Turbulence under the Microscope^{*}

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Abstract. Our objective was to observe a new form of turbulence caused by biological effects – biological micro-turbulence and explore its process and controlling factors. The methods used were proteus mirabilis CGCs micro-cultured to render the occurrence of the specific movement on micro-organic suspension and its controlling factors were determined by comparison with the control trials. The results showed that turbulence under the microscope was generally in a mass but partially regular. It was also confirmed that the turbulence under the microscope exhibited hollow effect, temperature-dependent switching on of occurrence and self-control of suspension quantity. It is clarified that this new form of turbulence is a spontaneous and self-control process, which provides an experimental model with controllable conditions for studies of turbulence and a new way for researches on the mechanism and physiological functions of the flow of body liquid.

Key words: Bacterial movement, chaos, cryptic growth cell, proteus mirabilis, turbulence

1. Introduction

During the growth of bacteria on media surface, a specific phenomenon of bacterial movement appears. This has received more and more attention in recent years [1–4]. J. Henrichsen [5] divided this movement into five types: swimming, gliding, twitching, darting and swarming. Furthermore, during the growth of some kinds of bacteria, they absorb water from vapor to form suspension or even exhibit unique forms of movement. We conducted bio-wave studies using proteus mirabilis (PM) as the subject and found that the vegetable cell of this bacterium could turn into cryptic growth cell (CGC). Here, bio-wave refers to the self-organization process of rhythmic growth of bacterial population. Compared with the vegetable cell, CGC possesses special biological characteristics, namely, it is filament-shaped, active and potentially proliferative but the amount of nuclear substances increased and no marked *in situ* proliferation is seen. During the formation of CGC, its interstitia absorbs water to form suspension, which results in the generally confused but locally regular movement. We term this phenomenon as bio-turbulence or turbulence under microscope. No report has been found on it. In physics, many studies have

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been conducted on the turbulence of macro-fluid. Turbulence [6], a special type of movement in nature, is non-fixed and highly dissipative fluctuations in which both large and small whirlpools exist. It is generally composed of subsystems interacting with each other with a dimension as high as 10^{10} – 10^{20} . It is difficult to deeply understand it for it is random and in a mass so that it becomes one of the irritating problems in natural sciences. We systematically observed the bio-turbulence with microscopy and studied its controlling factors, which explores turbulence from another level and a new angle and provides an experimental model with controllable conditions for the study of turbulence. Under the microscope, the turbulence exhibits both biological and microscopic characteristics, which may provide a new way of understanding the mechanism and physiological functions of the movement of body fluid in the organisms.

2. Materials and Methods

2.1. MATERIALS

2.1.1. *Bacterial Strain:*

Proteus mirabilis, a clinically isolated strain, kept in our laboratory, with stable biological features.

2.1.2. *Preparations of Culture Media*

The culture media for the wave growth of the bacterium were prepared according to the method reported by Qiwan Hsu et al. [7], the media contains: Tryptone 10 gram, Yeast Extract powder 5 gram, NaCl 10 gram, glucose 1 gram, Distilled water 1 liter, pH 7.2; the content of agarose varied from 5 gram per liter when the media is used for the micro-culture of bacteria to 20 grams when used to prepare the CGCs of PM and 0.1 gram per liter triphenyltetrazolium chloride (TTC) is needed.

2.1.3. *Preparation of CGC of PM*

It was conducted in the following procedure: After PM was inoculated on the central part of the culture media mentioned above, with 20 gram per liter agarose, the media was put into the specific culture system under the conditions as following: 40% relative humidity, average temperature 34 °C (31 °C–39 °C), time for temperature ranging from 31 °C to 39 °C 1 h and continuous air flow in the culture system. Eight hours after inoculation, colonies with a diameter of 1–2 mm were seen on the media and extended outward in the form of red-yellow alternative concentric rings at a speed of 1 ring/3 h, which is called rhythmic wave growth. The appearance of the red colony rings resulted from being reduced of TTC by bacteria. Whereas, the yellow ring of the colony represents the negative reductive reaction to TTC. The yellow ring consists of CGC of PM. CGC in the distal yellow ring was selected as the sample.

Figure 1. Coupled oscillation movement of CGCs ($\times 1000$).

2.1.4. *Micro-Video Recording System*

It includes an opton El-Einsatz phase-contrast fluorescent microscope, a Panasonic CL320 micro-video camera, a Sharp monitor (the word 'sharp' is the name of a Company), a Panasonic HD100 video recorder, a computer and image-processing software.

2.2. METHODS

The CGC of PM was prepared as $1 \times 10^8/\text{ml}$ suspension and cultured following the method as follows: Using a sterilized dropper, add the melted culture media with 5 gram per liter agarose, to the hollow of a clean chamber, covered with a lid, after the media solidification move the lid slightly and inoculate a loop of the suspension of CGC of PM on the surface of the media, recover the lid on the media surface. Keeping a suitable environmental temperature, and observing under the microscope, the movement of the suspension was dynamically observed and recorded with the micro-video recording system.

Figure 2. The view of the CGC suspension's flow ($\times 1000$).

3. Results and Discussion

3.1. COUPLED OSCILLATION AND OSCILLATION GROUP OF CGC

CGC could freely bend and swing in the suspension. Several CGCs tended to parallel to each other and move synchronously, which is named Swarm and fully studied [8–11]. What we found is that the swarm of *Proteus mirabilis* realized via synchronous movement of bacterial flagella (unpublished data), this work is similar to that of Strogatz, S.H. [12] who study the relationship between the coupled oscillators and biological synchronization. We borrow the term of 'coupled oscillation' in physics to describe the phenomenon, and for the purpose of exploring the mechanism by this way. We named the phenomenon of synergetic movement of several CGCs as coupled oscillation movement of CGCs and the CGC synergetic movement group as coupled oscillation group (Figure 1).

3.2. TURBULENCE AND HOLLOW EFFECT UNDER MICROSCOPE

Using microscopy, we found that several coupled oscillation groups collided with each other to result in the flow of the suspension at a high speed when the number and density of CGCs from vegetable cells increased to certain degrees. The flow of the suspension was non-oriental and showed the view of the suspension's flow, which might change at any time (Figure 2). The size of the coupled oscillation groups is various, i.e., the small one only contains 2–3 CGCs while the larger

Figure 3. Self-revolving structure caused by convergence of the CGC suspension's flow ($\times 400$).

contains tens of them and can produce much more power. Microscopic observation showed that the large coupled oscillation groups were in dominant position and could lead the small ones to result in orderly flow, which is stable-flow. However, in a certain region, two or even more large groups flowing in different directions usually co-existed. The convergence of the flows caused the change of the flow directions and appearance of discontinue flow. Rendered the image under microscope became mass generally and caused slender CGCs to revolve to form a self-revolving structure with an S-shaped center. The center was just part of one CGC and surrounded by the other parts shaped like rings. During the self-revolving, the center was continuously adjusted to adapt to the strike of the 'tide' of the suspension's flow (Figure 3). This is similar to the image of the flow form of the Taiji as it appeared at the convergence of the Luo River and its branches in ancient China. The appearance of the self-revolving structure reflects the local regularity during the fluid movement, which conforms to chaos characteristics [13]. This is consistent with the understanding of chaos in the world today.

The result showed that the flow under the microscope is a kind of movement of continuous media, the media contains bacteria, fluid and the products of bacteria. Generally, it is a random phenomenon, and locally there are a good many vortexes due to the flow of coupled oscillations groups containing more or less bacteria. These vortexes process different scale and characteristics of the flow under the microscope conforms to the description of turbulence made by Ruelle D. [14].

On the edge of the randomly distributed hollows on the surface of the media, gush of the suspension 'tide' was seen. CGCs went up and down with the gush, which propel the local suspension's flow. Sometimes, the flow surged over the surface of media far away from the hollows and then withdrew to expose it. This process repeatedly occurred at irregular intervals. All these are consistent with the results of the study on effects of projection on the object's surface on turbulence reported by L. Sirovich et al. [15].

3.3. OBSERVATION ON SELF-CONTROL OF THE SUSPENSION AND EFFECTS OF THE PRIMARY CULTURE TEMPERATURE

After the CGC was micro-cultured in a chamber with maintained humidity at 37 °C for 1–3 h, vapor convection occurred in the culture chamber. When the interstitia of CGCs absorbed a suitable amount of water the CGCs suspension formed and dispersed in all directions on the surface of media, and ultimately resulted in the turbulence occurring. The amount of absorbed water could not be controlled artificially. In one of the control trials, the turbulence was immediately stopped when some liquid was added to the chamber in which the turbulence was occurring. In another control trial, the turbulence did not occur after the CGC suspension was mingled in the culture chamber at 37 °C. This is called the self-control phenomenon of turbulence occurrence. Whether the turbulence occurs or not depends on the liquid quantity in the suspension on the medium surface at a certain area. Excessive liquid quantity results in free movement of CGCs while deficient quantity in swarming to form micro-colonies at the end. Observation on the switching on of turbulence of CGC suspension demonstrated that this process was temperature-dependent and its initial temperature was 25 °C–37 °C. When the culture chamber in which turbulence had occurred was kept at 4 °C for 10 min, no changes of the turbulence were found. Moreover, the turbulence remained the same for 8 h after the culture chamber was kept at 20 °C and stopped only for the changes of ecological conditions. However, no turbulence was found when the chamber was kept at 20 °C at the beginning of culture in the control. These results suggest that the temperature is important for the switching on of bio-turbulence, which might be that temperature can render the occurrence and convection of vapor and provide suitable amount of water for the space between CGCs to result in the occurrence of turbulence. Furthermore, the ability of temperature is promoting movement of CGCs is also an important factor.

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