Psbpp103

Literature, Russian Literature

PSBPP103 Differential Gram’s staining EXPERIMENT NO. 1 AIM: THEORY: Page No: C1 Date: To Gram stain the given bacterial suspension and to differentiate between gram positive and gram negative organism. Visualization of microorganisms in the living state is very difficult, not just because they are minute, but because they are transparent and almost colorless when suspended in an aqueous medium. To study their properties and divide microorganisms into specific groups for diagnostic purposes, biological stains and staining procedures, in conjunction with light microscopy, have become major tools in microbiology. Chemically, a stain may be defined as an organic compound containing a benzene ring plus a chromophore and an auxochrome. Stains are of 2 types: 1. Acidic stains e. g., picric acid 2. Basic stains e. g., methylene blue. Types of staining techniques: 1. Simple staining. (Use of a single stain)This type of staining is used for visualization of morphological shape (cocci, bacilli, and spirilli) and arrangement (chains, clusters, pairs, and tetrads). 2. Differential staining. (Use of 2 contrasting stains)It is divided into two groups: (a) Separation into groups, Gram stain and acid-fast stain. (b) Visualization of structures, Flagella stain, capsule stain, spore stain, nuclear stain. The Gram Stain The Gram stain is the most widely used staining procedure in bacteriology. It is called a differential stain since it differentiates between Gram-positive and Gram-negative bacteria. Bacteria that stain purple with the Gram-staining procedure are termed Gram-positive; those that stain pink are said to be Gram-negative. The terms positive and negative have nothing to do with electrical charge, but simply designate 2 distinct morphological groups of bacteria. Grampositive and Gram-negative bacteria stain differently because

of fundamental differences in the structure of their cell walls. The bacterial cell wall serves to give the organism its size and shape, as well as to prevent osmotic lysis. The material in the bacterial cell wall that confers rigidity is peptidoglycan. PSBPP103 Page No: C2 In electron micrographs, the Gram-positive cell wall appears as a broad, dense wall 20—80 nm thick and consists of numerous interconnecting layers of peptidoglycan. Chemically, 60% to 90% of the Gram-positive cell wall is peptidoglycan. Interwoven in the cell wall of Grampositive are teichoic acids. Teichoic acids that extend through and beyond the rest of the cell wall are composed of polymers of glycerol, phosphates, and the sugar alcohol ribitol. Some have a lipid attached (lipoteichoic acid). The outer surface of the peptidoglycan is studded with proteins that differ with the strain and species of the bacterium. The Gram-negative cell wall, on the other hand, contains only 2—3 layers of peptidoglycan and is surrounded by an outer membrane composed of phospholipids, lipopolysaccharide, lipoprotein, and proteins. Only 10%—20% of the Gram-negative cell wall is peptidoglycan. The phospholipids are located mainly in the inner layer of the outer membrane, as are the lipoproteins that connect the outer membrane to the peptidoglycan. The lipopolysaccharides, located in the outer layer of the outer membrane, consist of a lipid portion called lipid A: embedded in the membrane, and a polysaccharide portion extending outward from the bacterial surface. The outer membrane also contains a number of proteins that differ with the strain and species of the bacterium. PRINCIPLE The Gram-staining procedure involves 4 basic steps: 1. The bacteria are first stained with the basic dye crystal violet. Both Gram-positive and Gramnegative

bacteria become directly stained and appear purple after this step. 2. The bacteria are then treated with Gram’s iodine solution. This allows the stain to be retained better by forming an insoluble crystal violet-iodine complex. Both Gram-positive and Gramnegative bacteria remain purple after this step. 3. Gram’s decolorizer, a mixture of ethyl alcohol and acetone, is then added. This is the differential step. Gram-positive bacteria retain the crystal violet-iodine complex, while Gramnegative are decolorized. 4. Finally, the counterstain safranin (also a basic dye) is applied. Since the Gram-positive bacteria are already stained purple, they are not affected by the counterstain. Gram-negative bacteria, which are now colorless, become directly stained by the safranin. Thus, Gram-positive bacteria appear purple and Gram-negative bacteria appear pink. PSBPP103 Page No: C3 With the current theory behind Gram-staining, it is thought that in Gram-positive bacteria, the crystal violet and iodine combine to form a larger molecule that precipitates out within the cell. The alcohol/acetone mixture then causes dehydration of the multilayered peptidoglycan, thus decreasing the space between the molecules and causing the cell wall to trap the crystal violetiodine complex within the cell. In the case of Gram-negative bacteria, the alcohol/acetone mixture, being a lipid solvent, dissolves the outer membrane of the cell wall and may also damage the cytoplasmic membrane to which the peptidoglycan is attached. The single thin layer of peptidoglycan is unable to retain the crystal violet-iodine complex and the cell is decolorized. It is important to note that Gram-positivity (the ability to retain the purple crystal violet-iodine complex) is not an all-or-nothing phenomenon, but a matter of degree. There are several factors that could

result in a Gram-positive organism staining Gram-negatively: 1. The method and techniques used: Overheating during heat fixation, over-decolorization with alcohol, and even too much washing with water between steps may result in Gram-positive bacteria losing the crystal violet-iodine complex. 2. The age of the culture: Cultures more than 24 hours old may lose their ability to retain the crystal violet-iodine complex. 3. The organism itself: Some Gram-positive bacteria are more able to retain the crystal violetiodine complex than others. Therefore, one must use very precise techniques in Gram staining and interpret the results with discretion. PSBPP103 REQUIREMENTS: Sample used Stains used Given sample culture Page No: C4 Crystal violet (2gm Crystal violet + 20ml 95% ethanol + 0. 8gm Ammonium oxalet + 80ml distilled water) Gram’s iodine (1gm iodine + 2gm potassium iodide + 100ml distilled water) Decolorizer (Mix 95% alcohol & acetone in equal proportion) Safranin (0. 34gm safranin + 10ml absolute alcohol + 90ml distilled water) Clean grease free glass slide Nichrome wire loop Dropper Filter papers Compound microscope Cedar wood oil Miscellaneous PROCEDURE: On a grease free slide prepare smear of bacterial suspension and allow it to air dry. After drying heat fix smear and allow slide to cool. Cover the smear with crystal violet stain and leave for 1 minute Drain stain and wash the slide carefully under running tap water. Flood the smear with Gram’s iodine solution and wait for 1 minute. Wash the slide under running tap water. 6. Decolorize the smear with alcohol-acetone for 20-30 seconds (continue till purple stain just stops coming off the slide). 7. Gently wash the slide under running tap water and drain completely. 8. Counter stain the smear with safranin for 1 minute. 9. Wash the slide with water and air dry (or

dry it carefully by using a blotting paper). 10. A drop of cedar wood oil is put over smear and the slide under oil-immersion lens. 1. 2. 3. 4. 5. PSBPP103 OBSERVATION: Page No: C5 PSBPP103 RESULT: Page No: C6 CONCLUSION: PSBPP103 Growth Curve Experiment No. 2A AIM: THEORY: Page No: C7 Date: EXPERIMENT NO. 2 Determine the growth curve of Escherichia coli by using colorimeter. The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time OR log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The

growth curve has four distinct phases 1. Lag phase When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesizing the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent. PSBPP103 2. Page No: C8 Exponential or Logarithmic (log) phase During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 20, 21, 22, 23..... 2n, n is the number of generations) This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms.

E. coli divides in every 20 minutes; hence its generation time is 20 minutes. 3. Stationary phase As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This results in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavorable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual. 4. Decline or Death phase The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavorable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores. PSBPP103 PRINCIPLE: Page No: C9 The increase in the cell mass of the organism is measured by using the Spectro-photometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of

microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass. The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value. REQUIREMENTS: Sample used Media Glassware Miscellaneous Overnight grown culture of Escherichia coli in sterile Luria Bertani broth (25ml) Sterile Luria Bertani broth in side arm flask (50ml) Sterile 10ml pipette (1) Rotary shaker at 37°C Colorimeter (O. D at 540nm) Graph paper PROCEDURE: 1. Inoculate 5 ml of overnight grown culture of Escherichia coli into fresh sterile nutrient broth so as to get O. D 0. 05. 2. Determine the absorbance at 540nm after adjusting colorimeter. Use a sterile nutrient broth as the blank for adjusting 100% transmittance or 0% absorbance. 3. This is as used as ‘ 0’ hr reading. 4. Incubate flask on shaker at 37◦C. Take readings of inoculated flask for every half an hour interval till the stationery phase is reached. 5. Plot a graph of absorbance against time (minutes). PSBPP103 OBSERVATION: Time (minutes) 0 30 60 90 120 150 180 210 240 270 300 330 360 390 O. D (540nm) 0. 04 0. 04 0. 05 0. 08 0. 12 0. 15 0. 17 0. 20 0. 23 0. 24 0. 25 0. 25 0. 25 0. 23 Page No: C10 PSBPP103 RESULT: Page No: C11 CONCLUSION: PSBPP103 EXPERIMENT NO. 2B AIM: To determine the cell growth by plating method. Page No: C12 PRINCIPLE Aseptic technique and transfer of microorganisms INTRODUCTION In natural environments, microorganisms usually exist as mixed populations. However, if we are to study, characterize, and identify microorganisms, we must have the organisms in the form of a pure culture. A pure culture is one in which all organisms are descendants of

the same organism. In working with microorganisms, we must also have a sterile nutrient-containing medium in which to grow the organisms. Anything in or on which we grow microorganisms is termed a medium. A sterile medium is one that is free of all life forms. It is usually sterilized by heating it to a temperature at which all contaminating microorganisms are destroyed. Finally, in working with microorganisms, we must have a method of transferring growing organisms (called the Inoculum) from a pure culture to a sterile medium without introducing any unwanted outside contaminants. This method of preventing unwanted microorganisms from gaining access is termed aseptic technique. ASEPTIC TECHNIQUE The procedure for aseptically transferring microorganisms is as follows: 1. Sterilize the inoculating loop. The inoculating loop is sterilized by passing it at an angle through the flame of a gas burner until the entire length of the wire becomes orange from the heat. In this way, all contaminants on the wire are incinerated. Never lay the loop down once it is sterilized or it may again become contaminated. Allow the loop to cool a few seconds to avoid killing the inoculum. 2. Remove the inoculum. (a) Removing inoculum from a broth culture (organisms growing in a liquid medium): 1. Hold the culture tube in one hand and in your other hand; hold the sterilized inoculating loop as if it were a pencil. 2. Remove the cap of the pure culture tube with the little finger of your loop hand. Never lay the cap down or it may become contaminated. 3. Very briefly hold a flame to the lip of the culture tube. This creates a convection current that forces air out of the tube and preventing airborne contaminants from entering the tube. The heat of the gas burner also causes the air around your work area to rise, and this also reduces the chance of airborne microorganisms

contaminating your cultures. 4. Keeping the culture tube at an angle, insert the inoculating loop and remove a loopful of inoculum. 5. Again hold a flame to the lip of the culture tube. PSBPP103 Page No: C13 6. Replace the cap. (b) Removing inoculum from a plate culture (organisms growing on an agar surface in a petri plate): 1. Sterilize the inoculating loop in the flame of a gas burner. 2. Lift the lid of the culture plate slightly and stab the loop into the agar away from any growth to cool the loop. 3. Scrape off a small amount of the organisms and close the lid. 3. Transfer the inoculum to the sterile medium. (a)Transferring the inoculum into a broth tube: 1. Pick up the sterile broth tube and remove the cap with the little finger of your loop hand. Do not set the cap down 2. Briefly hold a flame to the lip of the broth tube. 3. Place the loopful of inoculum into the broth, and withdraw the loop. Do not lay the loop down. 4. Again hold a flame to the lip of the tube. 5. Replace the cap. 6. Resterilize the loop by placing it in the flame until it is orange. Now you may lay the loop down until it is needed again. (b)Transferring the inoculum into a petri plate: 1. Lift the edge of the lid just enough to insert the loop. 2. Streak the loop across the surface of the agar medium. These streaking patterns allow you to obtain single isolated bacterial colonies originating from a single bacterium or arrangement of bacteria. 3. In order to avoid digging into the agar, as you streak the loop over the top of the agar, you must keep the loop parallel to the agar surface. Always start streaking at the “ 12: 00 position" of the plate and streak side-to-side as you pull the loop toward you. Each time you flame and cool the loop between sectors, rotate the plate counterclockwise so you are always working in the “ 12: 00 position" of the plate. This keeps the inoculating loop parallel with the agar

surface and helps prevent the loop from digging into the agar. 4. Remove the loop and close the lid. 5. Resterilize the inoculating. In the future, every procedure in the lab will be done using similar aseptic technique. Different spread plate patterns PSBPP103 REQUIREMENTS: Culture Medium Glassware Overnight grown culture of Escherichia coli 1)Sterile Nutrient broth 2)Sterile Nutrient agar plates (14) 1) 1ml sterile pipettes (14) 2) 10ml sterile pipettes (1) 3) Sterile test tubes (112) 4)glass stirrer 1) Glass beaker containing absolute alcohol 2) Sterile saline (0. 85%) 3)Colorimeter (O. D at 540nm) Page No: C14 Miscellaneous 1) Inoculate 5ml of overnight grown culture of Escherichia coli into fresh sterile nutrient broth. After thorough mixing, withdraw 0. 5ml of aliquot & add into test tube containing 4. 5ml of sterile saline. Perform serial dilutions up to 10-8 dilution. Perform spread plate technique by spreading 0. 1ml of solution taken from 10-8 dilution tube on sterile nutrient agar plate. 2) After 0. 5ml aliquot is withdrawn, incubate the flask at 370 C. 3) After 30mins interval, withdraw aliquots of 0. 5ml &add it into test tube containing 4. 5ml sterile saline. Perform spread plate technique after doing serial dilutions. 4) Repeat this step for every 30mins interval. 5) In such way, take optical density readings up to 390mins & perform the serial dilutions & spread plate technique for each respective reading. 6) Keep all the plates for incubation at 370 C for 24 hrs. 7) Plot a graph of cell number against time on semi-log graph. PROCEDURE: PSBPP103 OBSERVATION: Page No: C15 Time (in minutes) 0 30 60 90 120 150 180 210 240 270 300 330 360 390 Cells forming units/0. 1ml 57 Ã—108 57 Ã—108 Cells forming units/ml 5. 7 Ã—1010 5. 7 Ã—1010 208 Ã—108 260 Ã—108 272 Ã—108 360 Ã—108 Uncountable 463 Ã—108 463 Ã—108 460 Ã

—108 450 Ã—108 462 Ã— 108 444 Ã—108 60 Ã— 108 2. 08 Ã—1011 2. 60 Ã —1011 2. 72 Ã—1011 3. 60 Ã—1011 Uncountable 4. 63 Ã—1011 4. 63 Ã— 1011 4. 60 Ã—1011 4. 50 Ã—1011 4. 62 Ã— 1011 4. 44 Ã—1011 6. 0 Ã— 1010 PSBPP103 RESULT: Page No: C16 CONCLUSION: PSBPP103 Cell Viability Testing by Dye Exclusion Method EXPERIMENT NO. 3 Date: AIM: To determine the cell viable count by dye exclusion method. Page No: C17 PRINCIPLE: The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, eosin, or propidium, whereas dead cells do not. in this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. Determination of the number of cells (total and viable): View the cells under a microscope at 100x magnification. Under the microscope, you should see a grid of 9 squares. Focus the microscope on one of the 4 outer squares in the grid. The square should contain 16 smaller squares. Count all the cells in the four 1 mm corner squares. If there are too many or few cells to count, repeat the procedure either concentrating or diluting the original suspension as appropriate. For an accurate determination, the total number of cells overlying one 1 mm2 should be between 15 and 50. If the number of cells per 1 mm2 exceeds 50, dilute the sample and count again. If the number of cells per 1 mm2 is less than 15, use a less diluted sample. If less dilute samples are not available, count cells on both sides of the Haemocytometer (8 x 1 mm2 areas). Keep a separate

count of viable and non-viable cells. If greater than 25% of cells are nonviable, the culture is not being maintained on the appropriate amount of media; reincubate the culture and adjust the volume of media according to the confluency of the cells and the appearance of the media. Include cells on top and left touching middle line. The cells touching middle line at bottom and right are not counted. Note: I. Trypan Blue is the " vital stain"; excluded from live cells. II. Live cells appear colorless and bright (refractile) under phase contrast. III. Dead cells stain blue and are non-refractile. PSBPP103 Page No: C18 Dye exclusion is a simple and rapid technique measuring cell viability but it is subject to the problem that viability is being determined indirectly from cell membrane integrity. Thus, it is possible that a cell's viability may have been compromised (as measured by capacity to grow or function) even though its membrane integrity is (at least transiently) maintained. Conversely, cell membrane integrity may be abnormal yet the cell may be able to repair itself and become fully viable. Another potential problem is that because dye uptake is assessed subjectively, small amounts of dye uptake indicative of cell injury may go unnoticed. In this regard, dye exclusion performed with a fluorescent dye using a fluorescence microscope routinely results in the scoring of more nonviable cells with dye uptake than tests performed with trypan blue using a transmission microscope. A more sophisticated method of measuring cell viability is to determine the cell's light scatter characteristics or propidium uptake (unit 5. 4). However, this technique is far more time consuming and is necessary only when precise measurements on the number of dead cells in a cell mixture must be obtained. Trypan blue exclusion, as described in the below protocol, can be

performed in 5 to 10min PSBPP103 REQUIREMENTS: Sample used Dye used Page No: C19 Pollen grains of 0. 4% Trypan blue (0. 4gm Trypan blue + 100ml Phosphate buffer saline) (pH= 7. 2) Phosphate buffer saline (PBS) (8gm NaCl + 0. 2gm KCl + 1. 44gm Na2HPO4 + 0. 24gm KH2PO4 + 800ml Distilled water) (pH= 7. 4) Centrifuge Haemocytometer slide Compound microscope Eppendroff tube Capillary tube Miscellaneous PROCEDURE: 1. Centrifuge an aliquot of cell suspension being tested for viability for 5 minutes at 100g and discard the supernatant. 2. The aliquot should contain convenient number of cells to count in Haemocytometer. 3. Mix one part of 0. 4% trypan blue and one part of the cell suspension. 4. Allow the mixture to incubate for 3 minute at R. T. (cells should counted within 3-5 minutes of mixing with trypan blue) as longer incubation period will lead to cell death and reduce viability count. 5. Take a drop of trypan blue and cell mixture on Haemocytometer. Place the Haemocytometer on stage of binocular microscope and focus on cells. 6. Count the unstained (viable) and stained (non-viable) cells separately in Haemocytometer. 7. To obtain total number of viable cells/ml of aliquot multiply total number of viable cells by 2 (1: 2). 8. To obtain total number of cells/ml of aliquot add up total number of viable and non-viable cells and multiply by 2. 9. Calculate percentage of viable cells by using following formula : total number of viable cells/ml of aliquot percentage of viable cells = Ã— 100 total number of cells/ml of aliquot PSBPP103 OBSERVATION: V V V N V V V N V V V V N V V V V N V V V V N V N Page No: C20 V= viable cells, N= non-viable cells CALCULATIONS: PSBPP103 RESULT: Page No: C21 CONCLUSION: PSBPP103 Cell Death by Physical Agent EXPERIMENT NO. 4 AIM: THEORY: Page No: C22 Date: To study the effect of

physical agent (temperature) on bacterial cell. Temperature is one of the most important physical factors affecting microbial growth. Every species of micro-organism grows over a temperature range from minimum temperature of growth to maximum temperature of growth (cardinal temperature points). The range of temperature preferred by bacteria is genetically determined resulting in enzymes with different temperature requirements. In between these temperatures limits, lies the optimum temperature (the temperature at which a species exhibits fastest growth). Growth rate increases form the minimum to the optimum because chemical reactions including enzyme catalyzed reaction proceed more rapidly as the temperature rises. Above the optimum temperature, the growth rate declines as the proteins (the most heat sensitive macro-molecules in the cell) get denatured. The causes of minimum temperature growth are more complex. Hydrophobic interactions between proteins become weaker as the temperature decreases and the shape of the proteins changes affecting their function. The influence of temperature on growth is actually the measure of the influence of temperature on the actions of the enzymes of the cells. As the temperature is lowered, the enzyme activity and thus the cell growth is slowed. At freezing point, essentially all the metabolic activity ceases, not only owing to the direct retardation of the enzyme activity but also because of the cell is deprived of water, which is essential for the update of nutrients as well as removal of waste products. When the temperature is raised above optimum for growth, metabolic activity is enhanced, but at the same time the rate of enzyme and protein breakdown markedly results in damage and death of cells. Bacteria may be divided into three major groups with respect to their

temperature requirement. 1. Psychrophiles : Optimum temperature between 00 C and 200 C. 2. Mesophiles : Optimum temperature between 200 C and 400 C. 3. Thermophiles : Optimum temperature between 400 C and 800 C. Thermophiles are of two types a. Facultative Thermophiles: Maximum growth at optimum temperature between 450 C and 600 C. b. Obligate Thermophiles: Maximum growth at optimum temperature above 600 C. PSBPP103 REQUIREMENTS: Sample used Media Glassware Page No: C23 Miscellaneous Overnight grown culture of Escherichia coli in nutrient broth Sterile saline Sterile nutrient broth Sterile 10ml pipette (1) Sterile 1ml pipette (2) Sterile test tubes (8) Sterile small test tubes (4) Water bath thermometer PROCEDURE: 1. Make a suspension of overnight activated culture of Escherichia coli by inoculating 1ml of culture in 9 ml of sterile saline. This is 10-1 dilution. 2. Prepare a serial dilution of the given dilution up to 10-8, using a sterile saline as diluent. 3. Pipette out 1ml of suitable dilution (10-6) in standard test tube and incubate at respective temperature for 20 minutes i. e. 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C. 4. Take 1 ml of incubated culture and transfer it in 4ml sterile nutrient broth. 5. Incubate tubes at 37°C for 24 hrs. 6. After incubation, take O. D at 540nm. 7. Plot the graph of absorbance against temperature on semi-log paper. PSBPP103 OBSERVATION: Page No: C24 Temperature (0C) 10 R. T (30) 37 50 90 Optical density (Î»= 540nm) 0. 176 0. 520 0. 544 0. 498 0. 237 RESULT: CONCLUSION: PSBPP103 Cell Death by Chemical Agent EXPERIMENT NO. 5 AIM: THEORY: Page No: C25 Date: To study the effect of chemical agent (NaCl) on bacterial cell. Nutrients are substances required by living organisms for their growth and development. Micro-organisms are diverse in

their specific physiological properties and hence in their specific nutrient requirements. The chemical composition of the cell determines the major requirement for growth. Owing to the diverse nutrient requirements of the bacteria, specific media are utilised to culture specific organisms. The compositions of these media vary considerably from one another. The elements in the medium have to be in the right amount so as to provide optimum conditions for the bacterial growth. Any shift from the optimum concentration can affect the growth of the bacteria or can even be lethal. Chemicals are used in the nutrient media to maintain pH, osmotic balance, etc. In order to study the effect of any chemical agent on cell death, varying concentration of the same can be used and bacteria be subjected to those concentrations. NaCl is used to maintain the balanced salt concentration in the medium as it confers the required osmolarity. By changing the concentration of NaCl, the osmotic pressure exerted can be changed, thus resulting in change of growth conditions. Higher concentrations of NaCl can result in plasmolysis causing cell death. REQUIREMENTS: Sample used Media Glassware Overnight grown culture of Escherichia coli in nutrient broth Stock- Sterile nutrient broth containing 20% NaCl Diluent-Sterile nutrient broth Sterile test tubes (13) Sterile 10ml pipette (2) Sterile 1ml pipette (1) Colorimeter (O. D at 540nm) Miscellaneous PROCEDURE: 1. Make a suspension of Escherichia coli in saline and prepare different concentration of NaCl using nutrient broth as diluent as given in the table. 2. Label the tubes according to the concentration of NaCl. 3. Inoculate these tubes with 0. 1 ml of culture and incubate at 37C for 24 hrs. 4. Observe the turbidity in tubes and note down O. D at 540nm. 5. Plot a graph of O. D against

concentration of NaCl. PSBPP103 OBSERVATION: Tube No. 1 2 3 4 5 6 7 8 9 10 11 12 13 Concentration Of NaCl (%) 0 2 4 6 8 10 12 14 16 18 20 + control control Stock (ml) 0 0. 5 1 1. 5 2 2. 5 3 3. 5 4 4. 5 5 0 0 Diluent (ml) 5 4. 5 4 3. 5 3 2. 5 2 1. 5 1 0. 5 0 5 5 Culture (ml) 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 0. 1 Incubate All the Tubes At 370C For 24 hours Page No: C26 Observation O. D (Î»= 540nm) 0. 721 0. 559 0. 448 0. 123 0. 085 0. 074 0. 064 0. 061 0. 059 0. 055 0. 050 0. 73 0. 035 +++ +++ ++ ++ + + - Stock- Sterile Nutrient broth with 20% NaCl Total volume- 5ml Diluent- Sterile Nutrient broth Culture- Escherichia coli PSBPP103 RESULT: Page No: C27 CONCLUSION: PSBPP103 Mitosis AIM: Page No: C28 Date: EXPERIMENT NO. 6 To prepare an acetocarmine squash of a root tip to demonstrate the process of mitosis in onion roots The genetic information of plants, animals and other eukaryotic organisms resides in several (or many) individual DNA molecules, or chromosomes. For example, each human cell possesses 46 chromosomes, while each cell of an onion possesses 8 chromosomes. All cells must replicate their DNA when dividing. During DNA replication, the two strands of the DNA double helix separate, and for each original strand a new complementary strand is produced, yielding two identical DNA molecules. DNA replication yields an identical pair of DNA molecules (called sister chromatids) attached at a region called the centromere. DNA replication in eukaryotes is followed by the process called mitosis which assures that each daughter cell receives one copy of each of the replicated chromosomes. During the process of mitosis, the chromosomes pass through several stages known as prophase, metaphase, anaphase and telophase. The actual division of the cytoplasm is called cytokinesis and occurs during telophase. During

each of the preceding stages, particular events occur that contribute to the orderly distribution of the replicated chromosomes prior to cytokinesis. THEORY: Mitosis: Mitosis is also called somatic cell division or equatorial division. The process of cell division, whereby chromosomes are duplicated and distributed equally to the daughter cells, is called mitosis. It helps to maintain the constant chromosome number in all cells of the body. PSBPP103 Stages of mitosis are as follows. Page No: C29 Prophase The first phase of mitosis is marked by the early condensation of the chromosomes into visible structures. At first, the chromatids are barely visible, but as they continue to coil, the chromosomes become thicker and shorter. The nuclear envelope is still present during this stage, as are any nucleolar structures. The centrioles are moving to the poles of the cell and spindle fibers are just beginning to form. Metaphase During the middle phase of karyokinesis, the chromosomes line up in the center of the cell, and form a metaphase plate. Viewed on edge, the chromosomes appear to be aligned across the entire cell, but viewed from 90° they appear to be spread throughout the entire cell (visualize a plate from its edge or from above). Each chromosome has a clear primary constriction, the centromere, and attached to each is a definitive spindle fiber. The spindle apparatus is completely formed, and the centrioles have reached their respective poles. The nucleolus and the nuclear envelope have disappeared. Anaphase The movement phase begins precisely as the 2 halves of a chromosome, the chromatids, separate and begin moving to the opposite poles. The centromere will lead the way in this process, and the chromatids form a V with the centromeres pointing toward the respective poles. Telophase The last phase is identified by the

aggregation of the chromatids (now known as chromosomes) at the respective poles. During this phase, the chromosomes uncoil, the nuclear envelope is resynthesized, the spindle apparatus is dismantled, and the nucleolus begins to appear. PSBPP103 PRINCIPLE: Page No: C30 Why use onion roots for viewing mitosis? - The roots are easy to grow in large numbers. - The cells at the tip of the roots are actively dividing, and thus many cells will be in stages of mitosis. - The tips can be prepared in a way that allows them to be flattened on microscopes slide (“ squashed") so that the chromosomes of individual cells can be observed. - The chromosomes can be stained to make them more easily observable. Regions of Onion Root tips There are three cellular regions near the tip of an onion root. 1. The root cap contains cells that cover and protect the underlying growth region as the root pushed through the soil. 2. The region of cell division (or meristem) is where cells are actively dividing but not increasing significantly in size. 3. In the region of cell elongation, cell are increasing in size, but not dividing. Viewing Chromosomes Fixing the Root Root is fixed with appropriate fixative in order to arrest their division states. For onion root tips ideal time to fix the root is 9am to 11am as the rate of division is maximum during this period. Staining the Chromosome Chromosomes generally are not visible as distinct entities in non-dividing cells, since the DNA is uncoiled, but the process of mitosis is facilitated by supercoiling of the chromosomes into a highly compacted form. Supercoiled chromosomes can be visualized in cells, particularly if they are treated with a DNA-specific stain, such as the Acetocarmine stain. PSBPP103 REQUIREMENTS: Sample Reagents Miscellaneous 1M HCL 45% acetic acid Aceto-carmine stain Microscope

Microscope slides Cover slips Watch glass Glass rod Forceps Scalpel Page No: C31 Onion or garlic roots PROCEDURES: Root-tip squash preparations for mitotic chromosome analysis of regenerated plants 1. Use a clean pair of forceps to collect healthy roots into a small vial containing distilled water. 2. Transfer the roots to a suitable pre-treatment (eq. 8-hydroxyquinoline 0. 29g per L ± dissolve at 60ºC) as quickly as possible and incubate for the appropriate time and temperature. 3. Fix the roots by transferring them into a fresh mixture of 3: 1 absolute alcohol: glacial acetic acid and incubate for at least 24 hours at 4ºC. (Steps 1-3 prepared beforehand) 4. Hydrolyze the roots by incubation in 1M HCL at 60ºC for 10-15 minutes. 5. Wash the roots briefly in distilled water. 6. Place the root onto a clean glass slide. Remove the translucent root cap at the extreme tip and place the root-tip in a small drop of 45% acetic acid. Mix in a small drop of aceto-carmine. 7. Heat very gently over a small flame for 2-3mins. 8. Place cover slip over the drop without creating air bubbles. 9. Tap the root-tip thoroughly in the drop with a flat ended glass rod. Remove remaining large pieces with a needle. 10. Squash under filter paper, vertically downwards using the thumb. Do not rock your thumb during the squash as this will roll the cells. 10. Examine under the microscope, first under lower magnification power (20X) and gradually under high magnification power (100X). PSBPP103 OBSERVATION: Page No: C32 CALCULATION: PSBPP103 RESULT: Page No: C33 CONCLUSION: