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Short Communication

HIGHER SPEED OF YEAST ADHERENCE IN COMPARISON WITH SPEED OF BACTERIA ADHERENCE TO SILICA MICROPARTICLES

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The current study explored the possibility to attach bacteria and yeasts to micro-silica particles. The aim of the study was to determine possible differences in the speed of rates of turbidity (or speed of adherence) between suspensions of bacteria and yeasts with and without silica microparticles. Some important findings were demonstrated, which might be promising for developing of a new diagnostic approach to distinguish bacteria from yeasts. Addition of SiO_2 beads to bacterial suspensions resulted in a significantly faster decrease of turbidity rates in comparison with corresponding suspensions without SiO_2 beads. However, yeasts adhered to silica micro particles quicker in comparison with the speed of adherence of bacteria to silica.

Key words: *silica microparticles, turbidity rate, yeasts, bacteria.*

Adhesion is a key issue for researchers of various fields, including in studies aimed at developing new diagnostic methods. Furthermore, over the past decades, a paradigm shift occurred in the field of microbiology with the understanding that microorganisms present in biological systems exist in biofilms, rather than in a free-living state. This changed the way in which we studied microorganisms *in vitro* and resulted in the development of new experimental models that replicate biofilm environments. Micro particles are being used for sorption of microorganisms. There are two approaches to do this: the microorganisms are pinned to the micro particle chemically (Matsen, 1985) or physically (Vincent *et al.,* 1985). Using the latter approach, it is assumed that adherence does not influence chemical bonds of the microorganism's membrane and gives an opportunity to dispatch the microorganisms easily, because the physical interaction does not develop strong chemical couples. Therefore, the current study focuses on engineering of microparticle surface to pin the microorganisms physically.

A surface of a particle attached to a microorganism is a pedestal to forming a biofilm. This process is controlled by properties of the surface. Specificity of proteins provides opportunity for specific microorganisms to be attached. Attachment of proteins to substrate might be considered in the frame of the adhesion theory formulated by Nobel Prize winner Landau and his coworker Derjaguin (Derjaguin and Landau, 1941). Adhesion is provided when a minimum of the superposed attracting and repulsing potential energies influencing the microorganism is reached at the adhering surface. An attracting force has typically a van der Waals origin, while the repulsing force is provided by electrostatics. The electrostatic force supplies stronger interaction between the adhered particle and the substrate than the van der Waals force. Moreover, the electrostatic force is characterised by several times longer interaction distance. Therefore, attachment of proteins could control the substrate surface electrical potential. Moreover, because of a very thin attached protein molecular layer, the substrate is electrically polarised due to an electrical field provided by the electrical potential of the particle surface. This also promotes cell attachment. Experimental results have provided evidence in favour of the proposed theory (Valdescu *et al.,* 2016).

An external membrane/shell of specific microorganisms is characterised by a particular electrical charge value. This gives an opportunity to select/collect the specific microorganisms at the substrate surface, if the latter is supplied with the adequate electrical potential (Skrastina *et al.*, 2014). Studies have shown that silica is a good model substrate to study attracting force (Loskill *et al.,* 2012). To reach the electrical potential of the substrate, surface radiation (Aronov *et al.,* 2007), electrical polarisation (Kobayashi *et al.,*

2001), and reconstruction of the chemical couples of the surface (Ratner *et al.,* 1996) can be employed.

The current study explored the possibility to attach bacteria and yeasts to micro-silica particles. Optical densitometry was used to identify assembling of particles with microorganisms.

Reference cultures of the following bacteria were used: *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 33591 (MRSA — Methicilin resistant *Staphylococcus aureus*), *Staphylococcus aureus* ATCC 25923 (MSSA — Methicilin sensitive *Staphylococcus aureus*), and yeast — *Candida albicans* ATCC 10231. Pure cultures of the following yeasts were used: *Kluyveromyces marxianus, Candida krusei, Candida glabrata* from Microbiology Laboratory, Traumatology and Orthopaedics Hospital, Rīga. The micro silica particles were hollow SiO_2, spherical nonporous glass beads with mean particle size 9–13 μm and density 1.05–1.15 g/ml (manufacturer Sigma-Aldrich, USA).

A DEN-1 densitometer (suspension turbidity detector, manufacturer Biosan, Latvia), was used for measurement of cell suspension's turbidity in the range of 0.3–15.0 McFarland units (100×10^6 – 150×10^7 cells/ml).

Suspensions in the range of 10.0 McFarland of bacteria and yeasts in the sterile water with and without SiO_2 beads were prepared. Measurement of suspension turbidity was performed at 30 minutes intervals in a period of 4 hours 30 min. Measurements of turbidity were made for emulsion particles assembled with microorganisms and silica, and for suspensions with only microorganisms.

Significant differences between treatments were determined using the paired 2 tails Student's t-test ($\alpha = 0.05$, as significant level of confidence).

Bacterial suspensions with SiO_2 beads showed a significantly faster decrease of turbidity rates in 4 hours 30 minutes at intervals in 30 minutes, in comparison with corresponding suspensions without SiO_2 beads: *E.coli* with SiO_2 beads and *E.coli* (from 10.0 McFarland units till 8.6 McFarland units and correspondingly, from 10.0 till 9.4 McFarland units, $p = 0.00037$), *P. aeruginosa* with SiO_2 beads and *P. aeruginosa* (10–6.6, 10–8.9, $p = 0.00017$), *E.faecalis* with SiO_2 beads and *E.faecalis* (10.0–8.5, 10.0–9.6, $p = 0.00008$), MRSA with SiO_2 beads and MRSA (10.0–7.7, 10.0–9.4, $p = 0.00015$), MSSA with SiO_2 beads and MSSA (10.0–8.7, 10.0–10.0, $p = 0.00005$). Yeasts suspensions with SiO_2 beads did not show significant difference in rate of decrease of turbidity rates in comparison with corresponding suspensions without SiO_2 beads at the significant level of confidence: *C.albicans* with SiO_2 beads and *C.albicans, Kluyveromyces marxianus* with SiO_2 beads and *Kluyveromyces marxianus, C.krusei* with SiO_2 beads and *C.krusei, C.glabrata* with SiO_2 beads and *C.glabrata* (see Table 1).

In all cases, yeasts suspensions showed a significantly faster decrease of turbidity rates in the period of 4 hours 30 minutes, in comparison with bacterial suspensions at the significant level of confidence: *Kluyveromyces marxianus* in comparison with *E.coli* (from 10.0 McFarland units till 3.0 McFarland units and correspondingly, from 10.0 till 9.4

Table 1

SUSPENSION TURBIDITY IN MCFARLAND UNITS AT 30 MIN INTERVALS

No	Microorganisms	0:00	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	4:30
1	*E.coli*+SiO_2 beads	10.0	8.9	8.8	8.8	8.7	8.6	8.6	8.6	8.6	8.6
	E.coli	10.0	9.6	9.6	9.6	9.6	9.6	9.5	9.5	9.5	9.4
2	*P. aeruginosa*+SiO_2 beads	10.0	7.8	6.8	7.0	7.5	7.5	7.0	6.7	7.4	6.6
	P. aeruginosa	10.0	9.5	9.4	9.4	9.3	9.4	8.9	8.9	9.0	8.9
3	*E.faecalis*+SiO_2 beads	10.0	9.2	8.6	8.9	8.6	8.9	8.3	8.8	8.6	8.5
	E.faecalis	10.0	10.0	10.0	9.9	9.9	9.7	9.7	9.7	9.6	9.6
4	MRSA+SiO_2 beads	10.0	8.0	8.8	8.3	8.2	7.8	7.8	7.7	7.8	7.7
	MRSA	10.0	9.7	9.7	9.7	9.6	9.6	9.4	9.5	9.4	9.4
5	MSSA+SiO_2 beads	10.0	8.9	8.8	8.8	9.2	9.4	8.6	8.5	8.8	8.7
	MSSA	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
6	*C.albicans*+SiO_2 beads	10.0	6.9	4.1	2.9	2.5	1.9	1.9	1.4	1.6	1.5
	C.albicans	10.0	5.6	6.2	5.0	4.7	4.5	3.9	3.6	3.0	2.7
7	*Kluyveromyces marxianus* +SiO_2 beads	10.0	6.4	4.4	4.0	4.2	4.3	2.8	2.8	2.2	1.8
	Kluyveromyces marxianus	10.0	5.6	5.5	4.5	5.0	4.6	4.0	3.8	3.3	3.0
8	*C.krusei*+SiO_2 beads	10.0	8.1	7.4	6.9	5.7	4.8	3.7	3.2	2.8	2.7
	C.krusei	10.0	7.3	7.3	6.7	6.1	5.7	5.0	4.6	4.3	3.9
9	*C.glabrata*+SiO_2 beads	10.0	7.9	6.8	5.1	3.8	3.1	2.4	2.2	1.8	1.6
	C.glabrata	10.0	4.7	6.3	5.3	5.0	4.3	4.1	3.4	3.3	2.6

McFarland units, $p = 0.00004$), *C.krusei* in comparison with *E.coli* (10.0–3.9, 10.0–9.4, p = 0.00020), *C.glabrata* in comparison with *E.coli* (10.0–2.6, 10.0–9.4, $p = 0.00006$), *C.albicans* in comparison with *P.aeruginosa* (10.0–2.7, 10.0–8.9, $p = 0.00007$), *Kluyveromyces marxianus* in comparison with *P.aeruginosa* (10.0–3.0 and 10.0–8.9, $p = 0.00005$), *C.krusei* in comparison with *P.aeruginosa* (10.0–3.9 and 10.0–8.9, $p = 0.00030$), *C.glabrata* in comparison with *P.aeruginosa* (10.0–2.6, 10.0–8.9, $p = 0.00007$), *C.albicans* in comparison with *E.faecalis* (10.0–2.7, 10.0–9.6, $p = 0.00004$), *Kluyveromyces marxianus* in comparison with *E.faecalis* (10.0-3.0, 10.0–9.6, $p = 0.00003$), *C.krusei* in comparison with *E.faecalis* (10.0–3.9, 10.0–9.6, $p = 0.00013$), *C.glabrata* in comparison with *E.faecalis* (10.0–2.6, 10.0–9.6, $p = 0.00004$), *C.albicans* in comparison with MRSA (10.0–2.7, 10.0–9.4, $p = 0.00006$), *Kluyveromyces marxianus* in comparison with MRSA (10.0–3.0, 10.0–9.4, $p = 0.00004$), *C.krusei* in comparison with MRSA (10.0–3.9, 10.0–9.4, $p = 0.00020$), *C.glabrata* in comparison with MRSA (10.0–2.6, 10.0-9.4, $p = 0.00006$), *C.albicans* in comparison with MSSA (10.0–2.7, 10.0–10.0, $p = 0.00003$), *Kluyveromyces marxianus* in comparison with MSSA (10.0–3.0, 10.0–10.0, $p = 0.00002$), *C.krusei* in comparison with MSSA (10.0–3.9, 10.0–10.0, $p = 0.00008$), *C.glabrata* in comparison with MSSA (10.0–2.6, 10.0–10.0, $p = 0.00003$) (see Table 1).

In all cases, yeasts with SiO_2 beads suspensions showed a significantly faster decrease of turbidity rates in the period 4 hours 30 minutes, in comparison with bacterial suspensions with SiO_2 beads: *C.albicans* in comparison with *E.coli* (10.0–1.5, 10.0–8.6, $p = 0.00023$), *Kluyveromyces marxianus* in comparison with *E.coli* (10.0–1.8, 10.0–8.6, $p = 0.00016$), *C.krusei* in comparison with *E.coli* (10.0–2.7, 10.0–8.6, $p = 0.00220$), *C.glabrata* in comparison with *E.coli* (10.0–2.6, 10.0–9.4, $p = 0.00111$), *C.albicans* in comparison with *P.aeruginosa* (10.0–2.7, 10.0–8.9, $p = 0.00158$), *Kluyveromyces marxianus* in comparison with *P.aeruginosa* (10.0–1.8, 10.0–6.6, $p = 0.00245$), *C.krusei* in comparison with *P.aeruginosa* (10.0–2.7, 10.0–6.6, $p = 0.04585$), *C.glabrata* in comparison with *P.aeruginosa* (10.0–1.6, 10.0–6.6, $p = 0.01079$), *C.albicans* in comparison with *E.faecalis* (10.0–1.5, 10.0–8.5, $p = 0.00015$), *Kluyveromyces marxianus* in comparison with *E.faecalis* (10.0–1.8, 10.0–8.5, $p = 0.00016$), *C.krusei* in comparison with *E.faecalis* (10.0–2.7, 10.0–8.5, $p = 0.00217$), *C.glabrata* in comparison with *E.faecalis* (10.0–1.6, 10.0–8.5, $p = 0.00110$), *C.albicans* in comparison with MRSA (10.0–1.5, 10.0–7.7, $p = 0.00045$), *Kluyveromyces marxianus* in comparison with MRSA (10.0–1.8, 10.0–7.7, p = 0.00045), *C.krusei* in comparison with MRSA (10.0–2.7, 10.0–7.7, $p = 0.00880$), *C.glabrata* in comparison with MRSA (10.0–1.6, 10.0–7.7, $p = 0.00265$), *C.albicans* in comparison with MSSA (10.0–1.5, 10.0–8.7, $p = 0.00019$), *Kluyveromyces marxianus* in comparison with MSSA (10.0–1.8, 10.0–8.7, $p = 0.00013$), *C.krusei* in comparison with MSSA (10.0–2.7, 10.0–8.7, $p = 0.00165$), *C.glabrata* in comparison with MSSA (10.0–1.6, 10.0–8.7, $p = 0.00090$) (see Table 1).

An interesting finding from this study was confirmation of the proposed original hypothesis that yeasts spp. adhere to silica micro particles quicker in comparison with the rate of adherence of bacteria to silica. There was a faster decrease of turbidity estimated by densitometer due to faster sedimentation of formed bigger complexes of yeast caused by gravitation force (fungal spp. have 10–12 μm in diameter in comparison with 1–5 μm of studied bacteria). This phenomenon can be used as a background to develop a new diagnostic tool to distinguish yeasts from bacteria.

Results of the current study clearly demonstrated that bacterial suspensions with SiO_2 beads caused significantly faster decrease of turbidity rates in comparison with suspensions without SiO_2 beads, as shown in a previous study (Loskill *et al.,* 2012) but yeasts suspensions did not show this difference. This difference also gives the opportunity to develop new diagnostic tool to distinguish bacteria from yeasts.

The conclusions are:

1. Bacterial suspensions with SiO_2 beads had significantly faster decrease of turbidity rates in comparison with corresponding bacterial suspensions without SiO_2 beads.

2. Yeasts suspensions with SiO_2 beads did not show significantly faster turbidity rates in comparison with corresponding suspensions without SiO_2 beads.

3. Yeasts suspensions showed significantly decreased turbidity rates in comparison with bacterial suspensions.

4. Yeasts spp. adhere to silica micro particles significantly faster in comparison with the speed of adherence of bacteria to silica.

Our study could assist to develop a new diagnostic tool and is useful to better understand biofilm formation processes.

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RAUGA SĒŅU ADHĒZIJAS ĀTRUMS PIE SILĪCIJA DIOKSĪDA MIKRODAĻIŅĀM IR LIELĀKS NEKĀ BAKTĒRIJĀM

Šajā darbā ir pētīts baktēriju un rauga sēņu adhēzijas ātrums pie silīcija dioksīda mikrodaļiņām. Pētījuma mērķis bija noteikt iespējamo atšķirību attiecībā uz turbiditātes pakāpes izmaiņām noteiktos laika intervālos (jeb adhēzijas ātrumu) dažādu baktēriju un rauga sēņu suspensijās ar un bez minētajām daļiņām. Pētījumā ir konstatēti vairāki svarīgi fakti, kas dod iespēju izstrādāt jaunu diagnostikas metodi baktēriju diferencēšanai no rauga sēnēm, proti: baktēriju suspensijās ar silīcija dioksīda mikrodaļiņām turbiditātes pakāpe mazinās ātrāk nekā bez daļiņām un tas ir statiski ticami, bet rauga sēņu suspensijās šis fenomens nav statiski ticams; rauga sēnes pielīp pie silīcija dioksīda mikrodaļiņām ātrāk nekā baktērijas, un šī atšķirība ir statistiski nozīmīga.