofilm cells showed greater
19 resistance at lower pHs (2.0-1.0) than the planktonic cells in both the logarithmic and
20 stationary phases (Fig. 6). Comparison of this result with that obtained at approximately pH
21 3, as shown in Fig. 2, reveals that the biofilm cells have enhanced resistance to both
22 hydrophobic undissociated molecules and proton (pH). From the scanning electron

1 microscopy observation of the cells after the survival and related tests, we hypothesized
2 that three mechanisms could explain the resistance of biofilm cells: the cell membrane
3 becomes more resistant; the biofilm is protected by extracellular polymeric secretions; and
4 the three-dimensional structure of the biofilm protects the inner cells (Kubota *et al.*, 2008).
5 No results of this study are consistent with any of these possible mechanisms. Van de
6 Guchte *et al.* (2002) reported that the acid resistance of LAB increases when adaptive
7 responses can be induced by incubation at a nonlethal acidic pH during the logarithmic
8 growth phase. Taranto *et al.* (2003) reported that the lipid composition of *Lactobacillus*
9 *reuteri* CRL 1098 grown with bile salts changed in terms of the ratio of
10 glycolipids/phospholipids. The changes in the lipid profile induced by bile salts, however,
11 did not improve the resistance of the bacteria to freezing and acid stress. In our study,
12 because the incubation pH of both biofilm and planktonic bacteria was 3.5 at both 12 h and
13 24 h, and there was no difference between biofilm and planktonic bacteria in terms of the
14 production of organic acids such as acetic acid, lactic acid, and citric acid at each time (data
15 not shown), the acids under growing conditions did not have much effect on the changes in
16 resistance.

17 The dilute suspensions of the bacterial cells in biofilms showed resistance to acetic acid
18 even though the diluted planktonic cells were less resistant. Although the physical
19 characteristics of the biofilm and planktonic bacterial cells were the same, the dilute
20 suspensions of only the bacterial cells in biofilms maintained their resistance to acetic acid.
21 It is suggested that not only the structure of the biofilms but also the individual cells in the
22 biofilms have an effect on the enhancement of the acid resistance of *L. plantarum*

1 JCM1149. Li *et al.* (2002) reported that a two-component signal transduction system
2 encoded by *hk11* and *rr11* represents a regulatory system involved in biofilm formation and
3 acid resistance in *S. mutans*. Moreover, in *L. plantarum* JCM1149, individual cells in the
4 biofilms could exhibit gene expressions and regulations that are different from those in
5 planktonic bacteria. Our future work should focus on elucidating the mechanisms
6 underlying stress responses of biofilms, developing the appropriate methods of controlling
7 LAB biofilms, and preventing LAB-related spoilages in food products.

8

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10

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14

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1 Figure legends

2

3 Fig. 1. Percent survivals of biofilm and planktonic *Lactobacillus plantarum* subsp.
4 *plantarum* JCM 1149 treated with acetic acid dissolved in 50 mM sodium acetate. Open
5 bars indicate data for bacterial cells treated with saline and closed bars indicated those for
6 the cells treated with 8.2% acetic acid. Error bars indicate standard deviations of three
7 independent experiments. a: below 0.0001%.

8

9 Fig. 2. Viabilities of biofilm (●) and planktonic *Lactobacillus plantarum* subsp.
10 *plantarum* JCM 1149 at stationary (◐) and logarithmic (○) phases in saline (0%) and
11 acetic acid dissolved in 50 mM sodium acetate (pHs 3.3-2.9). Error bars indicate standard
12 deviations of three independent experiments. a: below detection limit.

13

14 Fig. 3. Percent survivals of biofilm and planktonic *Lactobacillus plantarum* subsp.
15 *plantarum* JCM 1149 in diluted cell suspensions treated with acetic acid. Open bars
16 indicate data for planktonic bacterial cells and closed bars indicate those for the bacterial
17 cells originating from biofilms. Error bars indicate standard deviations of three independent
18 experiments.

19

20 Fig. 4. Viabilities of biofilm (●) and planktonic *Lactobacillus plantarum* subsp.
21 *plantarum* JCM 1149 at stationary (◐) and logarithmic (○) phases in acetic acid (pHs
22 2.5-1.7) (a), citric acid (pHs 2.0-1.4) (b), lactic acid (pHs 2.3-1.5) (c), and malic acid (pHs

1 2.1-1.5) (d). Error bars indicate standard deviations of three independent experiments. a:
2 below detection limit.

3

4 Fig. 5. Viabilities of biofilm (●) and planktonic *Lactobacillus plantarum* subsp.
5 *plantarum* JCM 1149 at stationary (○) and logarithmic (○) phases in ethanol (a) and
6 sodium hypochlorite (b). Error bars indicate standard deviations of three independent
7 experiments.

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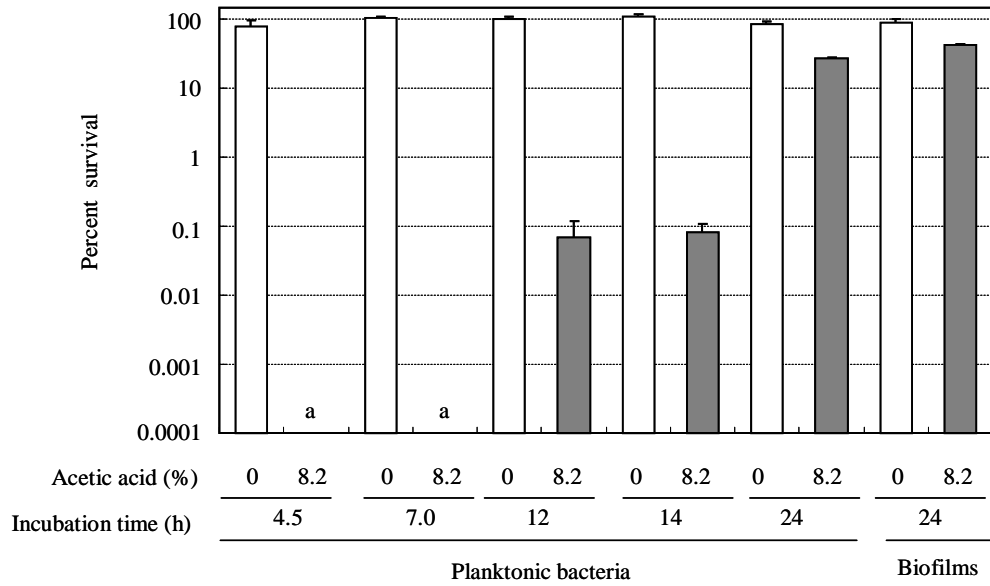
9 Fig. 6. Effect of pH on viabilities of biofilm (●) and planktonic *Lactobacillus plantarum*
10 subsp. *plantarum* JCM 1149 at stationary (○) and logarithmic (○) phases. Error bars
11 indicate standard deviations of three independent experiments. a: below detection limit.

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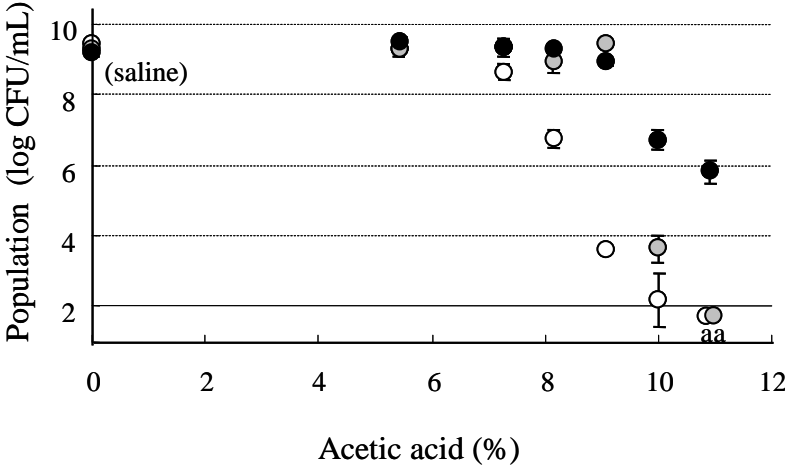
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1 Figures

Kubota H. *et al.*, Fig. 1.

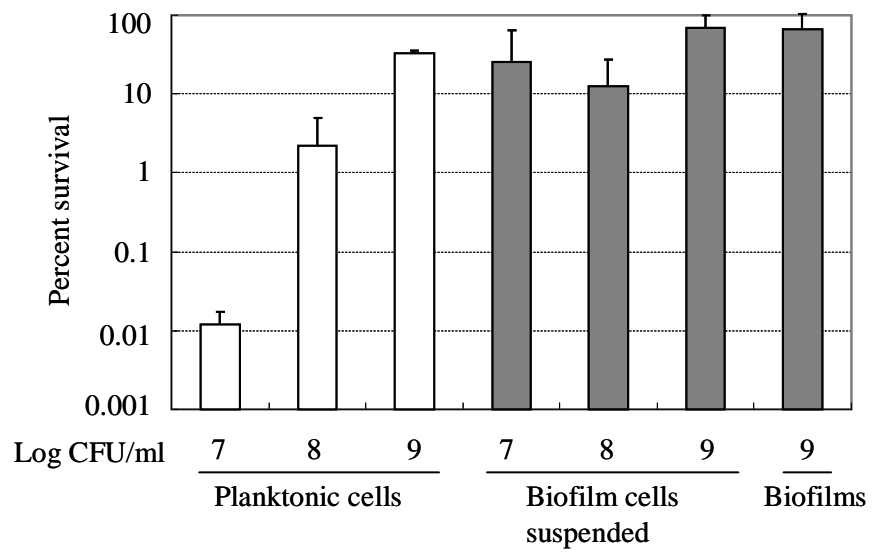


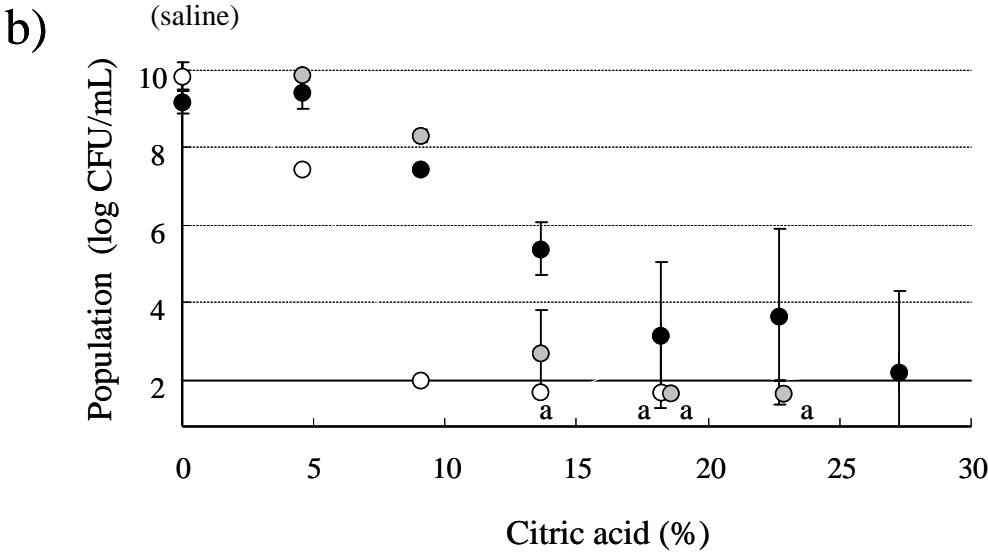
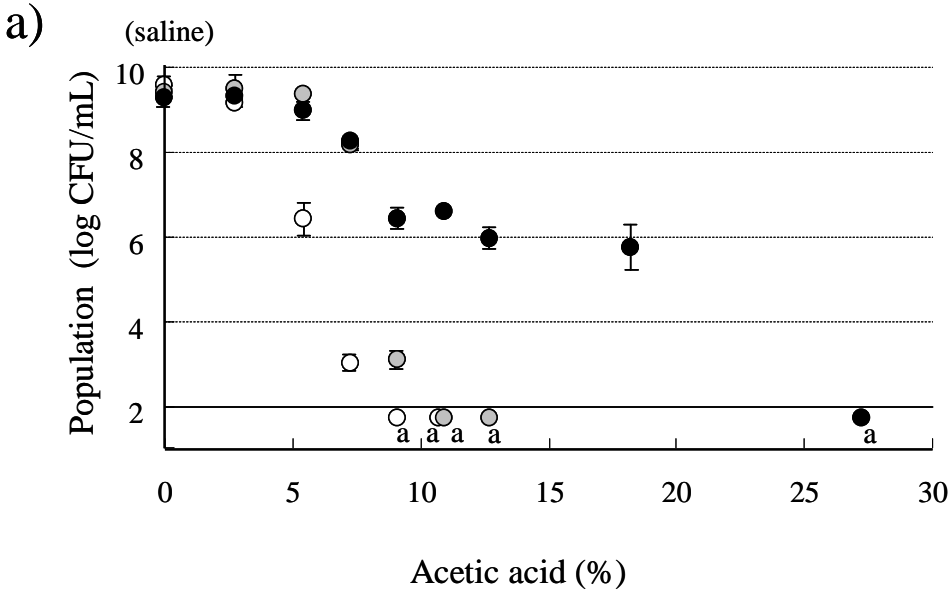
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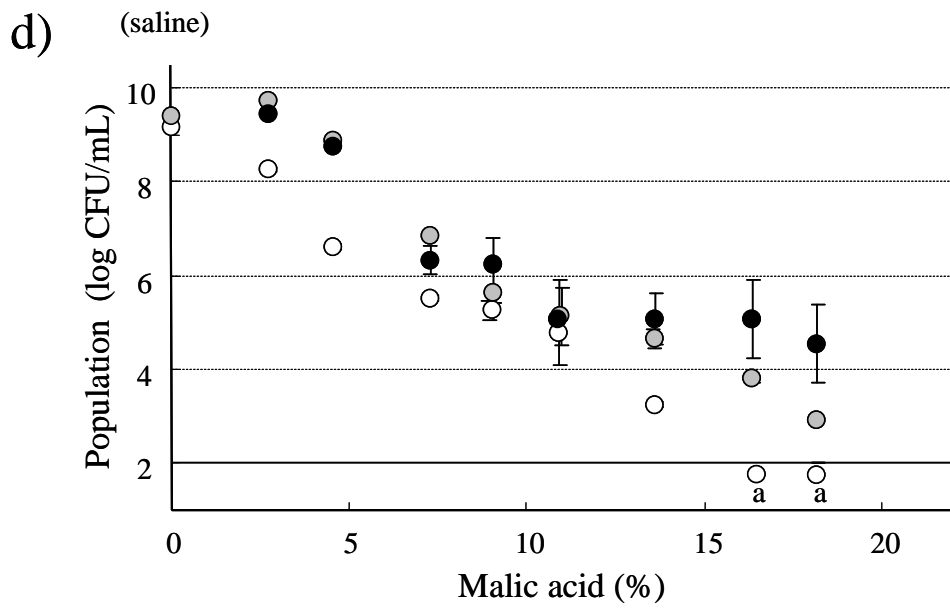
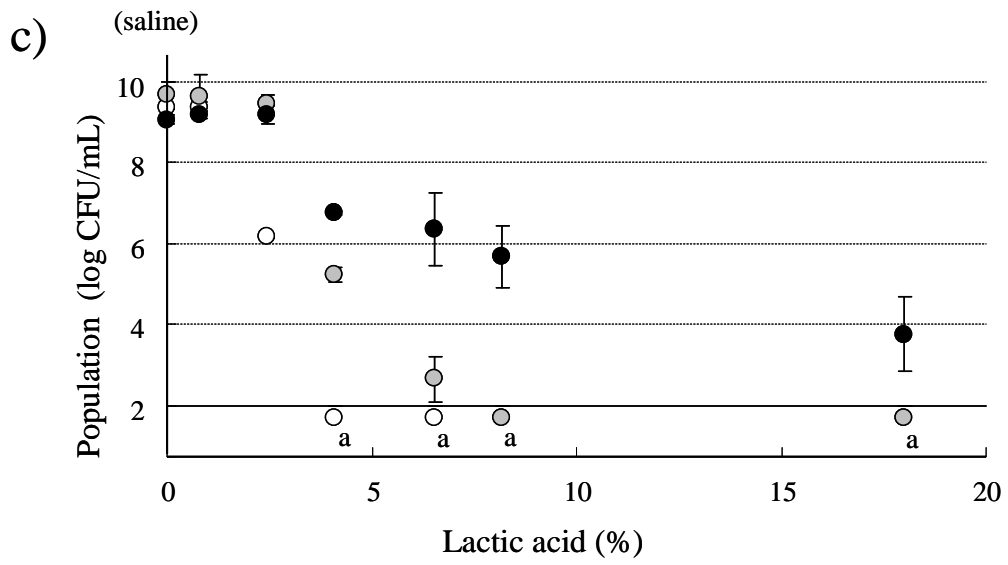


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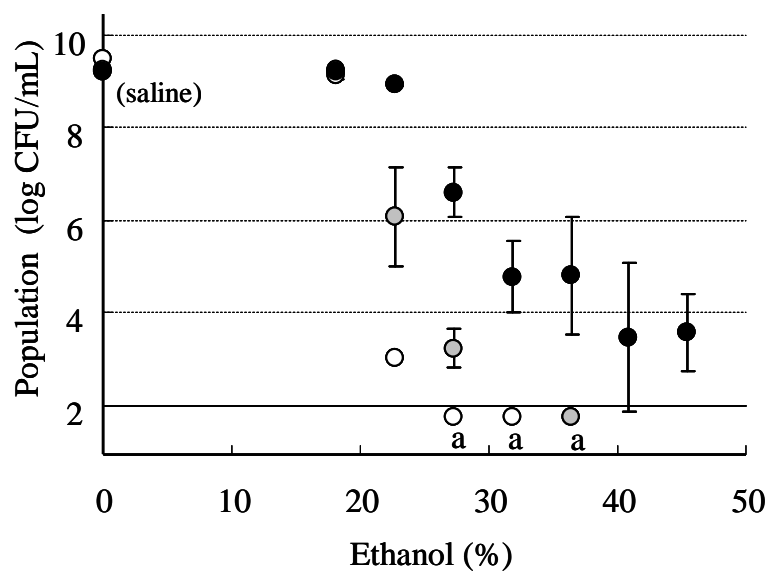
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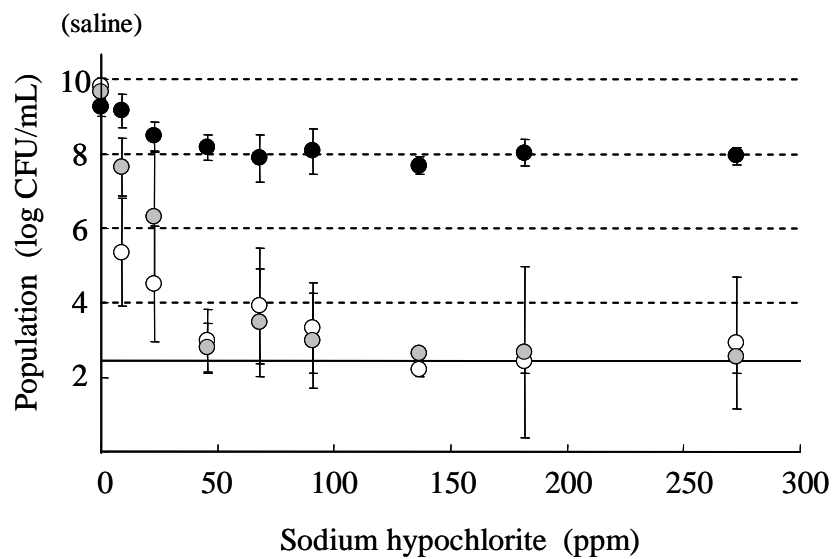




a)



b)



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