Bacteria identification procedure

ASSIGN BUSTER

According to Bacteria are simplest but complex and sophisticated organism as compare with other microorganisms. Bacteria are classified as prokaryote organisms due to lack of true nucleus and nucleus membrane, it chromosome lies freely in the cytoplasm. Morphologically, bacteria adopt variation of shape and size including irregular, circular, elevated, flat and punciform. However, bacteria are grouped in three main shapes namely rod - shaped (bacillus), spherical (coccus) and spiral (spirillus), these bacterial cell shapes are of fundamental significant in identification and classification of bacterial.

Bacteria can thrive aerobically or anaerobically everywhere in the environment; also can exist even in extreme temperature zones and high concentration of toxic chemical areas. Nutritionally, most bacteria metabolised their essential nutrient through several ways from the environment including feeding on host organisms (heterotrophs). Additionally, some bacteria metabolised their own food with the use of sunlight, water and CO2 through simple diffusion, and the ultimate goal is to retain intracellular equilibrium (Forbes et al., 2007).

Bacteria have been classified distinctively into Gram- positive and Gram negative organism depending on the composition of their cell wall and ability retain basic gram stain and counterstain. Gram-positive bacteria have thick peptidoglycan which enables it to take up crystal violet and reveals purple colour after gram stain. Alternatively, gram-negative bacteria have complex (thinner peptidoglycan) membrane with more than one layer and cannot retain basic crystal violet stain during decolouring stage but rather need counterstaining to reveal pink colour. However, few groups of bacteria have

waxy cell wall which does not pick up conventional gram stain easily but rather need to be heated for some minutes for dye to penetrate their cell wall. These bacteria resist acid or alcohol during the decolourization process (Madigan et al., 2009)

Bacteria prolifically reproduce asexually by binary fission when resources are available. During this process the cell divides resulting in the production of two daughter cells which are identical. Incidentally, some bacteria can reproduce by methods like budding and even create variance through recombination and exchange of genetic materials, this can occur when bacteria goes through the process of conjugation, transformation and transduction. In fact, this process makes bacteria more susceptible to some antibiotics ( ).

Some activities of bacteria is of high beneficial despite the fact that certain strains can be pathogenic and also destructive in terms of food (spoiling food). Economically, existence of bacteria has contributed significantly in food processes including preparation of fermented food such as yogurt, soya source, cheese and wine respectively. Moreover, bacterial has keep pharmaceutical and chemical manufacturing from collapsing; these industries used bacterial in preparing antibiotic, vaccines, steroid and agrochemical.

Common (commensal flora) flora living in the gut helps in the digestion of food and also synthesis of nutrient such as vitamin B. On the other hand pathogenic bacteria cause variety of diseases in animals and plants. Commonly bacteria found in the normal flora such as skin, mouth and

intestine can be pathogenic due to homeostatic imbalance of the immune system.

Accurate and definitive bacteria identification is important for accurate disease diagnoses; administer certain antibiotic and giving other treatments of infections. It’s also vital in trace back pandemic outbreak associated with bacterial infection. In addition identification of bacteria is employed in a wide range of application such as criminal investigation, food production and environmental studies.

Primarily, bacterial identification is the observation of some characteristic of unknown strain with registered bacteria strain or specie for example Escherichia coli. A number of specialised biochemical tests usually performed in bacteria identification includes carbohydrate test, enzyme test and test for specific end-products (API 20E kit). These tests often based on combination of morphology and metabolic by-product. Conversely, immunological tests, Protein and Nucleic acid sequences and typing method can also be used in obtaining specific and accurate conformation of true identities of strains of bacteria in a specialized controlled laboratory procedure. However, for the purpose of routine bacterial identifications, these techniques are very expensive and time consuming as compared to primary biochemical test.

Therefore performing accurate, constituent and definitive bacteria identification procedure is important to identify the disease causing agent which helps in making accurate diagnoses of certain diseases and also giving other treatment of infection.

AIMS

This practical is aiming to establish negative and positive reaction to different biochemical test employed in bacteria identification, also to discover unknown groups of bacteria and compare with known registered bacteria base on morphology analysis and other biochemical test furthermore to acquire the skill of biochemical technique of identification of bacteria.

DISCUSSION

The results obtained from the experiment indicate variation in growth as a result of favourable conditions provided for each organism throughout the incubation stage. Difference in bacteria colony appearance including shape, elevation, edge colour and texture were observed on the growing organism and this can be attributed to type of bacteria use in the experiment. (Greenwood et al, 2001) stated that bacteria thrive well when placed in an appropriate nutritious atmosphere maintained under right chemical and physical environments. This suggests that, the cell division leading to the multiplication of cell colonies on the growth medium was under suitable condition.

According to (Madigan et al, 2009), gram staining method reveals the cell morphology of various bacteria such as the rod, cocci and sh . Gram staining can also assist in grouping bacteria into gram positive and gram negative using the cell wall composition. This confirms the observation discovered under the microscope, some bacteria exhibit the characteristics of gram positive and gram negative. Out of 10 organisms inoculated in this practical,

one was observed to be mycelium and the rest were observed as rod shaped bacteria.

The organisms A, Band D were identified as gram positive bacteria as a result of similarities in their characteristic. The organism A, B and D were linked to bacillus due to the appearance of colonies present and other biochemical test. Large colonies, irregular margins, flat elevation with yellow colonies were observed for the three organisms. Also, gram positive rod shape bacterial were viewed under the microscope with the aid of gram stain technique. Again these organisms were tested positive for the following tests; endospore, gelatin because the medium was still liquidised, sucrose and lactose tends to turn the medium from its original colour to deep yellow, and catalyst forming bubbles due to the release of oxygen, but negative for oxidase. Additionally, these organisms reacted positively to citric because they utilised carbon as their energy source. There were some degree of growth on the plate and this concludes that these organisms are aerobic.

Based on morphological analysis, organism observed on agar plate label F was associated with Escherichia coli, the reason being that, the unknown organism formed medium sized colonies with circular shape, smooth texture with yellow colour colonies and convex elevated. Additionally, based on microscopic analysis carried out with gram stain, the unknown organism displayed gram negative rod shape bacteria. The unknown organism was catalase positive due to the presence of bubble formation which indicate the broken down of hydrogen peroxide to release oxygen , oxidase positive, indole positive, citrate negative, Methyl red positive, glucose positive, and

voges-proskasuer negative, therefore these result suggested that, the bacteria found on plate F can be confirm as E. coli.

Organism I was however identified as Salmonella bacteria because of the following observations, being gram negative rod, tested positive for catalase as a result of bubbles formation), responding negatively to gelatin since it solidifies, again this organism shows negative response to oxidase, urea, indole, glucose, lactose and sucrose.

Enterobacter was identified in plate G due to distinctive characteristic shown, the unknown organism appeared to be gram-negative rod which forms cream circular colonies, smooth edges, elevated with smooth texture. Also based on further test carried out, this organism appears positive for catalase test due to the expression of bubble, gelatine and oxidase results were negative; carbohydrate compounds test results including sucrose, lactose and glucose were negative and urea appeared to be positive. The organism came out colourless to indole which indicates negative result. For cross milk test, the organism was able to produce acid which turn the medium from blue to yellow with firm precipitation.

Klebsiella was also identified in plate C, again due to particular features expressed from the microbes, gram- negative rod was observed, and this organism formed small sticky red colonies that were circular, slight elevated with smooth edges. The enzyme catalase was able to degrade hydrogen peroxide and release oxygen by forming bubbles. However, gelatin remains liquid while oxidase synthesis turns to be negative. Urea was turn to pink as a re