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Download the PowerPoint PowerPoint Contents Figure 1: Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 2: Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 3: Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 4: Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 5: Strains of *Staphylococcus aureus* may or may not produce a golden yellow pigment. (beta hemolytic, non-pigmented strain. Compare with Figure 6). (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 6: Strains of *Staphylococcus aureus* may or may not produce a golden yellow pigment. (beta hemolytic, yellow-pigmented strain. Compare with Figure 5). (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 7: Non-hemolytic *Staphylococcus* species: *Staphylococcus epidermidis*. (Most species of coagulase negative *Staphylococcus* species are non-hemolytic). (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 8: Non-hemolytic *Staphylococcus* species: *Staphylococcus epidermidis*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 9: Non-hemolytic *Staphylococcus* species: *Staphylococcus epidermidis*. (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 10: *Staphylococcus saprophyticus*: non-hemolytic, bright white, creamy colonies (recovered almost exclusively from urinary tract infections in young, sexually active females). (Rebecca Buxton, University of Utah, Salt Lake City, UT) Figure 11: *Micrococcus luteus*: Dramatic bright yellow pigment (no hemolysis). (Rebecca Buxton, University of Utah, Salt Lake City, UT) The normal flora of the mucous membranes of man count among its members a variety of Gram-positive cocci. Two important genera, *Staphylococcus* and *Streptococcus* are presented in today's lab exercise. A. STAPHYLOCOCCUS The genus *Staphylococcus* is, for the most part composed of two noteworthy species: *Staphylococcus aureus* and *Staphylococcus epidermidis*. *S. aureus* is the major pathogen and can cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. *S. epidermidis* is normally non-pathogenic but can produce disease under certain conditions. The staphylococci produce the enzyme catalase that distinguishes them from the streptococci which do not. By definition, strains of staphylococci that produce the enzyme coagulase are *S. aureus*, thus differentiating them from all other staphylococci which do not. Most strains of *S. aureus* produce a non-diffusible golden yellow pigment and a zone of clear beta hemolysis on agar containing blood. Non-pathogenic strains of staphylococci usually produce white or grayish colonies and are coagulase negative. They may be hemolytic but usually are not. B. STREPTOCOCCUS The genus *Streptococcus* contains many species which are considered pathogens. Streptococci probably cause a greater variety of types of clinical diseases than any other genus. The most commonly isolated and/or the most virulent strains of streptococci are: Group A *Streptococcus* (*Streptococcus pyogenes*). These are beta-hemolytic, and bacitracin sensitive; cause septicemia, impetigo, pharyngitis, acute glomerulonephritis (AGN), rheumatic fever, wound infections, etc. Group B *Streptococcus* (*Streptococcus agalactiae*). These are beta-hemolytic, bacitracin resistant, CAMP test positive, and they are a prime cause of puerperal sepsis and neonatal meningitis. This organism is commonly isolated from the female genital tract. Group C *Streptococcus*. These are beta-hemolytic, bacitracin resistant, CAMP test negative and are often a complicating factor in wound infections. Group D *Streptococcus*. These are usually alpha or gamma, and generally reside in the intestinal tract. There are two sub-groups, divided on the basis of resistance to high concentrations of salt. Group D Enterococcus: can grow in high (6.5%) concentrations of salt. Group D Non-enterococcus: cannot grow in 6.5% salt *Streptococcus pneumoniae* is an oval diplococcus that is optochin sensitive, a-hemolytic and causes pneumonia, wound infections, meningitis, septicemia, and otitis media in children. The ability to hemolyze red blood cells varies among the species in the *Streptococcus* genus, and it is the first feature observed in species classification. The hemolytic reactions of streptococci on sheep blood agar (BAP) are: ALPHA HEMOLYSIS- a greenish discoloration of the blood agar in a zone surrounding or beneath the colonies. The word "viridans" means green and these a-hemolytic streptococci are often called "viridans" streptococci. BETA HEMOLYSIS- the complete lysis of red blood cells in a zone around the individual colonies. In this zone the medium becomes almost transparent. GAMMA HEMOLYSIS- a lack of reaction (no visible changes around the individual colonies). No hemolysis is produced. II. LAB WORK Materials supplied: (work individually) 1 Blood Agar Plate (BAP) with 1 *Staphylococcus* unknown 1 BAP with 1 streptococcus unknown 1 Bile-Esculin agar plate (pink dot) 1 6.5% NaCl broth (green cap) 1 BAP (divided in 2 parts by a marker line) 1 Rabbit plasma tube (purple cap) 1 petri dish with A (Bacitracin) and P (Optochin) differentiation discs 1 tube of *Staphylococcus aureus* (in broth for CAMP test) (yellow cap) 1 bottle of 3% Hydrogen Peroxide (H₂O₂) Procedure: Each student has his/her own: *Staphylococcus* unknown with a code number, *Streptococcus* unknown with a code number. Each student: should prepare a Gram-stain of his/her *Staphylococcus* and *Streptococcus* unknowns. should perform a Catalase test on his/her *Staphylococcus* and *Streptococcus* unknowns. This will identify which is which. should perform a Coagulase test on his/her *Staphylococcus* unknown. should inoculate his/her *Streptococcus* unknown to appropriate plates as described below. should determine the hemolysis of his/her *Streptococcus* unknown and set up the Bacitracin or Optochin sensitivity test, if necessary. (If this test is performed on a *Staphylococcus* unknown, points will be deducted.) CATALASE TEST: The Catalase test differentiates between groups of microorganisms on the basis of catalase production. This test is consistently positive for staphylococci and negative for streptococci. Catalase is an enzyme that splits hydrogen peroxide into water and oxygen. The oxygen released is seen as tiny bubbles. Pick up 1 or 2 isolated colonies with your sterile loop and spread on a slide. Do not add saline. Do not include any agar; blood cells have catalase in them. Add 1 drop of 3% hydrogen peroxide to the smear and look for vigorous release of bubbles. Bubbles indicate a positive reaction for catalase. GENUSCATALASE REACTION *Streptococcus* No bubbles (no catalase) *Staphylococcus* Bubbles (catalase present) COAGULASE TEST: The Coagulase test is the most reliable indicator of pathogenic, toxin-producing strains of *Staphylococcus*, i.e., for *Staphylococcus aureus*. Most pathogenic strains of *Staphylococcus* produce prostaphylocoagulase that reacts with an activator similar to prothrombin, to form the active clotting agent, coagulase. Coagulase in turn reacts with fibrinogen forming fibrin to produce the clotting of plasma. Use a sterile inoculating loop to remove a heavy inoculum from your presumptive *Staphylococcus* unknown (determined by the Gram stain and Catalase test). Emulsify this growth into 1 tube of Rabbit plasma, label with your name, and place in the 37°C incubator until the next lab period. After incubation, look for clotting of the plasma by tilting the plasma tube and observe whether the liquid is solidified or not (do not spill). ORGANISM COAGULASE PRODUCTION *Staphylococcus aureus* Clot (Coagulase produced) *Staphylococcus epidermidis* No Clot (No Coagulase) STREPTOCOCCUS GROUP IDENTIFICATION: To speciate your *Streptococcus* unknown, it is first necessary to categorize it on the basis of its ability to lyse sheep red blood cells (hemolysis). Examine your unknown BAP carefully (look only at isolated colonies for this determination) and decide based upon the following descriptions, and the demonstration plates at the front table, which of the 3 types of hemolysis your unknown exhibits. ALPHA HEMOLYSIS: Incomplete lysis of RBC's with the formation of a green discoloration surrounding the colony. BETA HEMOLYSIS: Complete disruption of RBC's with release of hemoglobin. A clearing around the colony. GAMMA HEMOLYSIS: No change in the medium at all. If your strep unknown exhibits a-hemolysis, then it could belong to Group D or be *Streptococcus pneumoniae* or a Viridans streptococcus. Streak your unknown to one half of the BAP and place one Optochin disc in the area of densest inoculation. Next, use a sterile loop to inoculate the Bile-Esculin Agar plate and the 6.5% NaCl broth with your Strep unknown. (Do this only if your unknown exhibits a-hemolysis.) If your strep unknown exhibits b-hemolysis, then streak your unknown to one half of the BAP and place one Bacitracin disc in the area of densest inoculation. Use the other half of the BAP for the CAMP test (see diagram). Streak your unknown Strep. in a straight line across the curved side of the plate. Sterilize your needle and streak the *Staphylococcus aureus* (broth, not plate) perpendicular to your unknown streak, leaving a 1cm space between them. If your strep unknown exhibits no hemolysis (g), then ignore steps b and c. Instead, use a sterile loop to inoculate the Bile-Esculin Agar plate and the 6.5% NaCl broth with your Strep unknown. Incubate your BAP plates in a candle jar at 37°C until the next lab period. Put the Bile-Esculin and 6.5% NaCl broth in the 37°C incubator. Be sure to put your name on all of your material. Note that if the streptococcus culture is sensitive to Bacitracin or Optichin, you will see an area of no growth surrounding the disc. Discard your unknown BAP plates. Materials kept longer than 24 hrs. often give inconclusive or erroneous results. CAMP TEST PROCEDURE: CAMP test performed on a full size Blood Agar Plate. Note the enhanced hemolysis in the shape of an arrow. NEXT LAB PERIOD Read and record the results of both your and your partner's unknown on the Report Sheet, using the table below: STREPTOCOCCUS IDENTIFICATION Group Hemolysis Bacitracin Sensitivity CAMP Test Bile-Esculin Agar 6.5% NaCl Broth Optochin Sensitivity ABeta+ BBeta+ CBeta- DEnterococcus Alpha or Gamma++ DNon-Enterococcus Alpha or Gamma+- Viridans Alpha+ Streptococcus pneumoniae Alpha++ At the end of this lab period, turn in your Report Sheet, labeled with your name and unknown #s. While every effort is made to ensure that this information is up-to-date and accurate, the statements found on this page are for informational purposes only. LSU-MC WWW Publication Policy, 9/23/96

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