Staphylococcus aureus: structure and function

Staphylococcus was first discovered in 1880 by Alexander Ogston. Currently, more than 30 different species of the genus has been identified. The name “ Staphylococcus” was derived from Greek, with the prefix “ Staphylo” referring to “ bunches of grapes” and the suffix “ coccus” referring to “ granule” (16). As the meanings suggest, bacteria from Staphylococcus are circular-shaped and their arrangement resembles bunches of grapes when observed under a microscope. Typically, a Staphylococcus has a diameter of approximately 1Î¼m (21).

The bacterial genus, Staphylococcus, will be isolated and identified in this project. This genus has been chosen to review because of its abundance on the skin of mammals and the pathogenic nature of one of its member, Staphylococcus aureus. Apart from skin infections, Staphylococcus aureus could mutate to form Methicillin-resistant Staphylococcus aureus (MRSA), which shows resistance to antibiotics. In both cases, these give rise to medical implications. In addition, the distinctive features of Staphylococcus aureus have increased the ease to isolate and identify it from other species in the genus via culturing and biochemical tests.

The aim of the project is to isolate Staphylococcus aureus from a bundle of cat hairs and verify its identity via microscopic examination and biochemical tests. No human specimen is used due to the potential pathogenic property of the bacterium. It is intended that a pure culture of pathogenic Staphylococcus aureus is obtained. For the purposes of this project, the importance of Staphylococcus aureus to humans, its classification in terms of morphology and physiological properties, methods of isolation with the use

of growth media and the technique of streak plating and identification by biochemical tests would be the four objectives to be addressed.

Objective 1: Importance of Staphylococcus aureus to humans

The importance of Staphylococcus aureus to humans would be outlined by a review of its cell structure, cell physiology and environmental niches, followed by the medical implications of Staphylococcus as a result of these properties.

Cell structure

As a member of the Bacteria domain, it is expected that Staphylococcus has bacterial cell structure. In other words, it lacks nucleus and membrane-bound organelles. The structural elements in a cell of Staphylococcus should include a cell membrane, cell wall, ribosome and nucleoid (6).

Moreover, being one of the five genera from the family of Staphylococcaceae, Staphylococcus possesses specific cellular properties that are unique to this family. In particular, it is a cocci and gram-positive bacterium and this indicates that its cell wall is essentially composed of a thick layer of peptidoglycan (21).

In addition to the above structures, Staphylococcus aureus possesses some special cellular structures that distinguish it from other species in the genus. This includes the possession of surface proteins that help attachment to proteins such as the fibronectin and fibrinogen-binding proteins involved in blood clotting (3). This cellular property may explain the pathogenic nature

of Staphylococcus aureus, as infections might be caused by invasion via wounds.

On the other hand, it is worthwhile to note that Staphylococcus does not have flagella and spores (16). That is to say, Staphylococcus aureus is non-motile.

Cell physiology

The cell physiology of Staphylococcus covers temperature, pH and oxygen requirements.

Most Staphylococcus can grow at 45°C, but it is reasonable to predict that its optimal temperature for metabolism would be close to the body temperature of humans, which is 37°C (5).

Concerning the optimum pH for metabolism, the enzymes in Staphylococcus work best in slightly alkaline medium, with a pH range of 7. 4 to 7. 6 (16).

As for oxygen requirement, Staphylococcus is facultative anaerobic (21). This implies that Staphylococcus can grow regardless of the presence of oxygen, but the presence of oxygen would be more favorable.

In the presence of oxygen, Staphylococcus utilizes glucose to carry out cellular respiration to generate energy for metabolism. Oxygen performs the role of a terminal electron acceptor and it is completely reduced to water (8).

When oxygen is lacking or absent, Staphylococcus may undergo fermentation and lactic acid is the usual product (21). In the process, glucose

is converted into substrate pyruvate, followed by its binding to the cofactor Nicotinamide Adenine Dinucleotide (NAD+) to produce lactic acid (6).

Moving on the ways Staphylococcus metabolize, as light is not readily available on skin surface and mucous membranes, it is proposed that Staphylococcus obtain energy via organic chemical compounds. Hence it is regarded as a chemotroph (21). The facultative anaerobic property of Staphylococcus may lead to a deduction that it utilizes organic carbon as the source of electron when oxygen is present. Though some Staphylococcus may use reduced forms of inorganic nitrates to generate electrons, its preference towards an aerobic atmosphere should define it as an organotroph (21). When comes to carbon source, Staphylococcus is a heterotrophy (12). That is to say, it attains its carbon source by utilization of organic substances such as sucrose for synthesis of metabolites (19). To summarize, Staphylococcus should be one of the members of the microbial group, Chemo-organotrophic heterotrophs.

Environmental niches

The environmental niches of Staphylococcus can be addressed by its interactions with the environment as to where it is found, the type of relationship it forms with other organisms and its capability of undergoing mutation.

Staphylococcus is commonly found on the skin and mucous membranes of animals with stable body temperatures, including humans (15). Typically, the skin temperature of humans is approximately 32°C, which is reasonably close to the optimal temperature of 37oC (22). This enhances the growth of

this microbe on skin. Moreover, the salty environment along skin surface due to the production of sweat may also account for the abundance of Staphylococcus in humans, since its enzymatic activity is optimal at more alkaline pH (17).

Staphylococcus aureus specifically colonizes in nasal cavity, larynx and on the skin surface of humans (2). The colonization of Staphylococcus aureus is principally achieved by fibrinogen-binding proteins adhering to the epithelial cells of the humans and thus this may outline a host-parasitic relationship between Staphylococcus and humans (10).

The interactions of Staphylococcus with the environment may also be underlined by mutation, which often occurs with Staphylococcus aureus. An example would be Methicillin-resistant Staphylococcus aureus (MRSA), a Staphylococcus aureus that is resistant particularly to the antibiotic, Methicillin (21). The mutation is caused by an alteration of the methicillin-resistance gene (mec A) coding for a penicillin-binding protein (4). This results in failure of antibiotics to cure infections caused by Staphylococcus aureus, which will be addressed in the medical implication section.

Medical implications of Staphylococcus

The features as in the cell structure, cell physiology and environmental niches of Staphylococcus can pose a great diversity of medical implications, which presents the importance of this bacterial genus.

Statistics show that Staphylococcus aureus is present in 79% of healthy people (14). Though Staphylococcus may colonize on the skin surface of the host without causing any harms, its ubiquity can still present various medical

issues. The MRSA mentioned previously would be one of the problems associated with Staphylococcus. Apart from methicillin, MRSA could show resistance against many other antibiotics such as penicillin and amoxicillin (1). The ineffectiveness of existing antibiotics to cure MRSA infections has resulted in fatality, and it is usually characterized by the incidence of septic shock and pneumonia (11). A rapid increase of MRSA infections has been observed over the decades. The rate of hospitalized MRSA infections was only 2% in 1974 but this figure increases dramatically to approximately 40% in 1997 (13). Consequently, this causes deaths of 19000 in the United States of America annually (11).

As Staphylococcus colonies on skin surfaces and mucous membrane, skin infections and diseases associated with mucous membranes could be another medical implication. It is known that Staphylococcus aureus may cause Scalded Skin and Toxic Shock syndromes. Moreover, it may cause urinary tract infections and food poisoning (9).

Objective 2: Classification of Staphylococcus

The classification of Staphylococcus can be reviewed in terms of its morphology and some of the physiological properties stated above.

Morphology

The morphology of Staphylococcus can be described as cocci gram positive bacteria arranged in a cluster, which can be readily observed under microscope with the application of gram stain. A purple color would be observed.

The reason for its cluster formation may be explained by its capability of undergoing binary fission in multiple planes with daughter cells remains proximal to each other (16).

Physiological properties

In terms of thermal requirement, Staphylococcus is classified as a mesophile. Regarding pH requirements, it falls into the category of neutrophile. Moreover, being a facultative anaerobe, Staphylococcus is catalase positive and it is generally considered a chemoorganotrophic heterotroph. In addition, Staphylococcus aureus is coagulase positive but not for other species in the genus. The absence of flagella indicates that Staphylococcus is a non-motile bacterium.

Objective 3: Methods of Isolation of Staphylococcus

The methods of isolation of Staphylococcus would include growing in medium followed by streak plating.

Growth media

To ensure optimum growth of Staphylococcus colonies, the sample of cat hairs should be enriched in nutrient broth with sodium chloride (NaCl) before plating on a nutrient agar. A nutrient broth normally consists of beef extract and peptone as fuels for growth (21). The temperature of incubation should be 37°C and the duration of incubation should be at least a day (20). This ensures that the Staphylococcus isolated can have sufficient time to grow at its optimum temperature. The addition of salt allows for a selective medium for Staphylococcus as it predominantly grows in salty environment. It also increases the pH of the medium to provide for a more alkaline environment to facilitate growth.

Alternatively, a growth medium can be done via a Mannitol salt agar (MSA), which consists of 7. 5% of NaCl and phenol red as a pH indicator. The medium is then incubated at 37°C for two days (14). MSA essentially acts as both a selective and differential medium. NaCl selects for saline-favored Staphylococcus and the pH indicator differentiates between Staphylococcus aureus and Staphylococcus epidermidis. Differentiation can be illustrated by the fact that Staphylococcus aureus utilizes mannitol in the agar for metabolism, and the generation of acidic product is indicated by a yellow color. However, this phenomenon does not apply to Staphylococcus epidermidis (21).

Streak Plating

Following enrichment, Staphylococcus in the medium can be transferred to an agar plate with nutrient broth and salt, by employment of aseptic techniques. At the same time, a transfer to an agar plate with only nutrient broth should be performed as a control set-up. This is to ensure the effectiveness of the selective media because other bacteria could grow on the agar plate if the medium was not set up properly.

Afterwards, the plates would be incubated for a week at 37oC for at least a day as in the incubation of sample in the nutrient broth. Plating and incubation should be repeated a few times to make sure that the colonies grown are pure.

Objective 4: Identification by biochemical tests

The identity of Staphylococcus cannot be confirmed by carrying out the gram reaction alone due to the fact that a great variety of bacteria from other

genus may also show gram positive reaction. Therefore, some biochemical tests have to be performed to verify that the bacteria isolated is in the genus of Staphylococcus and it is of the species Staphylococcus aureus. The catalase, Hugh and Leifson’s oxidation fermentation and cogulase tests are regarded as the standard tests for identification of Staphylococcus aureus (18). The mechanism of the tests is outlined below.

First of all, as Staphylococcus aureus is facultative anaerobic, it is expected that it contains enzymes to break down harmful products generated along the pathways of aerobic respiration. For instances, catalase breaks down superoxide radical hydrogen peroxide (H_2O_2) to oxygen and water, which are less harmful (8). Therefore colorless gas bubbles can be observed when H_2O_2 is added to a colony of Staphylococcus aureus.

Moreover, this property allows the Hugh and Leifson’s oxidation fermentation test to be performed. The bacterial sample is inoculated in a tube of Hugh & Leifson’s medium for five days to generate an anaerobic environment (18). As Staphylococcus can undergo fermentation in the absence of oxygen, growth can be observed throughout the tube. At the same time, it is necessary to implement positive and negative controls in order to confirm results. This can be achieved by inoculating bacteria that are known to be fermentative and oxidative respectively in the Hugh & Leifson’s medium along with the sample of Staphylococcus aureus.

Furthermore, the identification test between Staphylococcus aureus and other bacteria in the genus would be based on its reaction with coagulase. Staphylococcus aureus readily coagulates plasma but not for others species

in the genus (21). To ensure accuracy of the test, it is preferable to test on colonies extracted from culture plates that are known to contain coagulase positive Staphylococcus aureus and coagulase negative Staphylococcus epididymis respectively. The former acts as a positive control, while the latter acts as a negative control.

Conclusion

In conclusion, Staphylococcus is a bacterial genus that can pose various medical implications and it can be grown, isolated and identified based on its, environmental niches, morphology, physiological and structural characteristics. The aims of isolating Staphylococcus aureus as a pure culture and identifying by morphology and biochemical tests can be addressed by a review of the four objectives as summarized below.

Firstly, it is often found on epidermis of animal skins including humans and its ability to metabolize optimally at 37°C and at pH of 7. 4-7. 6 or salty environment makes it a potential pathogen to humans. In particular, the species Staphylococcus aureus can cause a great diversity of diseases and the mutated Methicillin-resistant Staphylococcus aureus could be fatal owing to its resistance to most antibiotics.

Secondly, it can be classified in terms of morphology and some of the physiological characteristics. Its morphology is gram positive and non-motile cocci bacteria growing in clusters. It is a mesophile, nuetrophile and facultative anaerobe. It is catalase positive and only Staphylococcus aureus is coagulase positive. The energy, electron and carbon sources of

Staphylococcus aureus can be described as chemo-organotrophic heterotrophic.

Thirdly, regarding growth medium, the sample of cat hair should be enriched in a medium of sodium chloride before incubating on an agar plate of nutrient broth and salt. In both cases, incubation should be at 37oC for a day. The colonies should be streaked plated a few times to remove contaminants so as to ensure culture is pure. This increases the efficiency of isolation of Staphylococcus aureus.

Lastly, Staphylococcus aureus can be identified by the catalase, Hugh & Leifson’s oxidation fermentation and coagulase tests. It is expected that bubbling is observed as a positive result in the catalase test. As for the Hugh & Leifson’s oxidation fermentation test, growth can be observed throughout the tube. In the coagulase test, clumping of plasma is seen as a positive result and this differentiates Staphylococcus aureus from other species in the genus. These tests establish the identity of Staphylococcus aureus.