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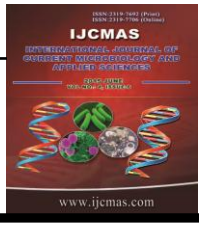

Original Research Article

Biological Treatment of Bacterial Biofilms from Drinking Water Distribution System in Lebanon

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A B S T R A C T

Drinking Water Distribution Systems provide opportunities for microorganisms that enter the drinking water to develop into biofilms. Antimicrobial agents, mainly chlorine, are used to disinfect drinking water, however, there are not yet standardized disinfection strategies with reliable efficacy and development of novel anti-biofilm strategies is still of major concern. In the present study the ability of *Lactobacillus acidophilus* and *Streptomyces* sp. cell free supernatants to inhibit the bacterial biofilm formation in Drinking Water Distribution System in Lebanon was investigated. Treatment with cell free supernatants of *Lactobacillus acidophilus* and *Streptomyces* sp. at 20% concentration resulted in average biofilm inhibition (52.89 and 39.66% respectively). A preliminary investigation about the mode of action of biofilm inhibition revealed that cell free supernatants showed no bacteriostatic or bactericidal activity against all the tested isolates. Pre-coating wells with supernatants revealed that *Lactobacillus acidophilus* cell free supernatant inhibited average biofilm formation (62.53%) by altering the adhesion of bacterial isolates to the surface, preventing the initial attachment step, which is important for biofilm production.

K e y w o r d s

Biofilm, Drinking water distribution system, *Lactobacillus acidophilus*, *Streptomyces* sp., Cell free supernatant, Adhesion

Introduction

Microorganisms that grow in the environment may enter the drinking water and attach to and grow on drinking water pipes and other surfaces, forming biofilms (WHO, 2014), as the most common means of growth in the environment. Bacterial socialization in the formation of biofilm has recently been described as a very successful form of life on earth (Flemming and Wingender, 2010) giving considerable advantages in terms of self protection for the microbial community from biological, physical, chemical and environmental stresses, including predation, desiccation, flux changes and disinfectants (USEPA, 2006; Wingender and Flemming, 2011).

Pathogens, even present below detection limit in water, can accidentally attach to biofilms which then can act as their

environmental reservoir and represent a potential source of water contamination, resulting in a potential health risk for humans if left unnoticed (Wingender and Flemming, 2011).

Most microorganisms present in drinking water distribution systems are harmless. However, infectious microorganisms may enter the distribution system and can survive and in some cases grow in the distribution system, increasing the potential for waterborne disease outbreaks (WHO, 2014).

Antimicrobial products, particularly chlorine, are used to disinfect drinking water. Although this strategy is widespread, there are not yet standardized disinfection strategies with reliable efficacy (Simões and Simões, 2013). Microorganisms may also develop resistance due to the indiscriminate use of disinfectants. As a result, bacteria will persist and drinking water quality may then deteriorate within the distribution system posing problems to public health (Kokare et al., 2009).

Development of novel anti-biofilm strategies is still of major concern. Secondary metabolites ranging from furanones to exopolysaccharides were proved to have anti-biofilm activity. *Escherichia coli* group II capsular polysaccharides, and marine *Vibrio* sp. exopolysaccharides were proved to have anti-microbial effect against bacterial biofilm formation (Abu Sayem *et al.*, 2011 and Jiang *et al.*, 2011). *Streptomyces sp. and Nocardiopsis sp.* isolated from the Arctic showed biofilm inhibitory activity against V. cholera due to the reactivity of the bioactive compound (exopolysaccharide or other adhesins) in the culture supernatant (Augustine et al., 2012) Selvin et al. (2010) and Abu Sayem et al. (2011), reported that bioactive compounds produced from marine invertebrates against biofilm-forming microorganisms might be produced by the associated microorganisms instead.

The aim of the present study is to investigate the ability of *Lactobacillus acidophilus* and *Streptomyces* sp. cell free supernatants to inhibit bacterial biofilm formation in Drinking Water Distribution System in Lebanon. A preliminary investigation about the mode of biofilm inhibition was evaluated.

Materials and Methods

Bacterial starins

Gram negative and Gram positive bacteria (31 isolates) that were used in the present investigation were isolated from drinking water distribution system in Lebanon, namely: *Citrobacter youngae, Citrobacter braakii, Enterobacter cloacae, Enterobacter intermedius, Klebsiella oxytoca, Klebsiella terrigena, Klebsiella pneumoniae ozaenae, Stenotrophomonas maltophilia, Pantoea spp., Hafnia alvei, Serratia marcescens, Serratia fonticola, Serratia plymuthica, Yersinia enterocolitica, Pseudomonas Flourescens/putida, Pasteurella pneumotropica/haemolytica, Pasteurella multocida, Chryseomonas luteola, Aeromonas hydrophila, flavimonas oryzihabitans, Acinetobacter, Alcaligenes, Staphylococcus aureus*, Coagulase negative *Staphylococcus* and seven different *E. coli* isolates that were shown to belong to the Shiga Toxin- producing *E. coli* and represented throughout the study as *E. coli* 1, *E. coli* 2, *E. coli* 3, *E. coli* 4, *E. coli* 5, *E. coli* 6 and *E. coli* 7. Gram negative bacteria and Gram positive *Staphylococci* were preliminary identified phenotypically.

Two different bacterial strains were used throughout the present work namely: *Lactobacillus acidophilus* was kindly provided from the microbiology lab of Ecole

Doctorale, Lebanese University, Tripoli and *Streptomyces* sp., kindly provided from cultural collection of microbiology lab at Faculty of Sciences, Alexandria University.

Detection of biofilm formation

Biofilm production of all the bacterial isolates under test was quantitatively investigated, one at a time, using the method of adherence to polystyrene microtiter plates proposed by Christensen *et al.* (1985), with some modifications. All the bacterial isolates were cultivated on Tryptic Soy Broth one at a time and incubated to initiate growth for 18 hours to reach an OD≤1 at 600 nm. The cultures were then diluted 1:100 with fresh medium (Hassan *et al.*, 2011), and 200 µl were used to inoculate sterile 96-well polystyrene microtiter plates. After incubation for 24 hours at 37ºC, the cultures were discarded and microtiter plates were washed with distilled water to eliminate the unattached cells. Attached cells were then fixed at 60ºC for 1 hour and stained with 1% crystal violet solution. Excess stain was removed by successive washings. The crystal violet in each well was solubilized with 200 µL of 96% ethanol, and microplate reader was used to measure the absorbance at 540 nm (Sosa and Zunino, 2009). Negative control wells contained sterile broth. The experiment was performed in triplicates and repeated three times. The interpretation of biofilm production was done according to the criteria of Stepanovic *et al.* (2007) (table 1).

Preparation of cell free supernatants

Lactobacillus acidophilus and *Streptomyces* sp. were allowed to grow, one at a time, at 37ºC for 24 hours in 100 ml MRS and GYM broth media respectively, dispensed in 250 ml Erlenmeyer flasks. At the end of the incubation period the cultures were centrifuged at 5000×g for 15 minutes to separate the cell pellets from the fermentation medium. Supernatants were then filtered through 0.45 µm PVDF filters. To ensure the sterility of the filtrates, 100 µl were spread onto Tryptic Soy Agar plates (Abu Sayem *et al.*, 2011). *Lactobacillus acidophilus* cell free supernatant was neutralized with 1 N NaOH to rule out the hypothesis of acid inhibition. To rule out the hypothesis of inhibition by the H_2O_2, CFS was treated with catalase (300 U/ml) then filter sterilized again (Ouali *et al.*, 2014).

Bacterial biofilm inhibition

Using cell free supernatant (CFS)

Cultures of bacterial isolates (200 µl each) were used to inoculate the sterile 96-well polystyrene microtiter plates along with the cell free supernatants of *Lactobacillus acidophilus* and *Streptomyces* sp. one at a time at concentrations of 5, 10 and 20% (v/v). Biofilm formation was detected after incubation for 24 hours at 37ºC. Negative control wells were inoculated with the same supernatant concentrations in sterile broth. The experiments were performed in triplicates and repeated three times.

Pre-coating the wells with cell free supernatant

Wells were treated with 200 µl of the *Lactobacillus acidophilus* and *Streptomyces* sp. cell free supernatants one at a time, for 24 hours at 37ºC. The un-adsorbed supernatant was withdrawn from the wells (Abu Sayem *et al.*, 2011). Pre-coated wells were inoculated with cultures of each of the tested isolates. The biofilm formation was detected after incubation for 24 hours at 37ºC. Negative control pre-coated wells were inoculated with sterile broth. The experiment was performed in triplicates and repeated three times.

Effect of cell free supernatants on established biofilms

Lactobacillus acidophilus and *Streptomyces* sp. supernatants were added, one at a time, to the 24 hours established biofilms of the different bacterial isolates in the microtiter plate and was then left at 37ºC in static conditions for another 24 hours.

Antibacterial activity of cell free supernatants using disc diffusion assay

Antimicrobial activity of the supernatants was assayed by disc diffusion susceptibility test (Clinical and Laboratory Standards Institute), using Muller-Hinton agar. Overnight cultures of the bacterial isolates under test in Tryptic Soy Broth were diluted to 0.5 McFarland (1×10^8 CFU/mL). The inocula were uniformly spread over the surfaces of agar plates and absorption of excess moisture was allowed to occur for 10 minutes. Sterile discs with a diameter of 10 mm were saturated with 50 µl of each of the supernatants and placed on inoculated Muller-Hinton agar plates. Plates were incubated at 37°C for 24 hours (Abu Sayem *et al.*, 2011).

Data analysis

The average biofilm formation was calculated and statistically significant decrease in biofilm formation was determined using paired sample test. The criteria for statistical significance was based on a (p<0.05).

Results and Discussion

Detection of biofilm formation

Ten out of the 31 tested isolates, were detected as strong (32.26%), 16 as moderate (51.61%) and 5 as weak (16.13%) biofilm producers. It is generally accepted that Drinking Water Distribution Systems are inhabited by different bacterial species that can form biofilms (Simões *et al.*, 2007 and Li *et al.*, 2010). Bacteria are typically the first microorganisms to colonize pipe surfaces. Once enough organic material adheres to the pipe surface - a process referred to as "conditioning" - bacteria can begin to attach (Mains, 2008).

Bacterial biofilm inhibition

Using cell free supernatant (CFS)

Results of the present investigation revealed that *Lactobacillus acidophilus* and *Streptomyces* sp. cell free supernatants inhibited biofilm formation of the bacterial isolates under test one at a time. Increase in CFS concentration showed an ascending trend in biofilm inhibition activity (tables 2 and 3), with maximum significant average inhibition (p<0.05) at 20% CFS concentration. *Lactobacillus acidophilus* and *Streptomyces* sp supernatants at 20% concentration showed average biofilm inhibition of 52.89 and 39.66% respectively (table 5). Ouali *et al.* (2014) reported that *Lactobacillus* sp. neutralized supernatant resulted in less adherence of *S. aureus* on the surfaces of glass slides and reported that Lactobacilli were considered as potentially antagonistic to spoilage and pathogenic bacteria because of their capabilities to produce inhibitory substances such as organic acids, H_2O_2 and bacteriocins. Hawthorn and Reid (1990) and Rodrigues *et al.* (2004) revealed that Lactobacilli compete with pathogens in urogenital and intestinal tracts and interfere with their adhesion on catheters device. On the other hand Augustine *et al.* (2012) also showed that 20% of the actinomycetes culture supernatant could inhibit up to 80% of the biofilm formation.

Table.1 Interpretation of biofilm formation

Average OD value	Biofilm production
≤ ODc	Non
ODc < ~ ≤ 2x ODc	Weak
2x ODc < ~ ≤ 4x ODc	Moderate
> 4x ODc	Strong

Table.2 Biofilm formation of the bacterial isolates under test using *Lactobacillus acidophilus* CFS

Isolate	Non treated ± SD	5% CFS ± SD	10% CFS ± SD	20% CFS ± SD
E.coli1	3.11 ± 0.11	2.38 ± 0.09	1.84 ± 0.07	1.18 ± 0.21
E. coli 2	3.41 ± 0.1	2.91± 0.12	1.65 ± 0.09	1.16 ± 0.19
E.coli3	5.77 ± 0.4	4.04 ± 0.14	2.46 ± 0.24	1.61 ± 0.15
E.coli4	2.24 ± 0.23	2.09 ± 0.17	1.24 ± 0.31	1.23 ± 0.08
E.coli5	2.14 ± 0.08	1.32 ± 0.11	1.20 ± 0.25	1.11 ± 0.06
E.coli6	2.73 ± 0.11	1.97 ± 0.13	1.54 ± 0.23	1.17 ± 0.18
E.coli7	4.15 ± 0.09	3.96 ± 0.15	2.22 ± 0.08	1.68 ± 0.22
Citrobacter youngae	1.85 ± 0.3	1.65 ± 0.21	1.24 ± 0.13	1.15 ± 0.07
Citrobacter braakii	1.63 ± 0.2	1.52 ± 0.11	1.32 ± 0.07	1.40 ± 0.1
Enterobacter cloacae	1.42 ± 0.5	1.36 ± 0.27	1.14 ± 0.16	0.90 ± 0.03
Enterobacter intermedius	3.08 ± 0.09	2.32 ± 0.13	1.60 ± 0.34	1.34 ± 0.16
Klebsiella oxytoca	4.37 ± 0.16	2.61 ± 0.15	2.53 ± 0.16	1.66 ± 0.25
Klebsiella terrigena	2.31 ± 0.2	1.26 ± 0.12	1.15 ± 0.18	1.06 ± 0.09
Klebsiella pneumoniae ozaenae	2.65 ± 0.07	2.43 ± 0.13	1.33 ± 0.11	1.24 ± 0.05
Stenotrophomonas maltophilia	1.88 ± 0.12	1.54 ± 0.15	1.43 ± 0.19	1.32 ± 0.09
Pantoea spp.	3.41 ± 0.05	2.71 ± 0.18	1.23 ± 0.21	1.17 ± 0.12
Hafnia alvei	2.75 ± 0.13	1.58 ± 0.09	1.19 ± 0.32	1.09 ± 0.09
Serratia marcescens	5.14 ± 0.14	5.11 ± 0.07	4.06 ± 0.1	2.10 ± 0.14
Serratia fonticola	2.81 ± 0.2	2.10 ± 0.4	1.81 ± 0.28	1.20 ± 0.23
Serratia plymuthica	2.59 ± 0.06	2.55 ± 0.18	1.79 ± 0.27	1.28 ± 0.11
Yersinia enterocolitica	3.78 ± 0.24	3.58 ± 0.21	2.71 ± 0.23	2.36 ± 0.07
Pseudomonas Flourescens/putida	2.52 ± 0.31	2.44 ± 0.09	1.26 ± 0.18	1.01 ± 0.04
Pasteurella pneumotropica/haemolytica	3.76 ± 0.12	2.93 ± 0.13	2.46 ± 0.22	1.91 ± 0.13
Pasteurella multocida	3.40 ± 0.07	3.38 ± 0.18	2.52 ± 0.25	1.96 ± 0.13
Chryseomonas luteola	8.51 ± 0.2	7.24 ± 0.08	6.29 ± 0.12	5.47 ± 0.21
Aeromonas hydrophila	1.67 ± 0.42	1.58 ± 0.27	1.53 ± 0.17	1.22 ± 0.1
Flavimonas oryzihabitans	4.61 ± 0.31	3.39 ± 0.21	2.86 ± 0.26	1.23 ± 0.23
Acinetobacter	8.51 ± 0.2	7.62 ± 0.14	5.73 ± 0.34	4.04 ± 0.33
Alcaligenes	4.87 ± 0.08	4.44 ± 0.34	3.42 ± 0.13	2.13 ± 0.14
Staphylococcus aureus	6.86 ± 0.26	6.66 ± 0.17	5.77 ± 0.24	3.70 ± 0.15
Coagulase negative *Staphylococcus*	4.48 ± 0.13	4.45 ± 0.09	3.42 ± 0.15	2.07 ± 0.26

Table.3 Biofilm formation of the bacterial isolates under test using Streptomyces sp. CFS

Isolate	Non treated ± SD	5% CFS ± SD	10% CFS ± SD	20% CFS ± SD
E.coli1	3.11 ± 0.11	2.11 ± 0.24	1.87 ± 0.15	1.32 ± 0.34
E. coli 2	3.41 ± 0.1	2.94 ± 0.14	2.56 ± 0.09	1.97 ± 0.23
E.coli3	5.77 ± 0.4	4.96 ± 0.22	3.63 ± 0.13	2.07 ± 0.39
E.coli4	2.24 ± 0.23	2.04 ± 1.10	1.86 ± 0.09	1.47 ± 0.28
E.coli5	2.14 ± 0.08	1.97 ± 1.05	1.62 ± 0.08	1.16 ± 0.13
E.coli6	2.73 ± 0.11	1.98 ± 0.24	1.68 ± 0.17	1.45 ± 0.21
E.coli7	4.15 ± 0.09	3.57 ± 1.2	3.16 ± 0.05	2.32 ± 0.15
Citrobacter youngae	1.85 ± 0.3	1.65 ± 0.21	1.52 ± 0.19	1.41 ± 0.08
Citrobacter braakii	1.63 ± 0.2	1.53 ± 0.09	1.42 ± 0.11	1.38 ± 0.14
Enterobacter cloacae	1.42 ± 0.5	1.45 ± 0.31	1.35 ± 0.27	1.23 ± 0.17
Enterobacter intermedius	3.08 ± 0.09	2.73 ± 0.17	2.36 ± 0.31	1.51 ± 0.24
Klebsiella oxytoca	4.37 ± 0.16	2.56 ± 0.21	2.43 ± 0.10	3.36 ± 0.18
Klebsiella terrigena	2.31 ± 0.2	2.17 ± 0.13	1.75 ± 0.25	1.53 ± 0.22
Klebsiella pneumoniae ozaenae	2.65 ± 0.07	2.29 ± 0.05	2.01± 0.16	1.46 ± 0.11
Stenotrophomonas maltophilia	1.88 ± 0.12	1.50 ± 0.09	1.45 ± 0.10	1.25 ± 0.12
Pantoea spp.	3.41 ± 0.05	2.83 ± 0.14	2.23 ± 0.21	1.26 ± 0.09
Hafnia alvei	2.75 ± 0.13	1.56 ± 0.19	1.29 ± 0.13	1.13 ± 0.07
Serratia marcescens	5.14 ± 0.14	5.17 ± 0.23	4.96 ± 0.08	3.83 ± 0.19
Serratia fonticola	2.81 ± 0.2	2.66 ± 0.17	2.31 ± 0.22	1.23 ± 0.30
Serratia plymuthica	2.59 ± 0.06	2.52 ± 0.09	2.12 ± 0.13	1.02 ± 0.43
Yersinia enterocolitica	3.78 ± 0.24	3.35 ± 0.12	3.21 ± 0.09	2.29 ± 0.17
Pseudomonas Flourescens/putida	2.52 ± 0.31	2.44 ± 0.16	2.26 ± 0.18	2.13 ± 0.07
Pasteurella pneumotropica/haemolytica	3.76 ± 0.12	3.10 ± 0.07	2.53 ± 0.24	2.13 ± 0.15
Pasteurella multocida	3.40 ± 0.07	3.53 ± 0.18	3.27 ± 0.16	2.86 ± 0.21
Chryseomonas luteola	8.51 ± 0.2	7.32 ± 0.27	6.61 ± 0.15	4.34 ± 0.46
Aeromonas hydrophila	1.67 ± 0.42	1.34 ± 0.25	1.28 ± 0.10	1.14 ± 0.07
Flavimonas oryzihabitans	4.61 ± 0.31	3.70 ± 0.29	3.21 ± 0.31	2.79 ± 0.14
Acinetobacter	8.51 ± 0.2	7.88 ± 0.08	6.83 ± 0.42	5.83 ± 0.26
Alcaligenes	4.87 ± 0.08	4.69 ± 0.17	3.98 ± 0.26	3.31 ± 0.13
Staphylococcus aureus	6.86 ± 0.26	6.42 ± 0.15	5.96 ± 0.31	4.99 ± 0.29
Coagulase negative *Staphylococcus*	4.48 ± 0.13	4.38 ± 0.09	3.87 ± 0.23	2.91± 0.21

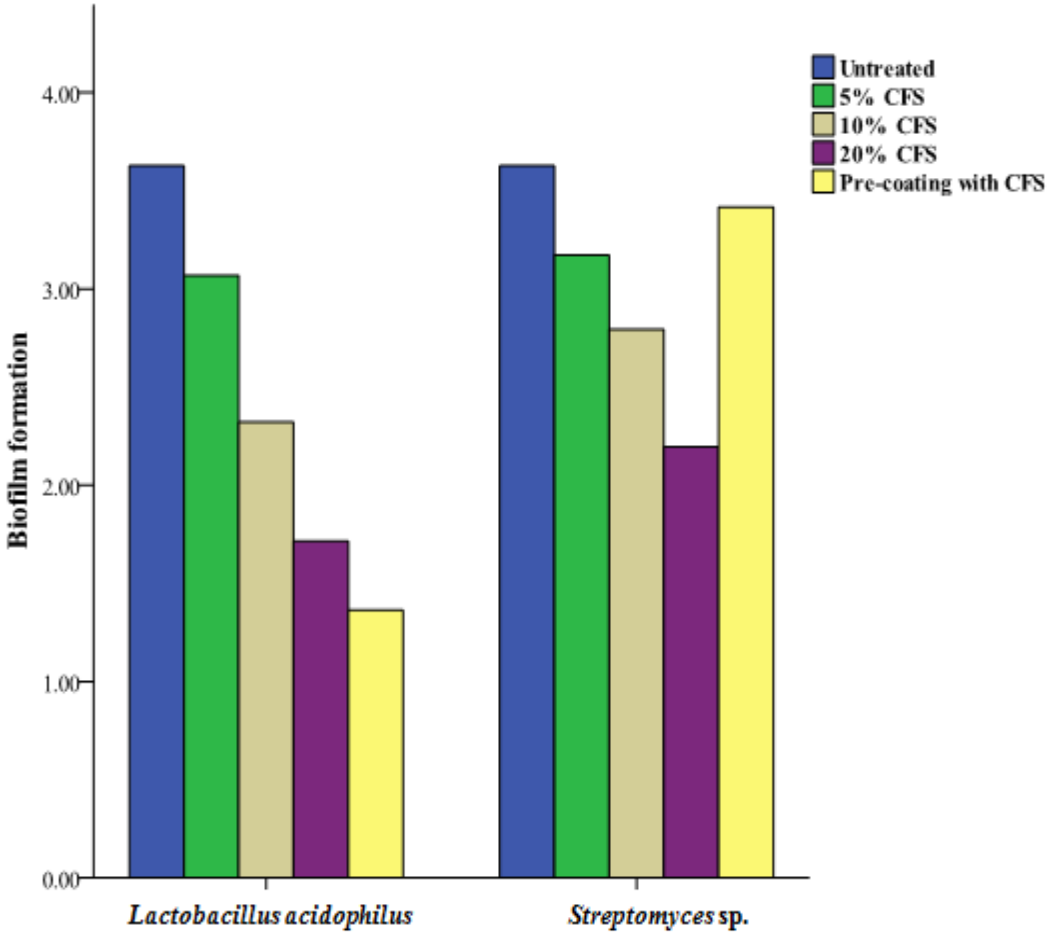
Table.4 Bacterial biofilm formation of the bacterial isolates under test after pre-coating with CFS

Isolate	Untreated ± SD	Pre-coating with *Lactobacillus acidophilus* CFS ± SD	Pre-coating with *Streptomyces* sp. CFS ± SD
E.coli1	3.11 ± 0.11	1.07 ± 0.06	2.42 ± 0.16
E.coli 2	3.41 ± 0.1	0.98 ± 0.04	3.58 ± 0.10
E.coli3	5.77 ± 0.4	1.10 ± 0.09	4.48 ± 0.21
E.coli4	2.24 ± 0.23	0.92 ± 0.07	2.42 ± 0.11
E.coli5	2.14 ± 0.08	0.97 ± 0.04	2.84 ± 0.09
E.coli6	2.73 ± 0.11	0.93 ± 0.08	1.84 ± 0.13
E.coli7	4.15 ± 0.09	1.09 ± 0.10	4.27 ± 0.27
Citrobacter youngae	1.85 ± 0.3	1.06 ± 0.05	1.91± 0.04
Citrobacter braakii	1.63 ± 0.2	0.96 ± 0.08	1.73 ± 0.18
Enterobacter cloacae	1.42 ± 0.5	0.99 ± 0.11	1.52 ± 0.06
Enterobacter intermedius	3.08 ± 0.09	1.10 ± 0.07	3.48 ± 0.12
Klebsiella oxytoca	4.37 ± 0.16	1.12 ± 0.13	4.14 ± 0.22
Klebsiella terrigena	2.31 ± 0.2	1.09 ± 0.04	2.13 ± 0.15
Klebsiella pneumoniae ozaenae	2.65 ± 0.07	1.03 ± 0.11	2.36 ± 0.09
Stenotrophomonas maltophilia	1.88 ± 0.12	1.18 ± 0.09	1.73 ± 0.12
Pantoea spp.	3.41 ± 0.05	1.02 ± 0.03	3.29 ± 0.23
Hafnia alvei	2.75 ± 0.13	1.12 ± 0.07	2.31 ± 0.15
Serratia marcescens	5.14 ± 0.14	1.54 ± 1.10	4.83 ± 0.29
Serratia fonticola	2.81 ± 0.2	1.13 ± 0.14	2.23 ± 0.21
Serratia plymuthica	2.59 ± 0.06	1.15 ± 0.06	2.19 ± 0.08
Yersinia enterocolitica	3.78 ± 0.24	1.60 ± 0.17	3.63 ± 0.14
Pseudomonas Flourescens/putida	2.52 ± 0.31	1.06 ± 0.03	2.53 ± 0.10
Pasteurella pneumotropica/haemolytica	3.76 ± 0.12	1.32 ± 0.14	3.58 ± 0.16
Pasteurella multocida	3.40 ± 0.07	1.79 ± 0.21	3.32 ± 0.19
Chryseomonas luteola	8.51 ± 0.2	3.50 ± 0.16	7.71 ± 0.31
Aeromonas hydrophila	1.67 ± 0.42	1.12 ± 0.05	1.82 ± 0.17
Flavimonas oryzihabitans	4.61 ± 0.31	1.42 ± 0.10	4.39 ± 0.23
Acinetobacter	8.51 ± 0.2	3.08 ± 0.23	7.91 ± 0.18
Alcaligenes	4.87 ± 0.08	1.42 ± 0.09	4.80 ± 0.25
Staphylococcus aureus	6.86 ± 0.26	2.56 ± 0.13	6.02 ± 0.13
Coagulase negative *Staphylococcus*	4.48 ± 0.13	1.86 ± 0.07	4.50 ± 0.21

Table.5 Average biofilm formation and inhibition percentage of isolates under test after treatment with CFS

Condition	Avarage biofilm formation	Inhibition %
Untreated	3.63	100
Lactobacillus acidophilus 5% CFS	3.07	15.42
Lactobacillus acidophilus 10% CFS	2.32	36.08
Lactobacillus acidophilus 20% CFS	1.71	52.89
Streptomyces sp. 5% CFS	3.17	12.67
Streptomyces sp. 10% CFS	2.79	23.14
Streptomyces sp. 20% CFS	2.19	39.66
Pre-coating with *Lactobacillus acidophilus* CFS	1.36	62.53

Figure.1 Average biofilm formation of all the tested isolates, treated and untreated



Pre-coating the wells with cell free supernatants

Pre-coating wells with CFS of *Lactobacillus acidophilus* resulted in biofilm inhibition in all the tested isolates (table 4) with highly significant average inhibition ($p<0.05$) 62.53% (table 5 and figure 1). However, no significant inhibition ($p>0.05$) was detected in the wells pre-coated with CFS of *Streptomyces* sp. *Lactobacillus acidophilus* CFS might reduce the adherence of cells to the surfaces of the wells. The optimum anti-adhesive properties of *Lactobacillus delbrueckii* against *E. coli* was reported by Abedi *et al.* (2013).

Vuotto *et al.* (2013) reported that *L. brevis* prevents the biofilm formation by *Prevotella melaninogenica*. Cell free extract of *Streptomyces akiyoshinensis* succeeded to reduce the cell surface hydrophobicity of *S. pyogenes* and inhibited biofilm formation (Nithyanand *et al.,* 2010). However in the present study *Streptomyces* sp. seemed to inhibit biofilm formation using another strategy. Other strategies for biofilm treatment were to interfere with the bacterial cell-to-cell communication ('quorum-sensing') system (Suga and Smith, 2003).

Augustine *et al.* (2012) reported that the bioactive compound present in *Nocardiopsis* sp. supernatant may be a source for the development of a potential quorum sensing inhibitor against *V. cholerae*. Small molecules and enzymes have been investigated also to inhibit or disrupt biofilm formation (Chen *et al.,* 2013). The biofilm inhibition property of AiiA enzyme from *Bacillus* sp. against *V. cholera*, and AiiA enzyme homologues has been detected in *Agrobacterium tumefaciens*, *Rhodococcus* sp., *Arthrobacter* sp. (Rasmussen and Givskov, 2006 and Augustine *et al.,* 2010).

Effect of cell free supernatants against established biofilms

Treatment of 24 hours established biofilm with *Lactobacillus acidophilus* and *Streptomyces* sp. supernatants showed no significant effect ($p>0.05$) on average biofilm formation. The effect was found to be much lower compared with that of the initial addition or pre-coating of the wells with the supernatant. This might be due to the modification of the target surface to prevent the initial attachment step, which is important for biofilm production (Abu Sayem *et al.,* 2011).

Antibacterial activity of cell free supernatants using disc diffusion assay

Lactobacillus acidophilus and *Streptomyces* sp. supernatants showed no bacteriostatic or bactericidal activity against all the tested isolates. Polysaccharides with nonbiocidal antibiofilm properties have been isolated from cell-free biofilm extracts of several species. Given their nonbiocidal mode of action, as well as their biocompatibility and biodegradability, antibiofilm polysaccharides could be a promising strategy suitable for the treatment and prevention of biofilm-related infections. Potential applications of such polysaccharides could be coating surfaces of indwelling medical devices or even using antibiofilm polysaccharide-producing bacteria in probiotics to outcompete pathogens (Rendueles *et al.,* 2013).

The present study highlighted the impact of *Lactobacillus acidophilus* CFS on biofilm formation of the bacterial strains isolated from the drinking water distribution system in Lebanon by altering their adhesion to the surface. Further studies are required for the identification of bioactive compound

responsible for the inhibition of biofilm formation and the possibility of its application in the drinking water pipes. However the mechanism of biofilm inhibition by *Streptomyces* sp. is still unclear and requires further research.

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