



# ISOLATION AND COMPARISON OF BIOFILM FORMATION BY *LACTOBACILLUS* SPECIES

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## Abstract

Infectious microorganisms if infected human require to be treated. Biofilm was accumulate of the colonies this study was done from period February 2018 to January 2019 in this research we collect 90 samples divided between 60 clinical samples and 30 healthy control all these samples collected from different gender randomly to detected lactobacillus species and them detected the ability of biofilm formation because there were association between biofilm, biofilm quantity and Pathogenicity of pathogens and classified these species from largest one in biofilm formation to smallest once according to measure mean and standard deviation of them *Lactobacillus acidophilus* followed with *L. plantarum*, *L. salivarius*, *L. casei*, *L. fermentum* and the list one was *L. brevis*.

**Key words :** Biofilm, Lactobacillus, Daman, Rogosa, Sharpe, Transposon.

## Introduction

In most time the adherence of microorganisms to the surface help to maintains microbial life skeleton. Surface adherence was a good way to some microorganisms in microenvironment to prevent keep out of them. The biofilm formation occurred in multiple steps this fact fixed with genetic research of single species biofilm formation (Watnick & Kolter1,1999).

The first seen of biofilm in seventeen century as form of caries in tooth (Costerton *et al.*,1999). Physical and chemical properties of biofilm not known well until beginning of 1970 (Reid & Burton, 2002). Growth and movement of marine bacteria show to be enhance when attached to sea surface this called Bottle Effect firstly seen with Heukelekian and Heller (Probert &, Gibson, 2002). On other way, characteristics of biofilm was study by curious in which used electron microscope in which give highly resolution if compared with light microscope. The employment of scanning with electron and transmission electron microscopy allowed to identify biofilm on trickling filters in a waste product water after that it becomes refer to accumulation of types of

microorganisms as biofilm (Stoodley *et al.*, 2002).

During the early 20th century, many of the early pioneer of microbiology discovered that some bacteria have ability for attachment to biotic and a biotic surfaces. Sessile bacteria are different from their planktonic counterpart; it has an altered phenotype and physiologically. The formations of bacterial biofilms on surfaces appear to be universal bacterial strategy for survival in both nature and disease (Costerton *et al.*, 1994). Steps of biofilm formation were studies genetically for many microorganisms like bacteria *Escherichia coli* (Pratt & Kolter, 1998), some pseudomonas species such as *Pseudomonas aeruginosa* (O'Toole & Kolter, 1994) and also *Vibrio cholera* was study (Watnick & Kolter, 1999). These genetic research include used simpler and on transposon mutants in which grown in 96 well plates (Cowan & Fletcher, 1987; Genevaux, *et al.*, 1996; O'Toole & Kolter, 1998).

## Materials and Methods

### Sample

This study we use 90 specimens divided to 30 healthy control and 60 clinical samples from period February 2018

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to January 2019, were taken randomly from both genders in different ages in Al-Kut, Wasite governorate. All persons don't receive any antimicrobial therapy, at least one week before sampling.

### Specimens collection and processing

Microbiologically, infection was evaluated by culture on different media which is used for isolation and cultivation of lactobacillus species and these included ((Nutrient agar, Schaedler broth, Mrs (De Man, Rogosa, Sharpe) agar and Mrs (De Man, Rogosa, Sharpe) broth)), after that microscopic examination was done. clinical specimens was collected and cultured immediately after collection stained with (Gram2 s) stain. this was done during the beginning of February 2018 to January 2019, samples were cultured immediately after that the plates were incubated at 37°C for 24-48 hours.

### Identification of Isolates

In this study O'Toole methods(O'Toole & Kolter,1998) was used for monitoring biofilm formation by used six lactobacillus species on polystyrene microtiter plates. Biofilms formed in hydrophilic treated wells, these techniques was done by following steps:

1. Transfer one isolated colony of *Lactobacillus* spp. from MRS agar to MRS broth and incubated for overnight under 5% CO<sub>2</sub> at 37°C to get a suspension broth of *Lactobacillus* spp.
2. Transfer 1ml of bacterial suspension to the wells of microtiter plate.
3. Incubated microtiter plate for 24-48h under 10% CO<sub>2</sub> at 37 °C.
4. Poured the suspension media and wash the wells

by distilled water to leave the biofilm which formed in the wells of microtiter plate.

5. Add 250ul of 1% solution of crystal violet to each well.
6. Microtiter plates were left to stand for 10 minute at room temperature
7. Rinsed the wells thoroughly with distilled water.
8. 1ml 95% ethanol added to each well, color extraction was allowed to process for 10minute
9. Transfer extraction ethanol solution to new microtiter plate, and the absorbance of the solution was determined by spectrophotometer 600nm.

## Results and Discussion

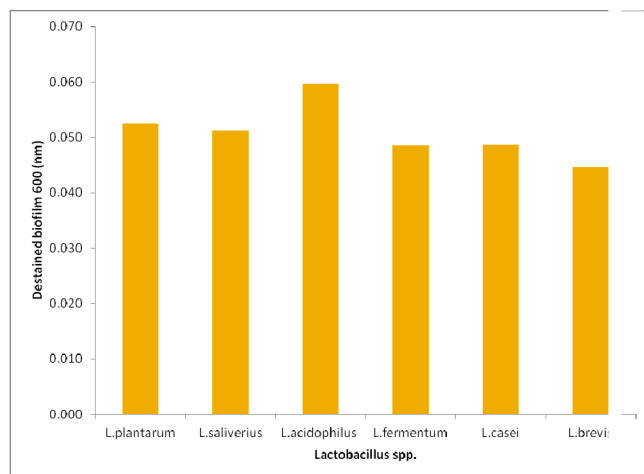
Biofilm was affected by many factors like type of bacteria in which formed of its, strength of bacterial attached to different surfaces and amount of extracellular slime formation (Jones & Versalovic, 2009; Fernández *et al.*, 2015). Biofilm formation affected with other factors like type and nature of these surfaces in which may be smooth or rough and physical factors such as electrical charge, hydrophobicity and hydrophilicity (Leccese *et al.*, 2015; De Angelis *et al.*, 2015; Rybalchenko *et al.*, 2015; Kiew *et al.*, 2014 ).

In this study we use 90 specimens divided to 30 healthy control and 60 clinical samples from period February 2018 to January 2019, these clinical specimens used in 12 experiments in each experiment we use six species of lactobacillus (*L. plantarum* followed by *L. acidophilus* after that *L. salivarius* then *L. casei* and *L. brevis*) isolated by O tool method, *L. acidophilus* appeared as big one species in biofilm formation with

**Table 1:** Spectrophotometer results of twelve experiments species, means and standard divisions of six lactobacillus species isolated in Al-Kut city of Wasit government.

	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5	Exp.6	Exp.7	Exp.8	Exp.9	Exp.10	Exp.11	Exp.12	Mean	SD
<b>L.plantarum</b>	0.0686	0.0539	0.0506	0.0608	0.0479	0.0329	0.063	0.0479	0.0594	0.0394	0.0567	0.0478	0.0524	0.010132
<b>L.salivarius</b>	0.0451	0.0509	0.0445	0.0778	0.0441	0.0513	0.0492	0.0441	0.0603	0.0436	0.0548	0.0494	0.0512	0.09776
<b>L.acidophilus</b>	0.061	0.064	0.0456	0.0742	0.0665	0.0632	0.0673	0.0665	0.0656	0.0459	0.0481	0.0485	0.0597*	0.009895
<b>L.fermentum</b>	0.055	0.0371	0.0419	0.0493	0.0497	0.0462	0.0588	0.0497	0.0588	0.0391	0.0449	0.0516	0.0485	0.09895
<b>L.casei</b>	0.0434	0.0552	0.0558	0.0388	0.0422	0.0488	0.0684	0.0422	0.0588	0.0375	0.0535	0.0393	0.0486	0.008283
<b>L.brevis</b>	0.0366	0.0395	0.06	0.0402	0.0437	0.0444	0.043	0.0437	0.0474	0.0489	0.0465	0.0411	0.0445	0.005977

Optical density at (600 nm) of destained biofilms of lactobacillus spp



**Fig. 1:** The lactobacillus species with biofilm formation for each one.

significant difference ( $P < 0.05$ ) compared with others species as seen in (Table 1 and Fig. 1).

According to report of united National Institutes of Health, “Biofilms are medically important, accounting for over 80% of microbial infections in the body”. The antimicrobial treatment no success in most time to prevent biofilm formation for this reason chronic infections may be occurred and required in some time for surgical removal of infected areas, according to that need to other researches for develop treatment against bacterial biofilm (Ignatova-Ivanova *et al.*, 2017). The research on microbial exopolysaccharides is attracting increased attention By the analysis of the microbial biofilm we recommend to use all methods in combination, as every of them provides different information about the type and the mechanisms of synthesis biofilm and the exopolysaccharides. In this study most biofilm producing strains of genus lactobacillus was identified, Further work is needed to investigate other applications and function in vitro of these biofilms.

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