

Identification of *Proteus Vulgaris* using Series of Biochemical Test and Staining Technique: Ecology, Pathogenicity (Enteric Pathogen), Description and Prevention of It's Diseases

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Abstract

Phenotypic identification of bacteria by staining technique and biochemical tests. This practical was carried out to identify *Proteus vulgaris* using biochemical test, this test includes, citrate utilization, indole, carbohydrate fermentation, methyl red, voges Proskauer, motility, production of hydrogen sulphide, catalase, and oxidase tests. Results obtained shows that *Proteus vulgaris* was positive to indole, motility, methyl red and urease. *Proteus vulgaris* was unable to utilize citrate for carbon and energy, and does not synthesis the enzyme amylase to break down starch. The bacteria fermented glucose to produce acid and gas.

Keywords: *Proteus vulgaris*, Biochemical tests, Staining techniques, Enteric pathogens, Ecology, Pathogenicity, Disease prevention, Urinary tract infections (UTIs), Antibiotic resistance, Environmental pollution, Niger Delta, Clinical microbiology, Bacterial identification, Human microbiota, Antibiotic susceptibility, Gram-negative bacteria

Introduction

A normal or healthy enteric sample contains a variety of bacterial Flora that helps aid digestion and absorption of nutrients while preventing the growth of harmful bacteria. they are generally referred to as flora or human microbiota³.

However, when bacteria that are consistent with a healthy enteric system are absent from a culture, pathogens can flourish, or toxins can be released by organisms like *E.coli* and *Proteus*¹. Disruption of normal enteric flora may be caused by use of antimicrobials, stress, or prolonged diarrhea. Animals that have been on antibiotic therapy may have their normal flora balance altered⁸. While it is

the job of an antibiotic to kill a pathogen, a side-effect is that other bacteria can be harmed in the process. Sometimes, even though healthy enteric flora has been inhibited, other non-enteric bacteria found on the skin may persist⁸.

Proteus species possess an extracytoplasmic outer membrane, this is commonly found in Gram-negative organisms. the outer membrane contains a lipid bilayer, polysaccharides, lipopolysaccharides and lipoprotein¹. Infection depends on the interaction between *Proteus vulgaris* and the host defense mechanisms. The first step in the infectious process is adherence to it's host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease. *Proteus vulgaris*, *E. Coli*

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and other gram-negative bacteria contain fimbriae (i.e., pili), which are tiny projections on the surface of the bacterium¹⁻⁷. Specific chemicals located on the tips of pili enable organisms to attach to the tissue of its host (eg, urinary tract endothelium). The presence of these fimbriae has been demonstrated to be important for the attachment of *Proteus vulgaris* to the tissue of its host. Stress in animals is a well-known contributor to a weakened immune system resulting to diseases caused by *Proteus vulgaris*. Environmental stressors include temperature changes or crowding are notable causes of stress¹⁰. Changes in nutrient content and quality of feed are often implicated as well. Stress can impact the release of digestion enzymes and cause altered rates of peristalsis, impacting the length of time for an organism to establish itself within the intestines. If an animal has an imbalance of normal flora, antibiotics are not usually the solution³. Instead, the focus can be on removing or easing the stressor to the animal.

Materials Used

MATERIALS PROVIDED BY THE LABORATORY

OXIDASE REAGENT

KOVAC'S REAGENT

METHYL RED

40% POTASSIUM HYDROXIDE (KOH)

3% HYDROGEN PEROXIDE (H₂O₂)

IODINE SOLUTION

STARCH AGAR PLATE

TRIPLE SUGAR - AGAR SLANT

MOTILITY MEDIUM

SIMMON CITRATE AGAR SLANT

PEPTONE WATER

MR-VP BROTH

FILTER PAPER

SUGAR FERMENTATION BROTHS (GLUCOSE, LACTOSE AND MALTOSE)

WIRE LOOP

UREASE BROTH

INOCULATING NEEDLE

BURSEN BURNER FLAME

70% ALCOHOL TEST TUBE RACKS

DISINFECTANT

SAMPLE OF URINE FROM INFECTED PATIENT

Methods

The work bench was disinfected using disinfectant

Catalase test: Two drops of catalase reagent (3% H₂O₂) was placed on a grease free slide, with the aid of a wire loop, a loopful of the urine sample was placed on the reagent, the absence of bubble indicated a negative result.

Oxidase test: A sterile filter paper was damped with the oxidase reagent, a loopful of the urine sample was taken using a sterile wire loop and rubbed on it. A none color change indicates a negative result.

Sugar fermentation test: the urine sample was inoculated into the fermentation tubes respectively (glucose, lactose and maltose) using a sterile wire loop to collect the sample into the broth, the tubes were inoculated at 37°C for 48hrs.

Triple sugar iron ager: using a sterile wire loop, the urine sample was collected and streaked on the TSIA slant while the butt was strapped with the urine sample using a sterile inoculating needle. The tube was inoculated at 37°C for 48hrs. After 48hrs, the tube was observed

Motility test: the urine sample was inoculated into the semi-solid medium using a sterile inoculating needle. The tube was incubated at 37°C for 48hrs. After the incubation period the tube was observed for diffusion growth indicating motility

Citrate utilization test: using a sterile wire loop, the urine sample was collected and streaked on the citrate agar slant while the butt was strapped with the urine sample using a sterile inoculating needle. The tube was incubated at 37°C for 48hrs. After 48hr, the tube was observed

MR-VP Test: the glucose phosphate broth was inoculated with the urine sample using a flamed

sterilized wire loop, the broth was incubated at 37°C for 48hrs. After 48hr., the broth was divided into two equal parts into sterile tube, 3 drops of methyl red indicator was added to one while 3 drops of 40%KOH and 5%alpha naphthol was added to the other and observed for results

STARCH hydrolysis test: the starch agar plate was streaked evenly with the urine sample using a

sterile wire loop. The plate was incubated at 37°C for 48hrs. After 48hr, the plate was flooded with the iodine solution and observed for results

Indole test: the urine sample was inoculated into the peptone water broth using a sterile wire loop. It was incubated at 37°C for 48hrs, after incubation 3 drops of KOVAC'S reagent was added and observed

Results

TEST	OBSERVATION	RESULTS	INFERENCE
Starch hydrolysis test	Absence of zone of clearance around the streaked line of the sample	Starch hydrolysis negative	Organism in urine sample does not have enzymes amylase to break down starch
Oxidase test	Absence of blue black color	Oxidase negative	Organism in urine sample does not possess the cytochrome c oxidase enzymes
Motility test	Diffuse growth in the medium	Motility test positive	Organism in the urine sample has organ of locomotion
Citrate utilization test	Agar did not change to blue color	Citrate negative	Organism in urine sample was not able to use the citrate as carbon and energy source
TSIA test	Butt-yellow Slant-yellow H ₂ S__+ve	Acid produced Acid produced H ₂ S produced	Fermentation Fermentation Ammonia was converted
Lactose broth test	There is no change in color or anything observed in the broth	Lactose fermentation negative	Organism in urine sample does not ferment lactose and does not produce acid or gas
MR test	Red coloration of the broth	Methyl red positive	Mixed acid fermentation Acid fermentation
VP test	Absence of brick red color	VP negative	The test is negative for acetoin, a precursor of 2,3-butanediol
Glucose test	Broth turn yellow and gas collected in Durham tube	Glucose fermentation positive	Organism in urine sample produced acid and gas
Indole test	Red ring was formed	Indole positive	Organism in urine sample was able to degrade the amino acid tryptophan to produce indole
Catalase test	Absence of bubble	Catalase negative	Organism in urine sample was unable to synthesize catalase enzyme to break down the hydrogen peroxide to water and oxygen

Gram reaction/cell morphology – Negative/Rod

From the above results organism is *Proteus vulgaris*

Discussion/Observation

Proteus vulgaris is a rod-shaped, nitrate-reducing, indole-positive and Gram-negative bacterium that inhabits the intestinal tracts of humans and animals². It can be found in soil, water, and fecal matter⁸. It is commonly found in the south-south, South-East and some part of North-West Zone of Nigeria. This is as the result of their regional area which is made up of water. The Niger Delta states which comprises of nine (9) states (Rivers state, bayelsa, Delta, Cross River, Abia, Imo, Edo, Akwa-ibom and Ondo state) suffer from this bacteria the most, this is as the result of the unhealthiness of the environment¹. The water and Environmental pollution is as a result of human factors which are by oil spillage during thievery of oil from pipe lines, sewage and improperly dumping of refuse. When it is rainy, the water washes this bacteria into the Rivers which is later consumed by humans and Animals. The shallow digging of the ground for borehole(water) installation also contributes to the verse spread of this diseases caused by *Proteus vulgaris*¹². Africa can be said to be the continent which suffer from this diseases the most, due to environmental conditions(pollution).



Niger Delta States

Proteus vulgaris is an enteric pathogen, it is grouped with the organelle and is an opportunistic pathogen of humans and animals². It is known to cause wound infections and other species of its genera are known to cause urinary tract⁹⁻¹¹. This bacteria is an intestinal bacteria (found in the intestine)². A normal or healthy enteric sample contains a variety of bacterial Flora that helps aid digestion and absorption of nutrients

while preventing the growth of harmful bacteria, this includes *Proteus vulgaris*. This bacteria can release toxins and can be reported as “Mixed bacterial growth consistent with disruption of normal enteric flora”. Disruption of normal enteric flora may be caused by use of antimicrobials, stress, or prolonged diarrhea. While it is the job of an antibiotic to kill a pathogen, a side-effect is that other bacteria can be harmed in the process³.



NGBOMBA ROAD, PORT HARCOURT, RIVER STATE, NIGERIA.

Proteus vulgaris has caused a wild spread of variety of infections, precisely urinary tract infections which ranges from Cystitis: the infection of the bladder, this is the most common Lower urinary tract infection found in male⁹

Vaginitis: this is the UTI which is associated with the infection of the vagina, found in females

Urethritis: This is also a common UTI that infects the bladder, this can be seen in the male and female reproductive system.

They have also be found to be the cause of wound infections in animals and sometimes humans, burn infections, bloodstream infections and most importantly Respiratory tract infections (RTI)¹². This infection is recorded to be transmitted through direct or indirect contact with contaminated persons and even Objects²⁻¹². Male according to this study were therefore found to be more vulnerable than Females in acquiring *Proteus* infections². *Proteus vulgaris* is highly resistant to antibiotics because of the plasmids present in the bacterium, making infections extremely difficult to cure¹¹. This is because the plasmids have varied drug resistant markers on them. It's symptoms can be a pain in flank, Abdomen, pelvic area or spinal cord. Pressure at lower part of the pelvic girdle is a

strong symptoms of a UTI caused by *Proteus vulgaris*. The most common signs are cloudy, foul-smelling or painful urination

A series of prevention such as environmental hygiene and most importantly personal hygiene is an essential factor in protecting oneself from diseases caused by *Proteus vulgaris*¹⁻⁴. The urination after sexual intercourse, staying hydrated, minimizing sprays or powders in genital areas which is common in this region, the practice of proper wiping technique, wearing of breathable underwear and proper balance diet are all way to hinder the wild spread of UTI caused by *Proteus vulgaris*¹⁻³. A patient suffering from these diseases can also apply this preventive measures, this can help to speed up the rate of recovery⁶. A recorded effective antibiotics has been listed by aggregate report of laboratories and hospital in Niger Delta region and this antibiotics therapy ranges from intravenous administration of Gentamycin, Floroquinolone, aztreonam, IMP-imipenCTX- cefotaxime; CRO-Ceftriaxone; CPXin Ciprofloxacin; AUG-Augmentin; AMXresult ,Amoxicillin; APX-Ampicillin; Gentamycin; SXT-Cotrimoxazole²⁻¹¹. There also African roots and herb which plays a vital role for the cure of such disease. they are Horsetail, Dandelion, Bearberry, Goldenseal, Boxwood and Vernonia amygdalina Delile popularly referred to as bitter leaf is also an essential herb for it's treatment¹. This is done by squeezing out it's bitter juice and drinking³.



Conclusion

This study discovered the key factor that enhances the wild spread of *Proteus vulgaris* in Niger Delta region and it's possible prevention and cure.

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