Swarming and Swimming Movement of Bacteria in Different Organic Wastes

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Abstract Motility plays an important role in biofilm formation and movement in different environmental conditions, colonization, and adhesion of bacteria to surfaces. The lowest swimming was 3 (mm) in agar medium and the highest value was 42.67 (mm) with the addition of WCW. The lowest swarming was carried out in agar medium with 7.66 (mm), while the highest value was found in N.A medium with the addition of 10% WCW 59.33 (mm). In all experimental conditions, an increase of 2.4 times (swimming) and 6.4 times (swarming) was observed after the addition of WCW to the controls.

Keywords: Organic wastes, Swarming, Swimming, Bacteria

1. Introduction

Bacterial growth and movement plays an important role in many different areas such as soil microbiology, water treatment and microbial pathogenesis [1].

Swarming is a social phenomenon that involves rapid coordinated movement of bacteria across a semisolid surface. The swarming requires the production of a functional flagellum and rhamnolipid surfactant. The sliding motion is separate from the swimming motion because swimming is the movement required to move on an aqueous, viscous semi-solid surface while allowing movement in a liquid medium of relatively low viscosity [2-10].

Swarming colonies of different bacterial genera share several common dynamic characteristics: the alignment of adjacent cells and their coordinated movement in multicellular rafts; the low curvature of their trajectories; the low frequency of cell tumbles; the formation of dynamic, circular vortices of cells; and the cooperative motility of cells across surfaces [11,12].

Swarming motility has been reported in a wide range of Gram (-) bacterial species belonging to the genera Proteus, Vibrio, Aeromonas, Serratia, Bacillus, Salmonella, Escherichia, Yersinia, and Pseudomonas. Pseudomonas aeruginosa has the potential to move on viscous surfaces by swarming motility [2-4,6,9,13-14].

Swarming is a multi cellular type of motility and is considered a model of bacterial social behavior [13]. However, chemotaxis is not strictly responsible for swimming motility [14,15].

Swimming in the aquatic environment and in low agar concentrations (0.3% agar) to flagellum; IV type pilus on solid surfaces mediated swimming and recently observed swimming over semi-solid (viscous) medium (0.5 to 0.7% agar). Swimming is generally defined as a dendritic-like colonial appearance and a social phenomenon that typically involves coordinated and rapid movement of bacteria along a semi-solid surface [10,16-19].

The swimming and movement of the bacteria along the surfaces using various mechanisms in aqueous environments is seen in many different forms. Swimming along a surface occurs when the liquid film is sufficiently thick and the morphological structure of the bacteria is not fully organized. When a surface liquid layer is examined or when cells are
inoculated to the surface of the agar medium, vegetative cells begin a differentiation process as multinucleated cells and prolonged, hyper flagellated cells [20].

Swimming motility provides a significant advantage for bacteria by allowing them to move away from toxins and alien species towards suitable conditions [21].

*P. aeruginosa* floats with a single, polar, monotric flagellum rotating with proton motivating power. In the context of the disease, this flagellar swimming motility is important in infection because the lack of swimming ability of *P. aeruginosa* mutant causes a decrease in pathogenesis [22,23].

*Escherichia coli* are a rod-shaped Gram (-) bacterium commonly found in the large intestine of warm-blooded animals [12]. It is a model organism for the behavior of bacterial cell movement in mechanical and mass fluids [11]. In particular, *E. coli* was used as a prototypic micro-swimmer. *E. coli* cells close motility is important in the early stages of biofilm formation and pathogenic infection [24]. *E. coli* cells have several extracellular helical thread-like structures called flagella [24,25].

*Bacillus cereus* is Gram (+), spore-forming, mobile, aerobic, rod-shaped and anaerobic bacteria. *Bacillus subtilis* is a soil bacterium that has a versatile metabolism and ability to survive in various habitats. It is known to enter the fusion motility as a cellular differentiation program in nutritional research when exposed to nutritional stress conditions [26,27]. Swarming migration has a bacterial action that can contribute significantly to the pathogenesis of *Bacillus* infection. Bacteria can be varied into prolonged, multi-core, hyper-flagellated swarmer cells that can move away from the colony in a coordinated manner along a moist, solid surface or in a viscous environment [28].

*Staphylococcus aureus* is anaerobic; Gram (-) has a coke structure and causes widespread infections [29]. It has been shown that *S. aureus* colonies can be passively propagated along the surface of the soft agar plates with the aid of the production of surfactant in a process called propagation [30].

*Enterococcus* is a genus of Gram (+) bacteria naturally found in the mammalian gastrointestinal tract. *Enterococci* are opportunistic pathogens with tolerance to various environmental conditions including extremes of temperature and pH, high salinity, detergents, and anti-biotics [31]. However, the characteristics of such ocular *E. faecalis* strains remain unknown [32].

### 2. Materials and Methods

#### 2.1. Microorganism

*P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. aureus* (ATCC BAA 1026) and *B. cereus* (ATCC 10876) obtained from the ATCC and used this study.

#### 2.2. Waste cheese whey

Waste cheese whey (WCW) was collected from commercial cheese factories in Malatya, Turkey.

#### 2.3. Waste frying oil

Waste frying oil (WFO) was obtained and collected from the food Restaurant Malatya, Turkey.

#### 2.4. Sugar beet molasses

Sugar beet molasses (SBM) was collected from Malatya Sugar Factories in Malatya, Turkey. These wastes were filtered for removing crude impurities and then, they’re autoclaved, and then used.

#### 2.5. Growth conditions

Bacteria were firstly cultured in Luria- Bertani (LB) broth medium (g l⁻¹): peptone (10), NaCl (10), and yeast extract (5). The final pH values of broth media was adjusted to 7.0. The same amounts of cells were grown at 37 °C, 0 rpm on incubator for overnight (O/N). 100 μl of over night cultures (OD₆₀₀ nm ~ 0,2-0,3) grown tube filled with 5 ml in 10 ml tubes was
inoculated, and incubated for 24 h of time. Phosphate-buffered saline (PBS buffer) (gl⁻¹: 8.0 NaCl; 0.2 KCl; 1.44 Na₂HPO₄; 0.24 KH₂PO₄ and pH 7.4) and PBS+10 % wastes. These cultures were subsequently incubated on an orbital shaker at 0, 100 rpm, 200 rpm and 37 °C for 24 h.

2.6. Motility

Swimming and swarming assays was determined using modified methods with WCW as the substrate. **Swimming.** Swimming plates were composed of 0.3% Nutrient Agar, supplemented with 10% wastes and sterile phosphate-buffered saline supplemented with 10% WCW. **Swarming.** Swarm plates were composed of 0.5% Bacto Agar and 8 g/L of nutrient broth, supplemented with 10% wastes and sterile phosphate-buffered saline supplemented with 10% wastes petri dishes. Petri dishes, dried overnight at room temperature. Cells were point inoculated with a sterile pipette 6 μl, and the plates were incubated at 25 and 37 °C for 24 hours, respectively. Motility was then assessed qualitatively by examining the circular turbid zone formed by the bacterial cells migrating away from the point of inoculation [3,9,13,17,18,23,33]. Each value is the average of three independent experiments.

3. Results

3.1. Agar Media Swarming

In the presence of melas in *S. aureus* with the highest swarming 21.66 mm, the lowest swarming motion was in the presence of WFO in *E. faecalis*. The biggest difference was observed in the presence of *S. aureus* and molasses with 17.33 mm. No movement was observed since there was no breeding of *B. cereus* in the presence of molasses. When WFO was added, there was no growth in *E. faecalis* and therefore no swarming motions were detected. The highest percentage difference was observed in *E. faecalis* with % 500 differences in the molasses environment (Figure 1-4).

3.2. Nutrient Agar Media Swarming

The highest swarming 59.33 mm with *P. aeruginosa* in WCW, while the lowest sliding motion was observed in *E. faecalis* with the presence of molasses 7 mm. The largest difference was observed in *P. aeruginosa* and WCW with 35 mm. The largest difference was observed in *P. aeruginosa* and WCW with 35 mm. The lowest difference was observed in the presence of molasses in *E. faecalis* with 0.7 mm. According to the control group in the presence of molasses, only *P. aeruginosa* decline. The highest rate of different was observed in 271 % *B. cereus* and WFO environment, while the lowest difference in molasses was observed in *E. faecalis* (111%). When all the results were taken into consideration, the sliding motion was more observed in the presence of molasses in agar medium; it was observed in the presence of WCW in the NA environment (Figure 1-4).

All bacteria were able to slip in the NA environment, while *B. cereus* and *E. faecalis* could not perform in agar medium.

3.3. Agar Media Swimming

In the presence of melas in *P. aeruginosa* with the highest swimming 19.33 mm in WCW, the lowest swimming motion (3 mm) was in the presence of WFO in *B. cereus*. The biggest difference was observed in the presence of *P. aeruginosa* and WCW with 16.33 mm. No movement was observed since there was no breeding of *B. cereus* and *E. faecalis* in the presence of molasses. When WFO was added, there was no growth in *P. aeruginosa* and *E. faecalis* and therefore no swimming motions were detected. The highest percentage difference was observed in *P. aeruginosa* with % 644 difference in the WCW environment. The lowest percentage difference was observed in *P. aeruginosa* with % 111 difference in the molasses environment (Figure 1-4).

3.4. Nutrient Agar Media Swimming

The highest swimming 55 mm with *S. aureus* in molasses, while the lowest swimming motion observed in *E. faecalis* with the presence of molasses 4.33 mm. The largest difference was observed in *S. aureus* and molasses with 47.33 mm.
The lowest difference was observed with *E. faecalis* and 1.1 mm in molasses. According to the control group in the presence of molasses swimming is decline. The highest rate of different was observed in 717% *S. aureus* and molasses environment, while the lowest difference in molasses was observed in *E. faecalis* (115%) (Figure 1-4).

When all the results were taken into consideration, the swimming motion was more observed in the presence of WCW in agar medium; it was observed in the presence of WCW in the N.A environment. Considering the Nutrient Agar medium, only *B. cereus* did not move.

**Figure 1.** Graphical representation of the movement at PBS.

**Figure 2.** Graphical representation of the movement at WFO.
Figure 3. Graphical representation of the movement at WCW.

Figure 4. Graphical representation of the movement in Molasses.

References

[1] Mannik J, Driessen R, Galajda P, Keymer JE, Dekker C. Bacterial growth and motility in sub-micron constrictions. Pnas 2009; 106 (35): 14861–14866.

[2] Semmler A B T, Whitchurch CB, Mattick J.S. A re-examination of twitching motility in Pseudomonas aeruginosa. Microb. 1999; 145: 2863–2873.
[3] Deziel E, Comeau Y, Villemur R. Initiation of Biofilm Formation by *Pseudomonas aeruginosa* 57RP Correlates with Emergence of Hyperpiliated and Highly Adherent Phenotypic Variants Deficient in Swimming, Swarming and Twitching. J Bacteriol. 2001; 183(4): 1195-1204.

[4] Tremblay J, Richardson AP, Lépine F, Déziel E. Self-produced extracellular stimuli modulate the *Pseudomonas aeruginosa* swarming motility behavior. Environ Microbiol. 2007; 9(10): 2622–2630.

[5] Overhage J, Bains M, Brazas M D, Hancock R.E.W. Swarming of *Pseudomonas aeruginosa* is a Complex Adaptation Leading to Increased Production of Virulence Factors and Antibiotic Resistance. J Bacteriol. 2008; 190(8): 2671–2679.

[6] Tremblay J, Déziel E. Improving the reproducibility of *Pseudomonas aeruginosa* swarming motility assays. J Basic Microb. 2008; 48: 509–515.

[7] Copeland MF, Weibel D.B. Bacterial Swarming A Model System for Studying Dynamic Self-assembly. NIH Public Access. 2009; 5(6): 1174–1187.

[8] Morris J D, Hewitt JL, Wolfe LG, Kamatkar NG, Chapman SM, Diener JM, Courtney AJ, Matthew Leevy W, Shrou Taylor A.M. Imaging and Analysis of *Pseudomonas aeruginosa* Swarming and Rhamnolipid Production. Appl Environ Microb. 2011; 77(23): 8310–8317.

[9] Wolska K, Szweda P, Lada K, Rytel E, Piechota M. Motility activity, slime production, biofilm formation and genetic typing by ERIC-PCR for *Pseudomonas aeruginosa* strains isolated from bovine and other sources (human and environment). Pol J Vet Sci. 2014; 17(2): 321–329.

[10] Yeung A T Y, Torfs E C W, Jamshidi F, Bains M, Wiegand I, Hancock R E W, Overhage J. Swarming of *Pseudomonas aeruginosa* Is Controlled by a Broad Spectrum of Transcriptional Regulators, Including MetR. J Bacteriol. 2009; 191(18): 5592–5602.

[11] Swiecicki JM, Slusarenko O, Weibel D.B. From swimming to swarming *Escherichia coli* cell motility in two-dimensions. NIH Public Access. 2013; 5(12):1490–1494.

[12] Goh S.N. Effects of Different Amino Acids on Biofilm Growth, Swimming Motility and Twitching Motility in *Escherichia coli* BL21. J Biol Life Sci. 2013; 4(2): 104-115.

[13] Inoue T, Shingaki R, Fukui K. Inhibition of swarming motility of *Escherichia coli* by branched-chain fatty acids. FEMS Microbiol Lett. 2008; 281: 81-86.

[14] Morris J D, Hewitt JL, Wolfe LG, Kamatkar NG, Chapman SM, Diener JM, Courtney AJ, Matthew Leevy W, Shrou J.D. Imaging and Analysis of *Pseudomonas aeruginosa* Swarming and Rhamnolipid Production. Appl Environ Microb. 2011; 77(23): 8310–8317.

[15] Yang A, Tang WS, Si T, Tang J.X. Influence of Physical Effects on the Swarming Motility of *Pseudomonas aeruginosa*. Biophys J. 2017; 112:1462–1471.

[16] Overhage J, Lewenza S, Marr A K, Hancock R.E.W. Identification of Genes Involved in Swarming Motility Using a *Pseudomonas aeruginosa* PAO1 Mini-Tn5-lux Mutant Library. Am Soc for Microb. 2007; 189 (5): 2164–2169.

[17] O’May C, Tufenkji N. The Swarming Motility of *Pseudomonas aeruginosa* is Blocked by Cranberry Proanthocyanidins and Other Tannin-Containing Materials. Appl Environ Microbiol. 2011; 77(9): 3061–3067.

[18] Murray TS, Kazmierczak B.I. *Pseudomonas aeruginosa* Exhibits Sliding Motility in the Absence of Type IV Pili and Flagella. J Bacteriol. 2008; 190(8): 2700–2708.

[19] Kohler T, Curty L K, Barja F, Delden CV, Pechere J.C. Swarming of *Pseudomonas aeruginosa* Is Dependent on Cell-to-Cell Signaling and Requires Flagella and Pili. J Bacteriol. 2000; 182(21): 5990–5996.

[20] Vicario JC, Dardanelli MS, Giordano W. Swimming and swarming motility properties of peanut-nodulating rhizobia. FEMS Microbiol Lett. 2015; 362: 1-6.

[21] Samad T, Billings N, Birjiniuk A, Crouzier T, Doyle PS, Ribbeck K. Swimming bacteria promote dispersal of non-motile Staphylococcal species. The ISME J. 2017; 1-5.

[22] Lovewell RR, Hayes SM, O’Toole GA, Berwin B. *Pseudomonas aeruginosa* flagellar motility activates the phagocyte PI3K/Akt pathway to induce phagocytic engulfment. Am J Physiol Lung Cell Mol Physiol. 2014; 306: 698-707.

[23] Murray TS, Kazmierczak B.I. FlhF Is Required for Swimming and Swarming in *Pseudomonas aeruginosa*. J Bacteriol. 2006; 188(19): 6995–7004.
[24] Lauga E, DiLuzio W R, Whitesides G M, Stone H A. Swimming in Circles: Motion of Bacteria near Solid Boundaries. Biophys J. 2006; 90: 400-412.
[25] Mittal N, Budrene E O, Brenner M P, Oudenaarden A V. Motility of Escherichia coli cells in clusters formed by chemotactic aggregation. PNAS 2003; 100(23): 13259–13263.
[26] Ng W. Retarded swarming motility in Bacillus subtilis NRS-762 and Pseudomonas aeruginosa PRD-10. Peer J Preprints. 2018; 1-10.
[27] Granum PE, Lund T. Bacillus cereus and its food poisoning toxins. FEMS Microbiol Lett. 1997; 157: 223-228.
[28] Callegan MC, Novosad BD, Ramirez R, Ghelardi E, Senesi S. Role of Swarming Migration in the Pathogenesis of Bacillus Endophthalmitis. IOVS 2006; 47(10): 4461–4467.
[29] Habib F, Rind R, Durani N, Bhutto A L, Buriro R S, Tunio A, Aijaz N, Lakho SA, Bugti AG, Shoaib M. Morphological and Cultural Characterization of Staphylococcus aureus Isolated from Different Animal Species. J Appl Environ Biol Sci. 2015; 5(2): 15-26.
[30] Pollitt EJG, Cruz SA, Diggle S P. Staphylococcus aureus forms spreading dendrites that have characteristics of active motility. Sci Rep. 2015; 1-12.
[31] Kim HS, Hahn H, Kim J, Jang DM, Lee JY, Back JM, Im HN, Kim H, Han BW, Suh S W. Structural basis for the substrate recognition of peptidoglycan pentapeptides by Enterococcus faecalis VanYB. Int J Biol Macromol. 2018; 119: 335–344.
[32] Todokoro D, Eguchi H, Suzuki T, Suzuki M, Imaohji HN, Kuwahara T, Nomura T, Tomita H, Akiyama H. Genetic diversity and persistent colonization of Enterococcus faecalis on ocular surfaces. Jpn J Ophthalmol. 2018; 62: 699–705.
[33] Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EBM, Horsman S, Lewenza S, Burrows L, Hancock R E W. Inhibition of Bacterial Biofilm Formation and Swarming Motility by a Small Synthetic Cationic Peptide. Antimicrob Agents Ch. 2012; 56(5): 2696–2704.