
The Investigation of Biofilm Formation by *Kluyveromyces lactis* and *Kluyveromyces lactis drosophilarum* on Biopolymer Composite Supports

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Abstract

The biofilm form of *Kluyveromces lactis* NRRL Y-8279 and *Kluyveromces lactis drosophilarum* NRRL Y-8278 were done mature on some biopolymer composite support (BCS) to count colony forming unites (CFU). These supports consisted of (w/w) polypropylene, (w/w) soybean hulls, (w/w) yeast extract, (w/w) soybean flour, and (w/w) bovine albumin. These composites were effectively on the growing of biofilm formation. This form of yeasts was analyzed to use Stripping-Sand method for each one on diverse composites such as BCS₁, BCS₂, BCS₃ and BCS₄. Yeasts were showed the visible biofilm formations on supports. Biofilm formation of *K. lactis* and *K. l. drosophilarum* were performed 1.2×10^9 and 1.6×10^{10} CFUml⁻¹ on the BCS₂. The materials especially polypropylene that were included by BCS₂ are critically to produce biofilm formation of yeasts on some applications such as food, biomedical, industries and laboratories. The results of this paper will be usefully on these applications and to notice about the form of polypropylene in the supports how it changes the formation of biofilm by yeasts.

Keywords: Biofilm, biopolymer, polypropylene, yeast

Kluyveromces Lactis ve *Kluyveromces Lactis Drosophilarum*'un Biyofilm Yapılarının Biyopolimer Kompozit Destekler Üzerindeki Araştırılması

Özet

Kluyveromces lactis NRRL Y-8279 ve *Kluyveromces lactis drosophilarum* NRRL Y-8278 mayalarının biyofilm yapısı bazı biyopolimer material destekler üzerinde geliştirilip, bu kültürlerin biyofilm ölçümleri birim alandaki koloni sayıları analiz edilerek hesaplanmıştır. Bu destekler ağırlık olarak (w/w) polipropilen, soya tanesi, maya özütü, soya tozu, sığır albumin ve mineral tuzlardan oluşmuşlardır. İlgili materyaller biyofilm gelişiminde önemli bir etkiye sahiptirler. Mayaların bu özelliği (biyofilm yapısı) Stripping-Sand metodu kullanılarak her bir destek için (BCS₁, BCS₂, BCS₃ ve BCS₄) ayrı ayrı hesaplanmıştır. Araştırmalar mayaların ilgili destekler üzerinde gözle görünebilir biyofilm oluşturdıklarını gösterdi. *K. lactis* ve *K. l. drosophilarum* kültürleri üzerinde biyofilm ölçümü sırası ile BCS₂ desteği üzerinde 1.2×10^9 ve 1.6×10^{10} CFUml⁻¹ olarak hesap edildi. Özellikle bu desteğin içerdiği polipropilenin kullanıldığı gıda, biyomedikal, endüstri ve laboratuvar gibi bazı alanlarda oluşacak biyofilm yapısında önemli bir yere sahip olduğu görülmüştür. Bu çalışmanın sonucu bazı uygulamalarda çok yararlı olacak ve günümüzde mayaların biyofilm yapıları oluşturmaları için polipropilen gibi destekler üzerine dikkatleri çekecektir.

Anahtar kelimeler: Biyofilm, biyopolimer, polipropilen, maya

INTRODUCTION

The formation of yeast is better described day by day with the developing technology. One of these descriptions is the biofilm formations. These forms are essential illuminated in biofilm properties. Therefore it is very important to know the biofilm formations. So biofilm can be defined as a community of living organisms with their own produced a adhering to the surface in a layer of jelly-type. Microorganisms receive necessary substrate to life and growth from outer surface or inside surface of film (Aktaş, 2006). These systems have more organisms so it is capable of producing a higher organic matter yield such as biofuels, enzyme, antibiotic nonetheless purification can be provided a significantly with these systems (Nasib et al., 2005). Also the biofilm properties of microorganism's resistance attained as a result it is important consequences in medicine. Biofilm formation also tends to increase with the hydrophobicity of the surface material. Biofilms form much more rapidly on teflon and other plastics than glass or metal. Possibly this is due to differences in hydrophobicity of the surfaces and ionic charges (Holzapfel et al., 1998). Biofilms are significant clinically as well as industrially. Clinically, biofilms are important as the source of persistent infections. They cause the dental caries and nosocomial infections, as well as a variety of other infections and diseases (Costerton et al., 1999). Biofilm is harmful in many area but they are beneficial in some cause at the industries. For instance, natural biofilm can reduce heat transfer in heat exchangers and cooling towers, foul reverse osmosis membranes and

contaminate food processing equipment (Mortensen and Conley, 1994; McDonogh, 1994; Carpentier and Cerf, 1993). Biofilm forms are used industrially to achieve several aims including the treatment of wastewater for removal of organics (Taras et al., 2005; Hall et al., 1987), and heavy metals (Meyer and Wallis, 1997). The biofilm formations have not detected any standard method. A variety of in vitro models using different substrates-devices have been described. Some investigators developed in vitro models on the basis of different plastics, glass slides, perfused biofilm fermenters, cylindrical cellulose filters, germanium substrata, microtiter plates and tissue culture flasks. These models have been used to investigate the effect of different variables, including flow, growth phase, nutrients and physiological conditions on fungal biofilm formation, morphology and architecture (Nett and Andes, 2006; Chandra, 2008; Uppuluri and Lopez-Ribot, 2010).

The biofilm formation of yeast carried out with some mixture supports such as polimer and agriculture materials such as this study. Polymers are light, cheap and easily shapeable organic compounds. Together with their superior properties such as being chemically inert and not being subject to corrosion, their mechanical properties being generally enough and their suitability for using in various purposes, they draw attention of not only chemists but various kinds of people working in different fields such as mechanical, chemical, textile, industrial and physics engineering. The importance of polymers is also big in terms of medicine, biochemistry and molecular biology (Saçak, 2004). The presence of

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multiple species allows for the treatment of waste streams that are diverse in composition and that fluctuate in component concentration. The BCS disc that consistently demonstrated the highest performance contained 50% (wt/wt) PP, 35% (wt/wt) soybean hulls, 5% (wt/wt) soybean flour, 5% (wt/wt) yeast extract, 5% (wt/wt) dried bovine albumin, and mineral salts (Yönten, 2010). Hence yeasts immobilize to the supports that they have some valuable nutrients.

The aim and objective of this present study is to analyze the biofilm formation capacity of *K. lactis* and *K. l. drosiphilarum* on the biopolymer supports that consisted of (w/w) polypropylene, (w/w) soybean hulls, (w/w) yeast extract, (w/w) defatted soybean flour, and (w/w) bovine albumin and to compare the ability of forming biofilm of these yeasts. Therefore these reports indicated that the biofilm population of industrial yeasts may analyze on this supports and it will be useful on formation of microorganism. As a result of this, *K. lactis* and *K. l. drosiphilarum* acquired resistance is important consequences in medicine and have significant and costly effects of corrosion on some valuable devices in the clinics, offices, and factories where cause rotting on the devices.

MATERIAL AND METHOD

Glucose, ACS grade and lactose KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$, NaCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, sulphuric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Sigma, Aldrich, USA) and agar, peptone, yeast, malt extracts were purchased from Acumedia (Michigan, USA).

Biopolymer supports was donated by Demirci, Iowa State University.

Yeast Strain and Maintenance

K. lactis and *K. l. drosiphilarum* used throughout this study, was kindly donated by NRRL (Northern Region Research Laboratories) culture collection (Peoria, IL, USA). These cultures were used in industrial area so they were chosen. First, lyophilized yeast was re-activated in 0.5 ml yeast malt extract medium (both at 3 g l^{-1} concentration) for 2–3 min., then culture was aseptically spread on solid agar slants involving 3 g l^{-1} yeast extract, 3 g l^{-1} malt extract, 5 g l^{-1} peptone, 10 g l^{-1} glucose and 20 g l^{-1} agar in distilled water which was previously autoclaved at $121 \text{ }^\circ\text{C}$ for 15 min. The inoculated solid medium was incubated at $30 \text{ }^\circ\text{C}$ for 4 days for appropriate growth and stored at $4 \text{ }^\circ\text{C}$ for further uses. The solid medium culture was prepared monthly for the maintenance (Yönten, 2010).

Biopolymer Supports

Biopolymer supports used in this work was showed Table 1 and which consisted of (w/w) polypropylene, (w/w) soybean hulls, (w/w) yeast extract, (w/w) defatted soybean flour, and (w/w) bovine albumin (Ho et al., 1997). BCS were manufactured; discs (3.2 mm I.D. , 12.7 mm O.D.) by methods described by (Demirci et al., 1997). The discs were given shape by high-temperature extrusion in a Brabender PL2000 co-rotating twin-screw extruder. The BCS pipes were prepared at Iowa State University and sent to our laboratory.

As shown in Fig. 1, the BCS pipes were then cut into approximately 3 mm slices with a band saw in the machine shop and the image of colonies of yeasts

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were given with various dilutions in Fig. 1.

Table 1. The per cent weight substances contained in the biopolymer composite supports.

Support	% (w/w) weight						
	PP	SH	SF	YE	RB	Ba	Salt
BCS ₁	50	40	10	–	–	–	+
BCS ₂	50	35	5	5	–	5	–
BCS ₃	50	40	5	5	–	–	+
BCS ₄	50	35	5	5	5	–	+

In the table PP (Polypropylene), Soybean heed (SH), Soybean Flour (SF), Yeast Extract (YE), R Bovine Enzyme (RB), Bovine Albumin (BA)

Repeated-Batch Fermentations Test-Tube Fermentations

2.0 g BCS were taken to 25×150 mm culture tubes. After they sterilized at 121 °C for 30 minutes in the autoclave. After finished of sterilization, BCS added 10 mL sterile % 5 (w/v) GM (glicose medium) 0.6 % (w/v) YE (Yeast extract) to sterile tubes. The

tubes were incubated to confirm an equilibrium with shaking at 30°C and 130 rpm for 24 h. The decantation was carried out aseptically to take some particulars. 10 mL sterile GM-YE medium was added to the tubes. After each tube was inoculated with 0.1 mL of yeasts at 30 °C for 24 h. The decantation system was carried out again to again in a day. This experimental process was carried out for each 6 days. After that 5 BCS disks were taken to analyze. Biofilm formation on BCS disks were determined by the Stripping -Sand method (Ho et al. 1997).

Biofilm Analysis

The analysis of CFUml⁻¹ and CFUg⁻¹ was done using Stripping- Sand method. In this method, the five BCS were taken to 100 ml sterile % 0.1 (w/v) peptone-waters for cleaning. The process of cleaning was carried out by the turning of the tubes.

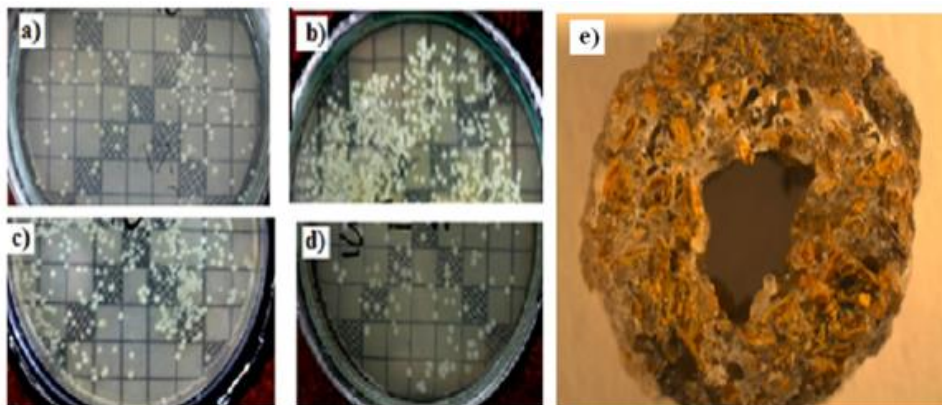


Figure 1. The image of culture colonies after inoculation to the glass plates. a) 10⁴ times dilution b) 10⁶ times dilution c) 10⁵ times dilution d) 10³ times dilution. e) The stereo microscope image of biopolymer composite support disk (3 mm).

Then 5 g of sterile sand and 9 ml 0.1% (w/v) peptone-waters added to the tubes. The culture tube was subsequently vortexed vigorously at 30

second intervals for a total of 1.5 min. The sample from each tube was serially diluted, and CFU of the 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ dilutions were determined by

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using yeasts MRS agar spread plates in duplicate by using expanding methods. CFU counts for the reliability of each planting were done two times. The plates were inoculated at 30 °C for 48 h. CFU counts of yeast were performed. The result of biofilm formation in the different dilutions of Fig. 2 as shown is determined by counting the colonies present.

According to the seed diluted in the material of the tubes the number of living cells was calculated by the formula (Halkman, 2005).

$$\frac{\text{Count}(CFU)}{\text{ml}} = \frac{CN \cdot DF}{V} \quad (a)$$

$$\frac{DF}{1} = \frac{1}{DR} \quad (b)$$

$$\frac{\text{Count}(CFU)}{g} = \frac{CN \cdot DF}{w} \quad (c)$$

In equation (a) *CN* is colony count, *DF* is dilutions factor and *V* is the volume (ml) that were transferred into Petri dishes with a dilution tube. In equations (b and c) *DR* is the ratio of dilutions *w* is the weight of 5 BCS disks (Halkman, 2005), according to reliable CFU counts should be between 50-250 as shown Fig. 2 BCS counts of the clamshell to the tube cultures at 70 °C taking 24 h was allowed to dry in the oven. After this period, 5 BCS disks weighed on precision scales for the account of biofilm formation.

RESULT AND DISCUSSIONS

The Analysis of Biofilm Formation

Recent development of biotechnology has reviled the need for the biofilm preparations and unique properties and advantages of its structure. In current study, biofilm formation of some yeast was investigated in terms of biofilm density and thickness. The biofilm formation for microorganisms was analyzed using Strapping Sand method.

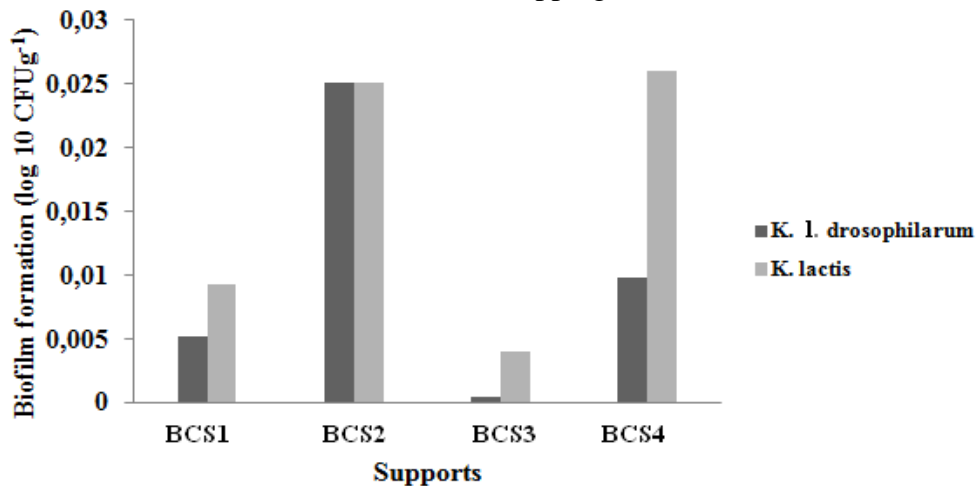


Figure 2. Biofilm formation (CFU g⁻¹) of *K. lactis* and *K. l. Drosophilarum* on biopolymer composite supports.

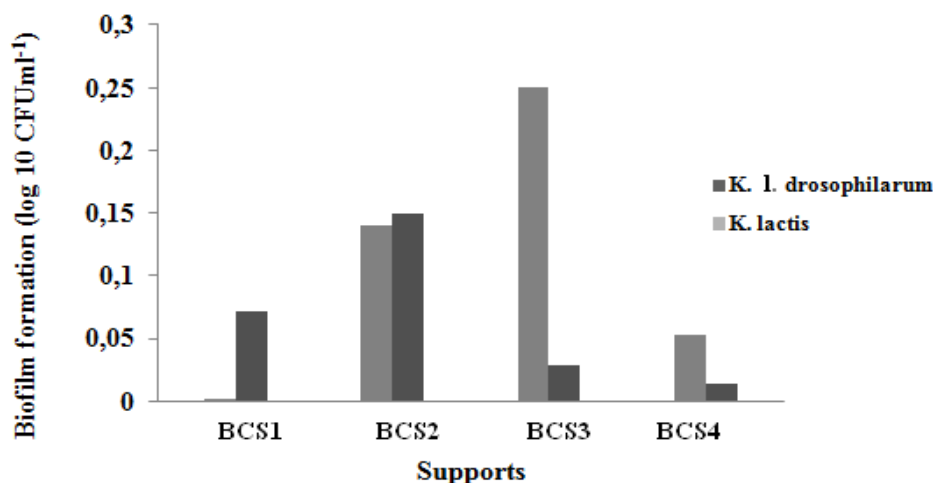


Figure 3. Biofilm formation (CFU ml⁻¹) of *K. lactis* and *K. l. Drosophilarum* on biopolymer composite supports.

These calculations were realized as repeated tube fermentation and then biofilm populations were reported after checking BCS analyses (Demirci et al., 1997; Halkman, 2005). The formed biofilm populations per unit amount and milliliter volume were given in Table 2 for yeast culture.

When was observed the number of colonies on BCS, it is occurred that best biofilm population is between 10⁹ and

10⁸. It was determined at the end of experimental analyses that culture concentrations have direct proportion with reported biofilm populations. It was showed in both Figure 2 and Figure 3 that *K. lactis* culture formed the best biofilm and had the most culture concentration.

In Fig. 2 best biofilm in liquid volume was determined to be originated from *K. lactis* culture with the support of BCS₂ as 1.5×10⁹ CFU ml⁻¹.

Table 2. The biofilm formation of yeasts CFUg⁻¹ and CFU ml⁻¹ on the biopolymer composite supports.

Supports	Yeast Cultures			
	K. lactis	K. l. drosiphilarum	K. lactis	K. l. drosiphilarum
	CFUg ⁻¹		CFU ml ⁻¹	
BCS ₁	9.2 ×10 ⁶	5.1×10 ⁶	7.1×10 ⁷	4×10 ⁶
BCS ₂	2.5×10 ⁷	2.5×10 ⁷	1.5×10 ⁹	1.4×10 ⁸
BCS ₃	4.0×10 ⁷	3.7×10 ⁶	2.8 ×10 ⁸	2.5×10 ⁸
BCS ₄	2.6×10 ⁷	9.8×10 ⁶	1.4×10 ⁸	5.3×10 ⁷

Again in a similar study while researching the biofilm property of *Klebsiella* culture it was found to be between 2×10⁷ CFU ml⁻¹ and 8×10⁸ CFU ml⁻¹ (Maldonado et al.

2007). On the other hand *K. l. drosiphilarum* formed a biofilm with a value of 2.5×10⁸ CFUml⁻¹ again with BCS₃ support. Therefore it was observed biofilm formation

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of *K. lactis* is better than *K. l. drosiphilarum* yeast per millilitre. In another studies, the cell concentrations after detachment from biofilms were 2.3×10^7 CFU ml⁻¹ for *C. glabrata* strains (Almshawit, 2014) and the biofilm formation of *C. parapsilosis* was observed 1×10^7 CFU ml⁻¹ on the teflon supports (Estivil et al., 2011).

When looking at Fig. 4, best biofilm population per unit weight amount was found to be *K. lactis* yeast with BCS₃ support as 4×10^7 CFUg⁻¹. Our other yeast *K. l. drosiphilarum* realized its best biofilm with BCS₂ support as

2.5×10^7 CFUg⁻¹. Therefore *K. lactis* yeast formed a better population compared to *K. l. drosiphilarum*.

The results obtained in a study were found to be similar to the results we found in our study. In this study the BCS's that we used in our system were used and lactic acid fermentation was realized. During fermentation the biofilm populations on BCS's, were calculated by taking colony numbers as a basis and found to be 1×10^8 CFU g⁻¹ (Estivil et al., 2011).

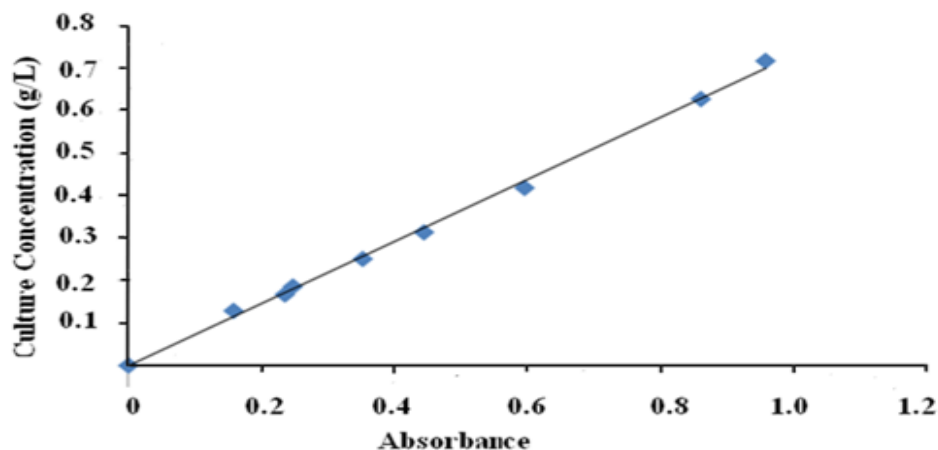


Figure 4. The calibration curve among the culture concentration and absorbance values.

In a study made on lactic acid production the number of colonies formed by *L. casei* culture on BCS's was found to be 5×10^8 CFUg⁻¹ (a-Ho et al., 1997). In another, performed with *L. lactis* culture biofilm was formed with the BCS's that we use for nisin production in a study and was calculated as 1×10^9 /CFU (Bober et al., 2004). Again in another study, the BCS that we use in biofilm reactors was used and lactic acid fermentation was realized. During fermentation, the biofilm populations on BCS's were calculated by taking colony numbers as

a basis and were found to be 1×10^8 CFUg⁻¹ (a-Ho et al., 1997). As shown in Table 3, our study was compared to the other works in the literature and various supports were chosen to immobilize the culture to these supports. The biofilm formation was analyzed using CFU and OD system on some supports such as teflon, polypropylene, PVC, dairy equipments, titanium, polyethylene terephthalate, stainless steel and polystyrene in the Table 3.

Table 3. Biofilm formation analyses of some cultures in literature and comparison to our study.

Cultures	Supports	Counting unite	References
<i>Candida tropicalis</i>	Polyurethane	5.75×10^5 CFU ml-1	Estivill et al. 2011
<i>Candida parapsilosis</i>	PVC	8.0×10^6 CFU ml-1	Estivill et al. 2011
<i>Candida parapsilosis</i>	Teflon	1.5×10^6 CFU ml-1	Estivill et al. 2011
<i>Candida albicans</i>	Titanium	9.588×10^8 CFU/disk	Li et al. 2012
<i>Candida albicans</i>	polyethylene Terephthalate	9.108×10^8 CFU/disk	Li et al. 2012
<i>Staphylococcus aureus</i>	Polystyrene	0.405 (OD590)	Ciccio et al. 2015
<i>Staphylococcus aureus</i>	Stainless steel	0.486 (OD590)	Ciccio et al. 2015
<i>Staphylococcus epidermidis</i>	Polystyrene	0.294(OD590)	Ciccio et al. 2015
<i>Staphylococcus epidermidis</i>	Stainless steel	0.145 (OD590)	Ciccio et al. 2015
<i>Lactobasillus casei</i>	Plastic composite supports	1.6×10^{10} CFU g-1	Ho et all. 1997
<i>Klebsiella</i>	Polystyrene	8×10^8 CFU ml-1	Maldonado et al. 2007
<i>Lactic Acid Bacteria</i>	Glass Cover Slips	1×10^9 CFU ml-1	Kubota et al. 2008
<i>Bacillus species</i>	Equipment in the dairy industry	$106 - 10^8$ CFU ml-1	Pasvolksy et al. 2014
<i>Lactobasillus casei</i>	Plastic compozite supports	$7.5 - 8.0 \times 10^9$ CFU/ disk	Demirci et al. 2003
<i>Kluyveromces lactis drosiphilarum</i>	BCS3	4×10^7 CFU g-1	In this study
<i>Kluyveromces lactis</i>	BCS2	1.5×10^9 CFU ml-1	In this study

CONCLUSIONS

K. lactis and *K. l. drosiphilarum* occurred the visible biofilm formations of each yeast on supports. Biofilm formation of *K. lactis* and *K. l. drosiphilarum* were performed 1.5×10^9 and 2.5×10^8 CFUml⁻¹ on the BCS₂. The materials especially polypropylene that were included by BCS₂ are critically to produce biofilm formation of yeasts on some applications such as food, biomedical, industries and laboratories. The results of this paper will be usefully on these applications and to notice about the form of polypropylene in the supports how it changes the formation of biofilm by yeasts. Therefore these

reports indicated that the biofilm population of industrial yeasts may analyze on this supports and it will be useful on formation of microorganism. As a result of biofilm formation properties of these two yeasts acquired resistance is important consequences in medicine. For example, many antibiotics are produced every year but they are ineffective against microorganism's resistant biofilm. Therefore, the biofilm is important in much area. However, most diseases due to the properties of culture those are difficult to treat. In addition these microorganisms have significant and costly effects of corrosion on some

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valuable devices in the clinics, offices, and factories where cause rotting on the devices.

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REFERENCES

Aktas, N., 2003. Treatment of whey wastewater in a fluidized bed biofilm reactor (FBBR) and modeling the system performance, *Ph.D. Thesis*, Hacettepe University, Ankara, 150s.

Almshawit, H., Macreadie, I., Grando, D., 2014. A simple and inexpensive device for biofilm analysis. *J. of Microbiol.Methods*, 98: 59–63.

Bober, J.A., Demirci, A., 2004. Nisin fermentation by lactococcuslactis subsp.lactis using biopolymer supportss in biofilm reactors. *J. of Sci. Res. Dev.* Vol. VI.Manuscript FP 04 001.

Carpentier, B., Cerf, O., 1993. Biofilm and their consequences with particular reference to hygiene in the food industry. *J. Appl Bacteriol .*, 75: 499-511.

Chandra, J., Mukherjee, P.K., Ghannoum, M.A., 2008. In vitro growth and analysis of Candida biofilms. *Nat. Protoc.*, 3(12): 1909–1924.

Costerton, J.W., Stewart, P.S., Greenburg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. *Science*. 284 (5418):1318-1322.

Demirci, A., Pometto, A.L., Ho, K.L.G., 1997. Ethanol production by *Saccharomyces cerevisiae* in biofilm reactors. *J. Ind.Microbiol.*, 19: 299-304.

Demirci, A., Cotton, J.C., Pometto, A.L., Harkins, K.R., Hinz, P.N., 2003. Resistance of *Lactobacillus casei* in plastic- composite-support biofilm reactors during liquid membrane extraction and optimization of the lactic acid extraction system. *Biotechnol.Bioeng.* 83(7), DOI: 10.1002/bit.10722

Ciccio, P.D., Vergara, Festino, A.R., Paludi, D., Zanardi, E., Ghidini, S., Ianieri, A., 2015. Biofilm formation by

Staphylococcus aureus on food contact surfaces: Relationship with temperature and cell surface hydrophobicity. *Food Control*. 50: 930-936.

Estivill, D., Arias, A., Torres-Lana, A., Carrillo-Muñoz, A.J., Arevalo. M.P., 2011. Biofilm formation by five species of Candida on three clinical materials, *J. of Microbiol Methods.*, 86: 238–242.

Halkman, K., 2005. Merc gıda mikrobiyolojisi uygulamaları. Mikroorganizma Analiz Yöntemleri. (Editör: Halkman, K), Başak matbaacılık, Ankara. pp. 358.

Hall, E.R., 1987. Biofilm reactors in anaerobic wastewater treatment. *Biotech. Adv.*, 5: 257-269.

Ho, K.L.G., Pometto, A.L., Hinz, P.N., Demirci, A., (a) 1997. Nutrient leaching and end product accumulation in biopolymer supports for L-(+)- lactic acid biofilm fermentation. *Appl. Environ. Microbiol.*, 63:2524-2532.

Ho, K.L.G., Pometto, A.L., Hinz, P.N., 1997. (b) Optimization of L-(1)-lactic acid production by ring and disc Biopolymer supports through repeated batch biofilm fermentation. *Appl. Environ. Microbiol.* 63: 2533–2542.

Holzappel, W.H., Petra, H., Snel, J., Schillinger, U., Huisint, Veld, J.H.J., 1998. Overwiev of gut flora probiotics. *In. J. Food Microbiol.*, 41:85-101.

Kubota, H., Senda, S., Nomura, N., Tokuda, H., Uchiyama, H., 2008. Biofilm Formation by Lactic Acid Bacteria and Resistance to Environmental Stress, *J.Biosci.Bioengin.*The Society for iotechnology, Japan, 106, 4; 381–386.

Li, J., Hirota, K., Goto, T., Yumoto, H., Miyake, Y., Ichikawa T., 2012. Biofilm formation of Candida albicans on implant overdenture materials and its removal. *J. Dent.*, 40: 686-692.

Maldonado, N.C., Silva, C.R. Cecilia, M., Macias, M.E.N., 2007. A simple technique to detect *Klebsiella* biofilm-forming-strains. Inhibitory potential of *Lactobacillus ermentum* CRL 1058 whole cells and products. Communicating Current Research anducational *Top.Trends. Appl.Microbiol.*, Formatex.

McDonogh, R., Schaule, G., Flemming, H.C., 1994. The permeability of biofouling layers on membranes. *J.Membr. Sci.*, 87: 199-217.

Research/Araştırma

- Mortensen, K.P., Conley, S.N.**, 1994. Film fill fouling in counterflow cooling towers: mechanisms and design. *CTI J.*, 15:10-25.
- Meyer, A., Wallis, F.M.**, 1997. Development of microbial biofilms on various surfaces for the treatment of heavy metal containing effluents. *Biotechnol Tech.*, 11(12): 859-863.
- Nasib, Q., Bassam, A.A., Thaddeus, C.E., Patrick, K., Lan, S.M.**, 2005. Biofilm reactors for industrial bioconversion processes: Employing potential of enhanced reaction rates. *Microb. Cell Factor.*, 4: 24 doi:10.1186/1475-2859-4-24.
- Nett, J.E., Andes, D.**, 2006. *Candida albicans* biofilm development, modeling a host-pathogen interaction. *Curr. Opin Microbiol.*, 9: 340-344.
- Pasvolsky, R., Zakin, V., Ostrova, I., Shemesh, M.**, 2014. Butyric acid released during milk lipolysis triggers biofilm formation of *Bacillus* species, *Int. J. Food Microbiol.*, 181: 19-27
- Saçak, M.**, 2004. Polimer kimyası. Gazi Kitabevi, Ankara.
- Taras, M., Hakansson, K., Guieysse, B.**, 2005. Continuous acetonitrile degradation in packed-bed bioreactor. *Appl. Microbiol. Biotechnol.*, 66: 567-574
- Uppuluri, P., Lopez-Ribot, J.L.**, 2010. An easy and economical in vitro method for the formation of *Candida albicans* biofilms under continuous conditions of flow. *Virulence*, 1: 483-487.
- Yönten, V.**, 2010. The investigation of species of yeasts formed biofilms. *Ph.D. Thesis*, Yüzüncü Yıl University, 2010, Van, 134s.