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SELECTIVE CULTURE MEDIUM FOR MICRO-ORGANISMS AND USE THEREOF

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This invention relates to a selective culture medium for microorganisms. More particularly, this invention relates to a culture medium for distinguishing *Staphylococcus aureus* and *Pseudomonas aeruginosa* from other pathogenic microorganisms and others that may be morphologically similar and from nonpathogenic staphylococci organisms.

The staphylococci are by far the commonest cause of skin infections such as boils, abcesses, carbuncles and similar suppurative processes in man. They are primarily significant as pathogens. They can be grown on culture media, such as agar or meat extract media, and the individual colonies are circular with entire edges. The pathogenic forms, that is, those isolated from suppurative processes, usually are *Staphylococcus aureus.*

Staphylococci ferment mannitol with the formation of acid, the latter being detected by a decrease in pH of the media, as evidenced by color change of a pH indicator such as brom thymol blue or brom cresol purple.

Pathogenic staphylococci, that is, the *Staphylococcus aureus,* cause clotting of decalcified blood plasma due to the production of staphylocoagulase, an active clotting agent. There is a high correlation between staphylococcal virulence for man and staphylocoagulase production. Coagulase-positive bacteria are usually virulent.

Plasma clotting (i.e., staphylocoagulase activity) can be demonstrated by mixing a bacterial culture with decalcified plasma, human or rabbit, incubating the plasma and observing clotting. The simple slide method is often used for qualitative purposes; the bacteria are suspended in a drop of plasma and observed on a slide for clumping.

Staphylococci constitute the normal flora of the human skin, mouth and upper respiratory tract and enter the body through the intact skin or breaks therein. They include the relatively avirulent *Staphylococcus epidermidis,* as well as the virulent *Staphylococcus aureus,* especially in the upper respiratory tract. The latter are the etiologic basis for a variety of skin lesions, as well as diseases of the respiratory tract. Staphylococcal infections have increased in prevalence and fatality in recent years and are the predominant bacterial infections in this country.

In the treatment of wounds and similar skin lesions it is often desirable, and sometimes necessary, to identify the microorganisms in the wound for proper selection of antibiotic treatment. In the customary practice, a culture is made of the wound or lesion in an agar medium and the microorganisms developed therein. *Staphylococcus aureus* is a common pathogenic microorganism in skin wounds and it is desirable to be able to identify these organisms promptly and accurately so that appropriate treatment can be instituted without delay. For this reason it is desirable to have a culture medium which contains an inhibitor which inhibits the growth of most microorganisms except *Staphylococcus aureus* and thus allows rapid and accurate identification of *Staphylococcus aureus* if present in the wound or lesion.

Pseudomonas aeruginosa is a common cause of hospital infections, particularly in burns, and it is desirable to have a culture medium which will aid in distinguishing this organism from other pathogens.

It is an object of this invention to provide a selective culture medium for microorganisms which inhibits the growth of nonpathogenic organisms, provides for the isolation of pathogenic staphylococci and distinguishes pathogenic *Staphylococcus aureus* from other staphylococcal organisms. It is another object to provide a selective culture medium which provides for the isolation and identification of *Pseudomonas aeruginosa.* These and other objects will be apparent from and are achieved in accordance with the following disclosure.

We have discovered that a culture medium containing a cadmium salt such as cadmium nitrate, phosphate or chloride is effective as a selective medium for the culture of pathogenic *Staphylococcus aureus.* This culture medium aids in the identification of pathogenic *Staphylococcus aureus* from other staphylococci and other microorganisms. The remaining components in the culture medium are conventional materials such as agar, proteose-peptone fractions, potassium phosphate, yeast extract, peptones, mannitol and pH indicators which indicate acid production.

The isolation of Staphylococcus from a wound is usually not a difficult matter. Inoculation of a blood agar medium with the organism from a purulent specimen followed by 24 hours' incubation at 37° C. gives a good growth of colonies. With *Staphylococcus aureus* 24 hours' incubation gives good growth of creamy colonies that are surrounded by the clear zones of β hemolysis. When the medium is rendered selective by the inclusion of the cadmium salt, it is possible to isolate *Staphylococcus aureus* from specimens heavily contaminated with other bacteria.

As some Pseudomonas strains can grow in the cadmium-agar medium, it may be necessary to distinguish them from *Staphylococcus aureus.* This can be done by the mannitol fermentation and plasma coagulation tests. The mannitol fermentation test can be conducted in the same medium in which the bacteria are grown. A drop in pH of the medium indicates that the mannitol has been converted to acid by fermentation. As Pseudomonas bacilli and nonpathogenic staphylococci (such as *Staphylococcus epidermidis*) do not ferment mannitol, they are readily distinguished from *Staphylococcus aureus.* The plasma coagulation test confirms this fact, as Pseudomonas bacilli and nonpathogenic staphylococci do not coagulate decalcified blood plasma.

Another and prefered way of distinguishing *Staphylococcus aureus* from Pseudomonas strains, such as *Pseudomonas aeruginosa,* is by addition of a soluble azide, such as sodium azide, to the culture medium. The azide inhibits pseudomonas strains while allowing the staphylococci to develop.

Pseudomonas aeruginosa can be distinguished from *Staphylococcus aureus* by addition of a bile salt, such as sodium desoxycholate or other soluble salt of desoxycholic acid, to the culture medium. Bile salts inhibit the staphylococci without significantly affecting the growth of Pseudomonas strains.

The culture medium can be a standard agar medium containing 0.002 to 0.4 gram of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per liter or equivalent amount of cadmium as cadmiun nitrate or cadmium phosphate. The culture medium can also contain D-mannitol, which has been used in other staphylo-

occus selective media, in concentration in the range of 5–20 grams per liter to aid in distinguishing *Staphylococcus aureus* from pseudomonas and from other staphylococci organisms. A suitable cadmium-mannitol-agar culture medium corresponds to the following formulation:

Formula in grams per liter of distilled water

Proteose-peptone No. 3 (Difco)	10
Phytone (BBL)	10
K_2HPO_4	2.5
Yeast extract	2.5
D-mannitol	10
$CdCl_2 \cdot 2\frac{1}{2}H_2O$	0.020 to 0.25
Agar	15

An indicator such as 0.04% brom thymol blue or 0.04% brom cresol purple may be added to indicate acid production by a color change.

The media of this specification can be used for the detection and enumeration of staphylococci. Most microorganisms with the exception of Pseudomonas are inhibited by the cadmium salt in the media. The exception to this are fecal samples where Proteus may grow. Plates can be heavily inoculated because of the inhibitory action of the cadmium salt. The inoculated plates are then incubated for 24–36 hours at approximately 37° C. Most of the colonies that grow on the media will usually ferment the mannitol. Organisms which grow and do not ferment the mannitol or coagulate plasma are nonpathogenic staphylococci or Pseudomonas. Those that grow, ferment mannitol and coagulate plasma are *Staphylococcus aureus* pathogens.

Selective culture media for the detection and enumeration of *Staphylococcus aureus* and for distinguishing it from *Pseudomonas aeruginosa* can be produced by the addition of 0.0004 to 0.4 gram per liter of sodium azide or other soluble azide to the media described hereinabove. The azide will prevent growth of Pseudomonas strains, so that the mannitol fermentation or plasma coagulation tests need not be used to distinguish *Staphylococcus aureus* from these strains. A typical selective culture medium for *Staphylococcus aureus* is the following, which will give a pure culture in purulent wounds:

Formula in grams per liter of distilled water

Proteose-peptone No. 3 (Difco)	10
Phytone (BBL)	10
K_2HPO_4	2.5
Yeast extract	2.5
D-mannitol	10
$CdCl_2 \cdot 2\frac{1}{2}H_2O$	0.02 to 0.25
Agar	15
Sodium azide	0.1–0.2

Selective culture media for the detection of *Pseudomonas aeruginosa* and for distinguishing this strain from staphylococci correspond to the foregoing formula with 0.005 to 10 grams per liter of a bile salt such as potassium desoxycholate (or other soluble salt of desoxycholic acid) in lieu of the azide. A typical formula is the following:

Formula in grams per liter of distilled water

Peptone	15
Phytone (BBL)	5
Sodium citrate	15
Potassium gluconate	5
$MgSO_4$	5
Glycerol	10
$CdCl_2 \cdot 2\frac{1}{2}H_2O$	0.3
Sodium desoxycholate	0.5

Media for isolation of Pseudomonas organisms can be incubated at temperatures from 20° C. to 42° C. for periods of 24 to 48 hours. Media for isolation of staphylococci can be incubated at 35° C. to 37.5° C. for periods of 24 to 48 hours. Such media are aqueous

media, whether in form of broths or agar gels, and are so described herein.

We claim:

1. A selective culture medium for the identification of microorganisms which comprises an aqueous medium containing a cadmium salt selected from the group consisting of cadmium nitrate, cadmium phosphate and cadmium chloride, the concentration of cadmium in said aqueous medium being equivalent to 0.002 to 0.4 gram of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per liter, and D-mannitol, the concentration of the D-mannitol being in the range from 5 to 20 grams per liter.

2. A selective culture medium for identification of microorganisms as defined by claim 1 containing a soluble azide in concentration of 0.0004 to 0.4 gram per liter.

3. A selective culture medium for identification of microorganisms as defined by claim 1 containing a bile salt in concentration of 0.005 to 10 grams per liter.

4. A selective culture medium for identification of microorganisms as defined by claim 3 containing a soluble desoxycholate in concentration of 0.005 to 10 grams per liter.

5. A method of identifying *Staphylococcus aureus* in a specimen of microorganisms which comprises inoculating a culture medium with said specimen, the culture medium containing cadmium in an amount equivalent to 2 to 400 parts of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per million and containing 5 to 25 grams D-mannitol per liter of culture medium, incubating the inoculated medium at approximately 37° C., measuring the change in pH of the medium, and removing from the culture medium a specimen of the colony grown therein and admixing said specimen with decalcified blood plasma, thereby identifying *Staphylococcus aureus* by its characteristics of growing in a medium containing cadmium, fermenting D-mannitol to form acid, and coagulating decalcified blood plasma.

6. A method of isolating *Staphylococcus aureus* in a specimen of microorganisms which comprises inoculating a culture medium with said specimen, the culture medium containing cadmium in an amount equivalent to 0.002 to 0.4 gram of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per liter and containing a soluble azide in concentration of 0.0004 to 0.4 gram per liter, and incubating the medium at a temperature in the range of about 35° to 37.5° C., whereby the *Staphylococcus aureus* is permitted to grow while other organisms are inhibited.

7. A method of isolating *Pseudomonas aeruginosa* in a specimen of microorganisms which comprises inoculating a culture medium with said specimen, the culture medium containing cadmium in an amount equivalent to 0.002 to 0.4 gram of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per liter and containing a bile salt in concentration of 0.005 to 10 grams per liter, and incubating the medium at a temperature in the range of about 20° to 42° C., whereby the *Pseudomonas aeruginosa* is permitted to grow while other organisms are inhibited.

8. A method of identifying *Staphylococcus aureus* in a specimen of microorganisms which comprises inoculating a culture medium with said specimen, the culture medium containing cadmium in an amount equivalent to 2 to 400 parts of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ per million and containing 5 to 25 grams D-mannitol per liter of culture medium, incubating the inoculated medium at approximately 37° C., measuring the change in PH of the medium, thereby identifying *Staphylococcus aureus* by its characteristics of growing in a medium containing cadmium and fermenting D-mannitol to form acid.

References Cited by the Examiner

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