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PATHOGENICITY AND ANTIGENIC STRUCTURE OF PROTEUS

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Abstract

The Proteus type are ubicuitary germs spread in the nature, being found in the soil, waste water, surface water, in the organic matters in putrefaction, in the human intestinal tube, in the food and in the pathologic products. Being germs spread very much in the nature, the infections with this microorganism depend on the immunologic status of the host, on the virulence of the incriminated strains, on the control of the infections and of the food.

The proteus ferments the glucose with a bit of gas and produce H2S phenylalanine deaminase. It doesn't ferment the lactose, don't produce lisindecarboxilase and β galactozidase. On the agar nutritive media 2%, without inhibitors it presents the phenomenon of invasion or migration, characteristic to the type.

All the species of Proteus are frequently met in the nature and among these Proteus vulgaris and Proteus mirabilis pretty frequent also in the human pathology. Proteus mirabilis is after E. Coli the etiologic agents most frequently met in the urinary infections and Proteus vulgaris was incriminated in the urinary infections but especially in gastroenteritis with aspect of food poisoning. P. Vulgaris is isolated frequently from the stool, being a component of intestinal microbiota.

Keywords: microbiota, gastroenteritis, food poisoning.

INTRODUCTION

The genetic identification of the colonies isolated on selective mediums is a perspective of wide applying in the establishing of the species. Pathogen conditioned, *Proteus* can outburst, alone or in association with other pathogen agents, infections on different levels of the host organism. Also it causes infections of the digestive tube, food poisoning and enteritis in newborn and small children, infections of the inferior respiratory tube, pneumonia is most of the times nosocomial.

The infections of the inferior urinary tube are represented by cystitis. In patients with urinary lithiasis, *P. Mirabilis* was frequently isolated in urine, the recurring bacteriuria being a complication without a solution for these ill people. The capacity of the *Proteus* bacteria to decompose the urea play a very important role in the inducing of the urinary lithiasis. The urease hydrolyzes the urea to the ammonia and carbon dioxide. The alkanizing of the urine by the increasing of the level of ammonia determines the suprasaturation of the phosphate magnesium and of the phosphate calcium and their crystallization forms calculi. The bacteria inside the lithiasis are

refractory to the antibiotics treatment. The lithiasis with large dimensions can affect the kidney function. The increase of the level of ammonia in the urine, due to the hydrolisis of the urea in the presence of urease, can produce also lesions of the epithelium of the urinary tube.

The resistance to the physical and chemical factors of the strains of Proteus is similar to the other enterobacteriacae. It can resist for a longer period in some antiseptic solutions, of detergent, and in perfusable solutions, in those that include glucose being able to multiply at the room temperature, this explaining the diffusibility of the bacteria in the hospital medium. The resistance to antibiotics of this bacteria is very large.

Proteus are bacilli gram negative, polymorphous, don't present capsule or spores. They are not demanding germs and on the simple agar and blood agar have a unique characteristic in the *Enterobacteriaceae* family to invade the medium, phenomenon named "phenomenon of escalade". From the place of inoculation, successive waves of culture migrates concentric up to the edge of the medium or up to the meeting of a migratory wave of another colony. If the migratory colonies belong to the same strain, the waves are intricating, forming a continuous web. If they belong to different strains, even from the same species of *Proteus*, the migrations are stopped at a distance of 2 mm, between them being traced a line of marking, phenomenon known also with the name of "Dienes phenomenon". This represents an important epidemic marker on the selective mediums that include biliary salts. *Proteus* grows under the form of S colonies, smooth, round, translucent, lactose-negative, with the color of the medium in "cat eyes".

MATERIAL AND METHODS

The analytic study was accomplished on pathologic products coming from excrements, performed at the Diaser laboratory, Oradea.

These being the products, it was used the technique of seeding for the isolation and identification.

Collection and transport of samples

The collection has to be made as close to the beginning of the disease and before the beginning of any antimicrobial treatment.

• The collection from the stool made spontaneously – it is preferred and is indicated in all the forms of acute diarrhea when the emission of excrements is frequent.

• For bacterial and parasite examinations, the collection is made with the "spoon" of the coproculture tube, concerning the liquid parts and especially, those mucous and/or sanguinolent, if they exist. The volume of the collection has to be of minimum 5 ml or 3-5 cm^3, if the stool is formed[3].

• For the isolations or virological exams is collected 5-10 cm^3 of excrements or minimum 5 ml, if the stool is not formed[3].

• The rectal collection – is recommended in:

– chronic shigellosis, where the curettage of the rectal mucous with the probe or the tampon offers greater chances to the isolation;

– the investigation of the carriers of *Shigella* and *Salmonella*, with the exception of the those with S. Typhi.

For this type of collection are used Nelaton probes (no.14-16) or adequate tampons, as the following: with the tampon, wet in saline isotone solution (not to be used lubricant gels), is penetrated the anal sphincter by slow rotation, introducing in the rectum approximately 15 cm. It will proceed identically also with the Nelaton probe, to which is adapted a syringe (10 ml) used for 1-2 aspirations. After the collection, the probes and tampons are introduced in sterile recipients that contain preservation medium, are labeled correspondently and are sent to the laboratory.

The *transport* of the samples and their processing is made in maximum 1h, if they were collected in recipient without medium of transport (with transport at the room temperature), or can be kept up to 24h at room temperature, if they were collected in recipients that contain *Cary-Blair* medium of transport which assures a good viability of the bacterial intestinal pathogens. An exception to these rules are the samples collected for the suspicion of infection with *Shigella spp, very sensitive bacteria, which needs seeding on the culture media immediately after collection*[3;4] .
For the viral etiology, the samples that are not processed immediately have to be kept at – 70°C[3] .

The isolation of the aerobe bacteria

• It is seeded the sample on two culture media, one weakly selective (Mac Conkey) and one moderately selective (Hektoen) and is incubated 24 h at 35-37ºC, following the cultures at 24 and 48 h for the appearance of characteristic colonies. For the *Vibrio* type, the recommended selective medium is BSA (bile salts agar), and for yeasts – the Sabouraud medium with Cloramfenicol.

• In order to increase the chances of isolation, the sample is sub cultivated on media of enriching that favors the multiplication of the pathogen (ex. selenite broth sodium acid for *Salmonella spp*., alkaline peptone water or broth with taurocholate and peptone at pH=8,0-9,0 for *Vibrio* where, after incubation can be made smears and cultures from the superior part of the medium). It is incubated 24 h at 35-37°C, then are made transmissions on the culture mediums.

• The colonies characteristic to each type will be transplanted in order to identify on the level of species and agglutination with specific serums.

RESULTS AND DISCUSSIONS

The *Proteus Mirabilis* colonies present a remarkable geometrical regularity. The microbiologic methods and the basic imagery techniques were used to measure the periodic macroscopic events in the morphogenesis of the migrating colonies, of escalade. We distinguished three initial phases (the phase of lag, the first phase of full and the first phase of consolidation), followed by the repeating of the further cycles of the consolidation phases, plus the consolidation. Each colony of *Proteus* corresponds to a cycle of swarming-plus-consolidation.

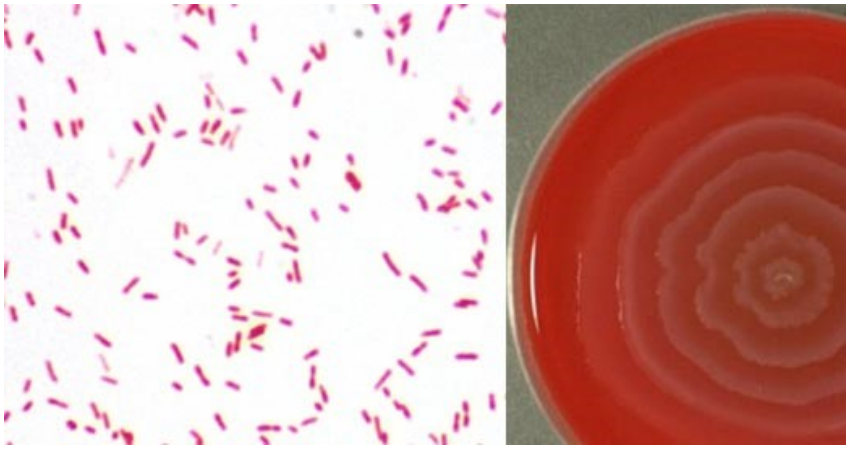

Fig. nr. 1. *Proteus mirabilis*.

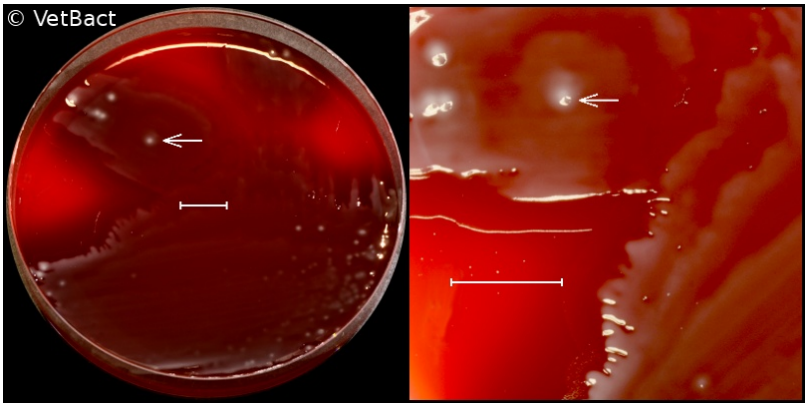

Fig. 2. *Proteus mirabilis*.

The duration of the phase of lag was dependent to the density of the inoculation in a way that indicates the functioning of the multicellular

effects of cooperation and inhibition. On our standard medium, the second and the further phases of the colony, it appears a structure with the form of internal waves visible with reflected illumination and darkness-field.

These internal waves have resulted from the organizing of the migrating bacteria in successive cohorts, thinker of the heated cells. The bacterial growth and motility were modified independently by the modification of the composition of the growth medium. By the variation of the concentration of glucose in the substrate, it was possible to be modified the production of biomass without affecting very much the kinetics of the extending of the colony surface. By the variation of the concentration of agar in the substrate, the initial production of bacterial biomass was not affected, but the dynamics of extension of the colonies was modified significantly. The greater concentrations of agar lead to slower phases, shorter, of the migrating colonies and the consolidation phases were longer.

Thus, the growth of the colony was limited by the greater concentrations of agar, but the wider view calendar of the cycles of consolidation-plus-consolidation remained constant. No variety of factors that had significant effects on the expansion of the colonies did alter the frequency of terraces at 34 ºC, but the length of the cycle of swarming-plus-consolidation was affected by the temperature and average enriching. Some clinical isolations presented significant differences in terraces at 34 ºC. The results have defined a number of parameters easily quantifiable in developing the colonies. The data did not show any connection between the running down of nutrients (glucose) and the beginning of different phases in morphogenesis of the colonies. More observations indicate the functioning of the thresholds dependent to the density in the control of the transitions between the distinct phases.

Proteus vulgaris, cultivated on agar that contains penicillin, suffers extraordinary morphological modifications, that vary depending on the temperature of incubation, the concentration of penicillin, the concentration of agar and the presence of small quantities of liquid between agar and the sliding-lip. The bacilli can be divided normally once or twice in elements that grow without division and which can develop in form of fantastic thread or inflated. In great concentrations of penicillin the fantastic forms are obtained by extending without division. In the beginning, the nuclei are divided as in the normal organisms. The forms of thread have nuclei condensed arranged in alternative model along the side of the cells. In inflations can be either nuclear material of cell inflating, a condensed central mass or a Reticulum. When the vacuoles are present, they replace the nuclear material.

The motility of the very wide organisms is slow and the flagella movement can be observed clearly by contrast of phase. The movement of the flagella of the organisms responds easily to the radiant heat and a careful study of these movements makes it impossible the accepting of the Pijper affirmations that the bacterial motility is due entirely to the wavy movements of the body and the flagellation is only for the mucoid threads following the motility.

In the study **"**A CYTOCHEMICAL LOCALIZATION OF REDUCTIVE SITES IN A GRAM-NEGATIVE BACTERIUM", accomplished by Woutera van Iterson, W. Leene they made a microscopic study of electrons of the sites of reduction of the cellular activity, which, in the life style, have incorporated tellurite. In the testing object *Proteus vulgaris*, the reduced tellurite proved to be stored in contiguous bodies with plasmatic membrane, but different in structure to those described in the bacilli Gram-positive (2). In fact the organisms proved to be composed on a conglomerate of elements that contained strong electrons-scattering reduced tellurite and a delicate granular "matrix". A limiting membrane was not observed around these complexes. In the serial sections the details of the complexes are presented.

The reduced tellurite was not stored in the plasmatic membrane at any important degree. Because there were no other places of deposit of the reduced products unveiled, it is presupposed that the complexes represent the mitochondria equivalents of the investigated organism. Moreover, the bodies could function as basal granules of the flagella.

CONCLUSIONS

Proteus vulgaris, cultivated on agar that contains penicillin, suffers extraordinary morphological modifications that vary depending on the temperature of incubation, penicillin concentration, agar concentration and the presence of small quantities of liquid between agar and sliding lid. The bacilli can be divided normally once or twice in elements that grow without division and which can develop in form of fantastic thread or inflated. In great concentrations of penicillin the fantastic forms are obtained by extending without division. In the beginning, the nuclei are divided as in the normal organisms. The forms of thread have nuclei condensed arranged in alternative model along the side of the cells. In inflations can be either nuclear material of cell inflating, a condensed central mass or a Reticulum. When the vacuoles are present, they replace the nuclear material.

The bacterial growth and motility were modified independently by the modification of the growth medium composition. By the variation of the concentration of glucose in the substrate, it was possible to be modified the production of biomass without affecting very much the kinetics of the

extending of the colony surface. By the variation of the concentration of agar in the substrate, the initial production of bacterial biomass was not affected, but the dynamics of extension of the colonies was modified significantly.

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