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Motility and chemotaxis of *Escherichia coli* in medium with attractant and repellent

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ABSTRACT

This study was conducted in order to satisfy the following objectives: (1) To compare the migration bands of *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus* in Sugar, Indole and Motility (SIM) medium + chemotaxis medium (CM), SIM + attractant (glucose), SIM + repellent (alcohol) and SIM only; (2) To observe and compare the swimming behaviour of *E. coli* under the microscope in the presence of attractant and repellent; and (3) To compare chemotaxis of *E. coli* in the presence of attractant and repellent by capillary assay. Motility bands were observed in tubes with SIM + CM, SIM + attractant, SIM + repellent and SIM only that were inoculated with *E. coli* and *B. megaterium*. However, the motility band in SIM + repellent travelled less far as compared to SIM + CM and SIM + attractant. No motility bands were observed in tubes inoculated with *S. aureus*. Highest number of colonies (CFU/mL) were observed in capillary tubes dipped in attractant (3.7×10^8), followed by CM (5.2×10^7) and least in repellent (7.6×10^6). Analysis of data showed that log CFU/mL in capillary tubes dipped in attractant (8.55±0.14) was significantly higher as compared to capillary tubes dipped in CM (7.64±0.29) and repellent (6.87±0.14).

Keywords: *Escherichia coli*, motility, chemotaxis, attractant, repellent

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INTRODUCTION

By the end of the 19th century, the motor responses of bacteria had been thoroughly characterized by numerous investigators including Wilhelm Pfeffer, a great German physiologist[1]. The research of Pfeffer established that bacteria move in response to changes in temperature (thermotaxis), light (phototaxis), salinity (osmotaxis) and oxygen (aerotaxis), and to specific metabolites and other signalling molecules (chemotaxis). It was not until the end of the 20th century, however, that the molecular mechanisms that underlie bacterial sensory-motor regulation had been established[1].

Bacterial chemotaxis is defined as a physiological response to environmental stimuli in which the organisms move toward a favourable medium or swim away from a hazardous medium[2,3]. Chemotactic bacteria use complex intracellular response systems as they are too small to sense changes in their biochemical environment relative to the length of their cell. *Escherichia coli* is the most widely studied chemotactic bacterium, along with *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Rhodobacter sphaeroides*[4].

Chemotactic bacteria rely upon three physiological components in order to detect and respond to their environment. According to Stock et al. (2000) and Wolanin et al. (2002), motile prokaryotes use a two-component signal transduction system with conserved components to regulate motor activity[5,6]. In general, a two-component system includes a histidine protein kinase (HPK) that catalyzes the transfer of phosphoryl groups from ATP to one of its own histidine residues and a response regulator that catalyzes transfer of phosphoryl groups from the HPK-histidine to an aspartate residue on the response regulator[7].

Objectives of the Study

The objectives of this study include: (1) To compare the migration bands of *E. coli*, *B. megaterium* and *Staphylococcus aureus* in Sugar, Indole and Motility (SIM) medium + chemotaxis medium (CM), SIM + attractant, SIM + repellent and SIM only; (2) To observe and compare the swimming

behaviour of *E. coli* under the microscope in the presence of attractant and repellent; and (3) To compare chemotaxis of *E. coli* in the presence of attractant and repellent by capillary assay.

MATERIALS AND METHODS

Demonstration of Bacterial Migration Bands

E. coli, B. megaterium and *S. aureus* (negative control, non-motile) were separately grown in Luria Bertani (LB) agar slant for 18-24 hours. SIM medium was prepared in tubes and autoclaved at 121 °C at 15 psi for 15 minutes. Chemotaxis medium (CM) which is composed of 10^{-3} M EDTA, 10^{-3} mannitol, 10^{-2} MgCl and 10^{-2} K_2HPO_4-KH_2PO_4 buffer (pH 7) was also prepared. Afterwards, CM, glucose (attractant) and absolute ethanol (repellent) were filter sterilized. The following tubes were prepared: (1) SIM only, (2) SIM + CM, (3) SIM + glucose as attractant and (4) SIM + ethanol as repellent. The CM, glucose and ethanol were added in SIM before it solidifies. The final concentration of glucose and ethanol in tubes with SIM was 1 M.

The 18-24 hours bacterial cultures were separately resuspended in 1 mL 0.0037 EDTA solution. Afterwards, 20 µL of resuspended *E. coli* was pipetted out and dropped into the surface of the prepared tubes with SIM + CM, SIM + glucose and SIM + ethanol. Meanwhile, stab inoculation was done in tube with SIM medium only. Each tube was replicated twice. The same procedure was done using *B. megaterium* and *S. aureus*. The tubes were then allowed to stand for 5-6 hours and migration bands were observed as indicators of motility.

Demonstration of Chemotaxis by Microscopic Assay

Five millilitre of filter sterilized CM was added into 18-24 hours culture of *E. coli* to resuspend the cells. Filter sterilized glucose and ethanol were set aside. Clean depression slides and cover slips were prepared through flame sterilization. The hanging drop technique was employed to observe the motility of the bacterium. Approximately 9 µl of resuspended *E. coli* cells were placed on a cover slip; the cover slip and drop were then inverted over the well of a depression slide. This procedure was repeated with addition of glucose or ethanol in the resuspended *E. coli* cells. The motility of *E. coli* was observed under the oil immersion objective of light microscope. The motility was also video-documented.

Demonstration of Chemotaxis by Capillary Assay

Freshly grown *E. coli* was resuspended in CM for 30 minutes. Afterwards, 200 µl of the cell suspension was transferred into three sterile Eppendorf tubes. Meanwhile, 200 µl of CM, 1 M glucose and 1 M absolute ethanol were separately placed in sterile Eppendorf tubes that contain two 1 µl capillary tubes. After 10 minutes, the capillary tubes were dipped into Eppendorf tubes with 200 µl cell suspension for 45 minutes to allow the entry of cells. After 45 minutes, the capillary tubes were removed from the cell suspension and its exteriors were washed with distilled water. The content of the capillary tube was resuspended in 100 µl diluent. The cells were serially diluted, and 100 µl of 10^{-2} 10^{-3} and 10^{-4} dilutions for CM, 10^{-3}, 10^{-4} and 10^{-5} dilutions for glucose, and 10^{-1} 10^{-2} and 10^{-3} dilutions for alcohol were spread plated on prepared LB plates. The plates were incubated at room temperature and the number of colonies was counted after 24 hours.

RESULTS AND DISCUSSION

In *E. coli* and *B. megaterium*, motility bands were observed in SIM + CM, SIM + attractant, SIM + repellent and SIM only. However, the motility band in SIM + repellent travelled less far as compared to SIM + CM and SIM + attractant. No motility bands were observed in *S. aureus* inoculated in SIM with CM, attractant or repellent, and SIM only (Figure 1). *S. aureus* served as negative control since this is a non-motile bacterium.

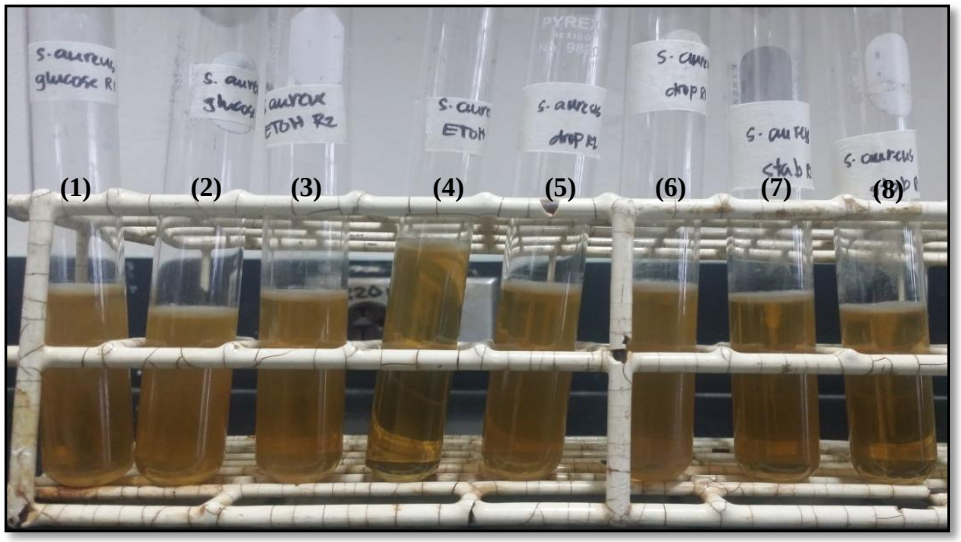

Figure 1A: *S. aureus* inoculated in SIM + glucose (tubes 1 and 2), SIM + alcohol (tubes 3 and 4), SIM + CM (tubes 5 and 6) and SIM only (tubes 7 and 8). No motility bands were observed.

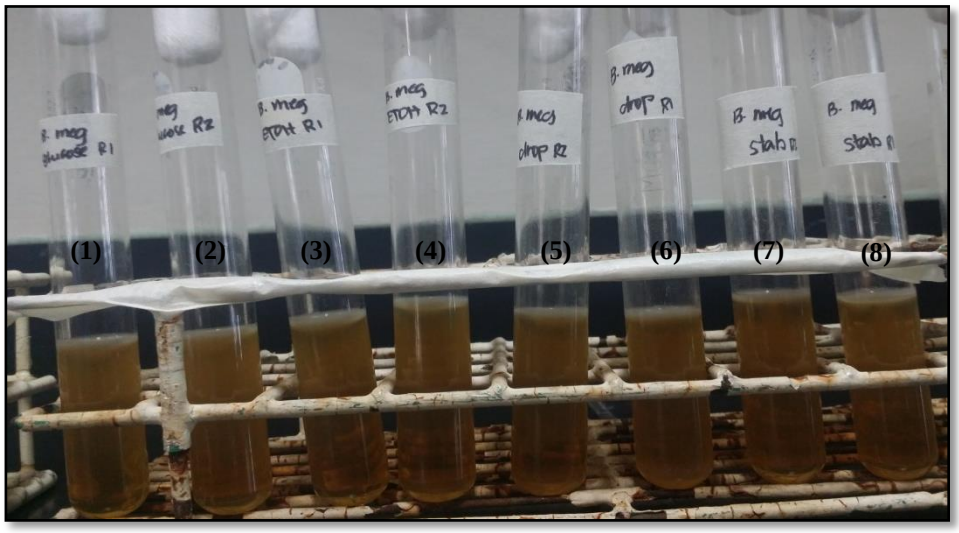

Figure 1B: *B. megaterium* inoculated in SIM + glucose (tubes 1 and 2), SIM + alcohol (tubes 3 and 4), SIM + CM (tubes 5 and 6) and SIM only (tubes 7 and 8). Motility bands were observed in all tubes.

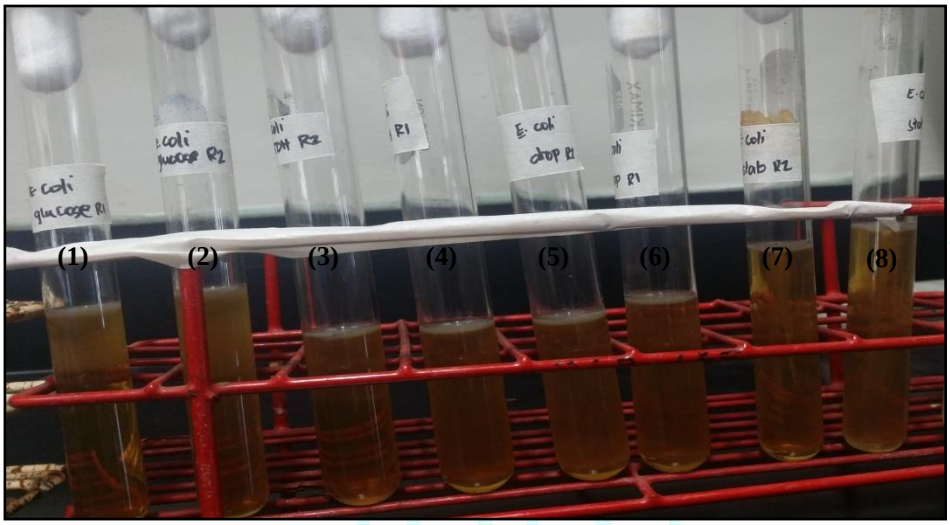

Figure 1C: *E. coli* inoculated in SIM + glucose (tubes 1 and 2), SIM + alcohol (tubes 3 and 4), SIM + CM (tubes 5 and 6) and SIM only (tubes 7 and 8). Motility bands were observed in all tubes.

Highest count (CFU/mL) was observed in capillary tubes dipped in attractant (3.7×10^8), followed by CM (5.2×10^7) and least in repellent (7.6×10^6) (Table 1). The colony counts were linearized (log) first before subjecting it to statistical analysis (One-way ANOVA, Tukey's test). Analysis of data showed that log CFU/mL in capillary tubes dipped in attractant (8.55±0.14) was significantly higher as compared to capillary tubes dipped in CM (7.64±0.29) and repellent (6.87±0.14) ($p<0.05$) (Figure 2).

Table 1. Number of *E. coli* colonies (CFU/mL) in capillary tubes dipped in CM, attractant and repellent.

	Dilution	No. of Colonies	CFU/mL	logCFU/mL
Chemotaxis Medium	10^{-4}	260	2.6×10^7	7.415
		923	9.2×10^7	7.964
		237	2.4×10^7	7.380
		645	6.5×10^7	7.813
Attractant	10^{-5}	416	4.2×10^8	8.623
		522	5.2×10^8	8.716
		247	2.5×10^8	8.400
		303	3.0×10^8	8.477
Repellent	10^{-3}	457	4.6×10^6	6.663
		834	8.3×10^6	6.919
		820	8.2×10^6	6.914
		934	9.3×10^6	6.968

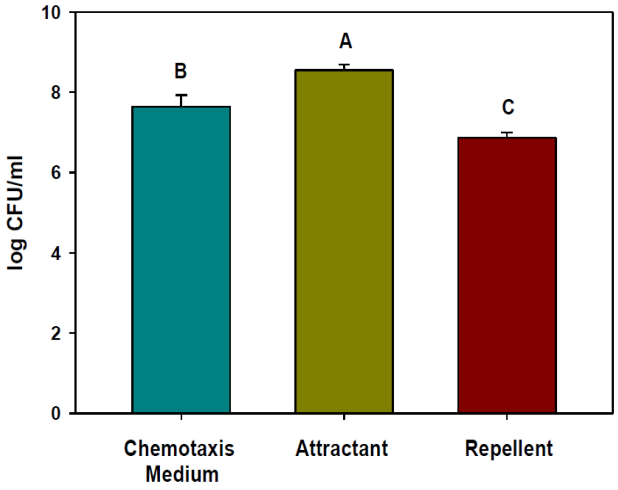

Figure 2. Linearized *E. coli* count (log CFU/mL) in capillary tubes dipped in CM, attractant and repellent (different letter showed significant difference at $p<0.05$).

Most bacteria such as *E. coli* bias their swimming motion towards specific attractants and away from repellents[2,8]. It was extensively studied that *E. coli* cells swim towards amino acids (serine and aspartic acid), sugars (maltose, ribose, galactose, glucose), dipeptides, pyrimidines and electron acceptors (oxygen, nitrate, fumarate). According to Adler et al. (1973), the most effective attractants are those that have thresholds near 10^{-5} M or below. Conversely, *E. coli* swims away from potentially noxious chemicals such as alcohols and fatty acids[9].

According to Berg and Brown (1972), the motion of *E. coli* resembles a random walk having periods of smooth swimming that is interrupted by brief tumbles that could change the swimming direction[10]. As observed under microscope, the tendency of *E. coli* to tumble was enhanced when the bacterium perceives conditions to be worsening, such as the addition of repellent, which in our case was absolute alcohol. Conversely, tumbling was suppressed and cells keep running when they detect that conditions are improving, in our case, when sugar was being added. Thus, when a bacterium runs up a gradient of attractants or down a gradient of repellents it tends to continue on course.

In addition, increasing the concentration of attractant and decreasing the concentration of repellent will result to decrease frequency of tumbling. Meanwhile, decreasing concentration of attractant and increasing concentration of repellent will result to increase frequency of tumbling[1].

E. coli response to attractant and repellent is mediated by protein network[5,8]. Using data from behavioural, genetic, biochemical and structural studies, the signal transduction pathway mediating *E. coli* chemotaxis has been extensively characterized[11,12]. *E. coli* can sense a variety of amino acids, sugars and dipeptides, as well as pH, temperature and redox state. Information about the chemical environment is transduced into the cells by chemoreceptors (e.g. aspartate receptor Tar), which span the membrane. The chemoreceptors form complexes inside the cells with the kinase CheA and CheW. CheA phosphorylates itself and then transfers phosphoryl groups to a diffusible messenger protein, the CheY. A tumble is induced when the phosphorylated CheY interacts with the flagellar motors[13,14]. The rate of CheY dephosphorylation is greatly enhanced by CheZ. Tumbling is reduced when attractants bind to the receptors, thus, decreasing the rate of CheY phosphorylation. Adaptation is provided by changes in the level of methylation of the chemoreceptors; methylation increases the rate of CheY phosphorylation[13,14]. Enzymes such as CheR and CheB are responsible in adding and removing methyl groups. In order to adapt to an attractant, methylation of the receptors must rise to overcome the suppression of receptor activity caused by the attractant binding. CheA enhances the demethylating activity of CheB by phosphorylating CheB on its amino-terminal domain[13,14].

CONCLUSION

The tendency of *E. coli* to tumble was enhanced when the bacterium perceives conditions to be worsening, such as the addition of repellent, which in our case was absolute alcohol. Conversely, tumbling was suppressed and cells keep running when they detect that conditions are improving, in our case, when sugar was being added. *E. coli* response to attractant and repellent is mediated by protein network.

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