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ORIGINAL ARTICLE

Designing and Constructing a Petri dish with High moisture retention capability, suitable for culture of Aerobic bacteria

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ABSTRACT
Petri dishes, as shallow cylindrical glass or plastic lidded dish, are used by biologists to culture cells, such as bacteria. They are consisted of container and lid that loosely attached to each other. This increases the probability of contamination and leads to drying out of culture media. We aimed to resolve the deficiencies through designing a new Petri dish. In this study, diagonal threads on the wall of lid and container were precisely created and a screw structure dish capped with a whole turn was constructed. Staphylococcus aureus sensitive to vancomycin and resistance to penicillin and cotrimoxazole was cultured in the designed (with a half turn rotation) and control Petri dishes for 24 h. Also, moisture retention capability of the designed Petri dish was evaluated by incubating at 50˚C for 120 h. The growth rate of aerobic bacteria and moisture retention capability were calculated by measurement of cell volume after centrifuging and evaluating macroscopically, respectively. The results of bacterial growth showed the same rates in the both dishes while the designed Petri dish indicated significant superiority in moisture preservation compared to the control Petri dish. Findings of the study suggested the designed Petri dish is suitable for biological researches.
Keywords: Petri dish, Screw structure, Aerobic bacteria, Moisture preservation

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INTRODUCTION

A pure culture was proposed by Robert Koch as the base of whole research in the field of infectious illnesses [1]. The isolation of a bacterium provides the opportunity to investigate virulence and to achieve Koch's criteria which in turn offers a connection between illnesses and microorganisms [2]. Besides, bacterial culture enables us to evaluate the antibiotic susceptibility and therefore provides the opportunity to treat effectively infectious diseases [3]. In addition, providing a bacterial culture allows us to determine the genome sequence of strains [4] and discern proteomic patterns to identify specific proteins and evaluate their antigenicity through immunoproteomic methods. This help us to provide the proteins as antigens for serologic tests [5]. Moreover, pure bacterial culture facilitates manipulation of genomic structure and makes it possible to us to distinguish the virulence, antibiotic resistance and the invasive capability of bacteria [6].

Clinical microbiology has been changed fundamentally since the invention of the Petri dish [7]. Using transparent lid in the structure of Petri dish makes it to us to watch colonies and limit the contamination. Adding kitchen substances, such as gelatin and agar has provided solid culture media [8], and this results in probable discrimination of bacterial species in pure culture [9]. Petri dishes are easy to use, inexpensive and constituted of base and lid [10]. However, as there is no grabbling between base and lid, they easily separate from each other. This causes some problems including drying out [11] and contamination [12] of cell culture media.

There are two main types of Petri dishes; those which have the projections on the inner surface of the lid and those which are free of projections on the inner surfaces of the lid. The projections are three small, (1

to 2 mm) high nub-like, located on the flat edge of container and prevent the dish from being completely capped [11].

Staphylococcus aureus -a gram-positive bacterium- belongs to Micrococcae family. The microorganism is characterized with a thick cell wall that contains nearly 50% peptidoglycan by weight. It is frequently distinguished as a commensal germ that lives on the surface of human anterior nostrils. Indeed, human is known as the main reservoir for *Staphylococcus aureus*. Also, *Staphylococcus aureus* could live on the other sites of body including throat, skin, vagina, perineum and gastrointestinal tract [13]. The bacterium is a non-motile, aerobic and facultative anaerobic microorganism [14].

In the present study, we aimed to construct a screw structure Petri dish to resolve the problems of drying out and contamination. The Petri dish was constructed successfully and characterized by bacterial culture and moisture retention capability methods.

MATERIALS AND METHODS

Materials

Sterile nutrient agar culture medium and control Petri dish were obtained from Sigma-Aldrich Company (USA). *Staphylococcus aureus* sensitive to vancomycin and resistance to penicillin and cotrimoxazole obtained from skin was prepared from Pasteur Institute of Iran, Tehran. Distilled water was used throughout the study.

Preparation of designed Petri dish

Firstly, a primary design of Petri dish was plotted, and the precise design was then prepared by Tarsh Tarash Company (Iran). Next, the proposed design was printed three dimensionally and constructed manually using plexy plates.

Evaluating bacterial growth on designed Petri dish

To evaluate the bacterial growth, the designed Petri dish was sterilized by UV irradiation under class II laminar hood. Next, 20 ml sterile nutrient agar culture medium were poured in both designed and control Petri dishes. The same concentrations of *Staphylococcus aureus* obtained from skin were then dispersed on the solidified nutrient agar culture media. The lid of designed Petri dish was rotated a half-turn clockwise and become attached. Also, the lid of control Petri dish was put on it. After that, both Petri dishes were incubated at 37˚C for 24h. The quality of bacterial growth was then evaluated by the measurement of cell volume after centrifuging and was compared to each other.

Evaluating moisture retention capability of designed Petri dish

The designed and control Petri dishes were filled with nutrient agar. The lid of designed Petri dish was rotated whole turn clockwise and capped completely. Also, the control Petri dish was covered via putting the lid on it. Next, they were transferred to incubator 50˚C and incubated for 120 h. Moisture retention capability of the both Petri dishes was then evaluated macroscopically.

RESULTS

Preparation of designed Petri dish

The primary design of Petri dish was consisted of horizontal shallow threads on the wall of both container and lid. Slim threads on the inner surface of container cause Petri dish to caulk. Due to the high cost of mold construction, plexy plates were used for shaving manually the designed Petri dish. This resulted in thicker walls of the designed Petri dish compared to the control one (Figure 1).

Evaluating bacterial growth on designed Petri dish

The results of bacterial culture showed the same growth rate of microorganism in the both Petri dishes after 24 h incubation at 37˚C (Figure 2).

Evaluating moisture retention capability of designed Petri dish

The study of moisture retention capability showed the considerable superiority of the designed Petri dish compared to the control Petri dish. While culture medium in the control Petri dish was completely dried and separated from the bottom of the Petri dish as a thin sheet, the culture medium in the designed Petri dish kept its appearance, and no change was seen. In other word, the culture medium water was retained in the designed Petri dish (Figure 3).

Low tilt of the threads in the experimental model limited rotating the lid on the container in specific sites. Therefore, a sophisticated design of Petri dish with more suitable tilt was desirable that could be achieved with the mold. Due to the lack of mold, we satisfied to a model of Petri dish prepared by three-dimensional printer (Figure 4).

Figure 1: The designed Petri dish. Upper part: the lid, underneath part: the container with caulking loop.

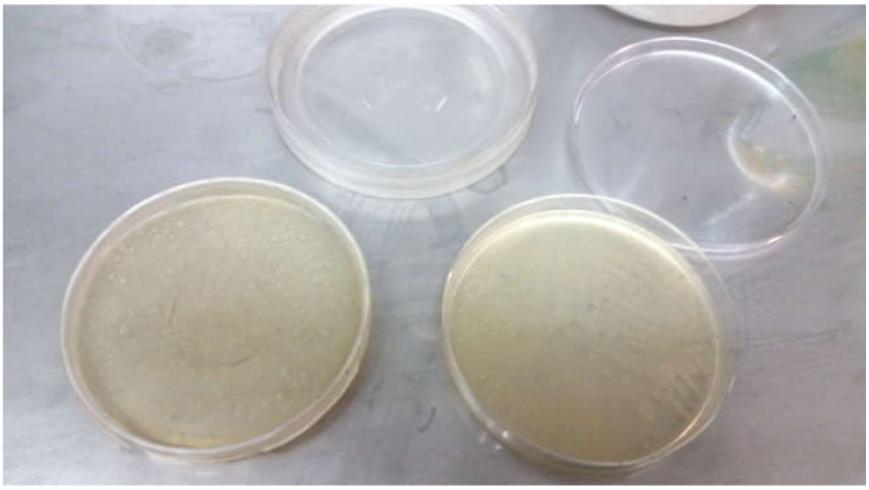

Figure 2: The rate of bacterial growth in the designed (left) and control Petri dishes (right).

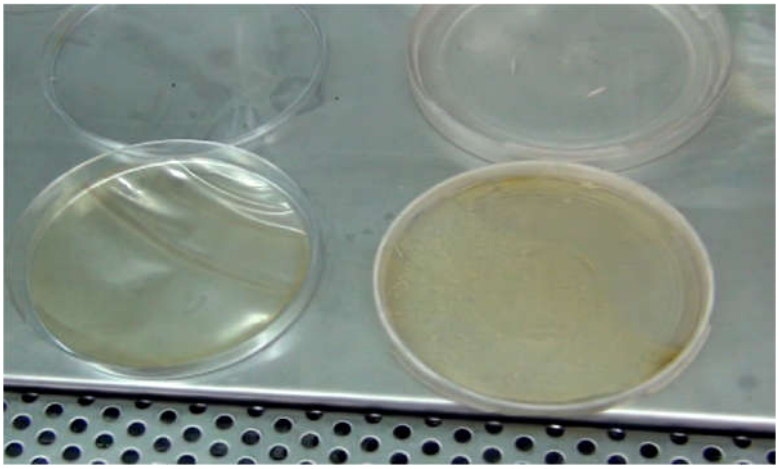

Figure 3: Moisture retention capability of the designed (right) and control (left) Petri dishes.

Figure 4: The proposed model constructed by three-dimensional printer.

DISCUSSION

In the present study, a Petri dish with high moisture retention capability and adjustable aeration was designed and constructed. In literature reviews including the United States Patent and Trademark Office, the Petri dish similar to our design was not reported [11,12,15]. Brusewitz prepared a Petri dish for culturing microorganisms. In this study, projections were created on the periphery of the inner surface of lid, and matching indents were prepared on the rim of container. This provides an adjusting gap between the lid and the container to regulate the gas exchange between the inside of Petri dish and atmosphere [11]. In another study, Henderson constructed a Petri dish equipped with a superior seal that was able to hold a sterile environment within the dish [12]. In addition, Ervin designed and constructed a Petri dish which had a bottom container member with a circumferential groove placed in the bottom corner of dish. The groove expedites distribution of liquid culture media throughout the filling operation. This results in holding the margin of the solidified media in situ that enhances the durability, and therefore provides an attractive visual view when the Petri dish is observed from the side. Also, the corresponding top and bottom walls of the lid and bottom container was 30% thinner than their related side wall divisions and this was cost effective to save the fabrication material [15]. However, in the conventional Petri dishes, the lid and the container are loosely attached to each other. This leads to the increase of probability of Petri dishes contamination and consequently the increase of likelihood of resterilization. Also, air inflow and outflow in Petri dishes enhance the risk of drying out of the culture media [11, 12]. Thus, making a Petri dish without the mentioned problems is desirable which was here prepared of plexy plates. The Petri dish was constructed through creating some diagonal threads in the outer and the inner part of walls of container and lid, respectively. The threads were developed in which the lid and the container were loosely linked to each other with a half-turn rotation. This provides an airflow which is necessary for aerobic bacteria growth. The growth of staphylococcus aurous confirmed this matter. Besides, a protruded loop in the inner surface of container wall caused Petri dish to caulk with a whole turn and as a result maintaining the moisture of culture media. Finally, it should be noted that the designed Petri dish in scale up production is economically affordable.

In conclusion, Petri dish is a fundamental tool in clinical microbiology consisting of a container and a lid that are loosely attached to each other. This enhances the probability of drying out of culture media and contamination of Petri dish that leads to resterilization of Petri dish. In the present study, a Petri dish was designed and prepared through creating the diagonal threads on the walls of the container and the lid. The screw structure Petri dish was characterized by bacterial culture and moisture retention capability

techniques. The results showed the competence of the designed Petri dish to resolve the Petri dish conventional problems.

COMPETING INTERESTS

The authors have declared that no competing interest exists.

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