هلء في عندي أكتر من طريقة
عشان اعمل culturing
للبكتيريا ، فحسب اختلاف ال
indication اللي أنا بدي إياه
من ال culture رح تختلف
ال method.

Experiment 3 زراعة البكتيريا
Culturing Methods and Plating Techniques

Culture methods:

- Culture methods employed depend on the purpose for which they are intended.
- The indications for culture are:
  - To isolate bacteria in pure cultures.
  - To obtain sufficient growth for the preparation of antigens and for other tests.
  - For bacteriophage & bacteriocin susceptibility.
  - To determine sensitivity to antibiotics.
  - To estimate viable counts.
  - Maintain stock cultures.

شرح ال indication
بالصفحة اللي بعد
هاي .

بدنا نحكي
عن ال method
اللي. ممكن
نستخدمها بال
culturing.

Culture methods include:

1. Streak culture
2. Lawn culture
3. Stroke culture
4. Stab culture
5. Pour plate method
6. Liquid culture
7. Anaerobic culture methods
8. Spread plate method.

ال streak هي اللي بدنا نعملها في التجربة هاي .
و ال lawn culture إحنا ما رح نعملها لكن هي بتكون agar plate و بصير ينصب على وجها floating للبكتيريا و بتركها عشان يصيرلها culture .
بالنسبة للطريقة الرابعة و التالتة هدولا بصيروا جوا test tube (حكينا إنه من الطرق اللي ممكن اعمل فيهم solidification لل agar كان في وحدة منهم ال slant و التانية ال stab)
بس الفرق إنه بال slant ال test tube بصيرله soldification و هو مايل بس بال stab
ال test tube بصيرله solidification وهو standing بطريقة عامودية، طيب ازا في عندي تست تيوب في agar معموله solidification على شكل slant بقدر اعمل عليه
ال strock culture يعني بجيب ال inculcation loop و بفرد البكتيريا العنب على سطح ال slant يعني ع الجهة المايلة اما بالنسبة لل stab culture بجيب ال incubation loop
و بدخله بشكل عامودي داخل التيست تيوب

The common plating techniques employed in microbiology are Streak Plate Method, Spread Plate Method and Pour Plate Method.

الطريقة الخامسة حنحطي عنها بالتفصيل كمان شوي اما ال liquid culture حمحضرها يتجربتنا هاي رح تكون عبارة عن broth media و نعمل culturing داخل ال broth و anaerobic culture method و أخيرا في عندي spread plat method و هلء رح نشرح عنها اكتر .

The indication for culture are:

1_To isolate bacteria in pure culture

اول indication :

يعني انا في عندي mixture من مجموعة بكتيريا بدي ازرعهم بطريقة معينة عشان يصير عندي كل نوع من البكتيريا على شكل colony منفصلة عن البكتيريا التانية،وبقدر اخد هاي ال colony و أحضّر pure culture بس من نوع واحد من البكتيريا

2_To obtain sufficient growth for the preparation of antigens and for other tests

ال indication التاني:

أنا بدي اعمل تكثير لكمية البكتيريا الموجودة عندي عشان بدي أعمل عليها tests مختلفة

3_For bacteriophage & bacteriocin susceptibility

هلء في فرق بين ال bacteriophage وال bacteriocin و لكنه همه biological tools الهم (microbial killing activity)

ال bacteriophage هي عبارة عن بكتيريا صايرلها infection بفيروس و هاي البكتيريا بتطلع material بتعمل killing ل other micro organism.

أما ال bacteriocin فهي بكتيريا بتطلع small peptide or protein و هاي البروتينات الها activity إنها تعمل killing ل other micro organism.

4_to determine sensitivity to antibiotics

رح نعمل في تجاربنا القادمة antibiotics sensitivity test

5_to estimate viable counts

أنا بدي ازرع حجم معين من البكتيريا و أشوف في هادا الحجم كم عدد البكتيريا الموجودة

6_maintain stock culture

يعني أنا بدي أحافظ ع ال source مثلاً لل E_coli فأنا بعمل platting و culture بطريقة معينة و منها بقدر اخد stock culture يحتوي مثلا بس على ال e_coli و استخدمها في test معين لقدام .

هلء ال streak plate method هي اكتر الطرق المستخدمة عشان أعملisolation للبكتيريا individually. و اعمل isolating colony from mixture of bacteria (يعني عشان اعمل pure culture).

طيب أنا هادا بشو يهمني ؟ لعدة أسباب

1) Streak Plate Method

The streak plate technique is the most widely used method of obtaining isolated colonies from a mix of cultures (pure cultures). Why? To characterize, identify, differentiate, propagate, study or perform antimicrobial susceptibility testing on a microorganism, one must first isolate the targeted microorganism from the other species to which it does not belong.

مثلا لو بدي ادرس بس نوع معين من البكتيريا فبدي culture تحتوي على نوع معين من البكتيريا مثلا لو بدي أدرس الantimicrobial activity لنوع معين من الانتيبيوتيك فبدي أشوف ازا بعملي killing لنوع معين من البكتيريا ولا لأ ،فبلزمني أول إشي أحضر isolate pure culture من هاي البكتيريا.

Principle: The streak plate technique is essentially a method to dilute the number of organisms, decreasing the density. This allows for individual colonies to be isolated from other colonies. Each colony is considered "pure," since theoretically, the colony began with an individual cell.

هلء بخصوص الprincipleهي طريقةعشان اعمل dilution لعدد البكتيريا اللي عندي و هيك بسمح لكل colony تكون بعيدة عن اللي جنبها و هيك بقدر اشوفها بشكل واضح و استفيد منها بعدين

In this method a sterilized inoculating loop or transfer needle is dipped into a suitable diluted suspension of microorganisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks. The process is known as streaking so the plate prepared is called a streak plate. A sterilized inoculating needle with a loop made up of either platinum or nichrome wire is used for streaking. One loopful of specimen is transferred onto the surface of the agar plate in a sterile petri dish and streaked across the surface in the form of a zig-zag line. This process is repeated to streak out the bacteria on the agar plate so that some individual bacteria are separated from each other. The first streak will contain more organisms than the second and the second more than the third and so on. The last streaks should thin out the culture sufficiently to give isolate colonies. The successful isolation depends on spatial separation of single cells. Each colony ~~usually~~ represents the growth from a single organism when such a plate is incubated colonies will appear on the surface of the medium. Pure colonies can be obtained from well isolated colonies by transferring a small portion of each to separate culture media.

أول خطوة اني بعقم ال loopعن طريق اني بحرقه و بعدين بداخله داخل التيوب اللي فيه بكتيريا سسبنشن و بتعلق البكتيريا على ال loop و بعدين بفردها ع الplate

بهاي الطريقة بكون في عندي agar plate (solidified و جاهز) و بجيب ال inoculcation loop و بدخله داخل السسبنسن من البكتيريا و بكون بتركيز معين و بعدها بناخد هادا ال loopو بتعمل ستريكينغ على سطح ال agar plate تاعي و بكون على شكل حركة zigzag و عشان هيك بنسمي ال plate: Streak plate

Streak Plate Method

و هادا شكل الplate بعد ما اعمله inoculcation

ال spread method بستخدمها لعدة اسباب بس بالبداية بكون عندي mixture of microorganisms موجودين مع بعض ، بالبداية ال plate و بنحط عليه بالسنتر volume معين من ال microorganismاو ال pure cultureتاعي و بعدها بفرده على سطح ال plate و بعدين بوزعهعلى الأطراف و بعدين بعمله incubation و بعدين بشوف ازا صار عندي growth of microorganism عل السطح
و بستخدم هاي الطريقة أكتر اشي ازا بدي أقيس ال viable count او بدي اعمل counting of microorganismعلى سطح ال plate

2) Spread Plate Method

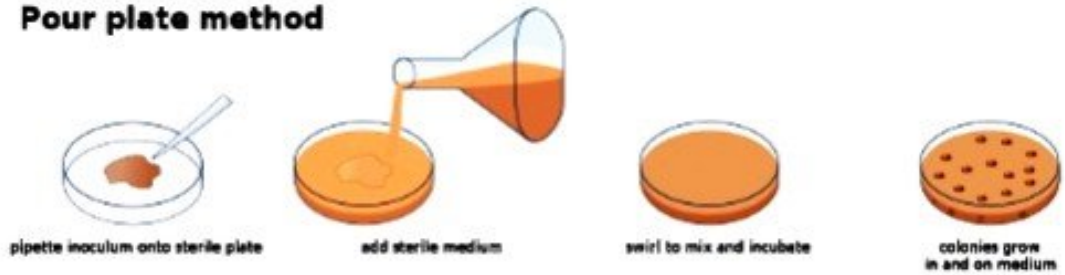
The spread plate technique is used for the separation of a dilute, mixed population of the microorganisms so that individual colonies can be isolated. In this technique, a small volume of dilute microbial mixture is transferred to the center of an agar plate and spread evenly over the surface with a sterile L-shaped bent glass rod, while the petri dish is spun, at some stage, single cells will be deposited with the bent glass rod on the agar surface. Incubate the agar plate at 37°C for 24 hours, in the inverted position. The dispersed cells will develop into isolated colonies. Because the number of colonies will be equal to the number of viable organisms in the sample spread plates can be used to count the microbial population. As we will see in another experiment.

بهاي
ة

ال pour plate method برضو بعملها عشان اعرف اعمل bacterial counting لكن شو بتفرق عن ال spread method انه بجيب البيتريتيش فاضي و بحط عليه البكتيريا سسبنشن او الpure culture و بعدها بجيب ال agar solution و بتركه يبرد شوي لدرجة حرارة 45و بديره فوق البكتيريا سسب و بحركه عشان تتوزع ال microorganism مع ال agar و بعدين بتركه عشان يصيرله 1 solidification و بعمله incubation

3) Pour Plate Method

In pour plate method, successive dilutions of the inoculum (serially diluting the original specimen) are added into sterile petri plate to which is poured melted and cooled (42°C - 45°C) agar medium and thoroughly mixed by rotating the plates which is then allowed to solidify. After incubation, the plates are examined for the presence of individual colonies. The pure colonies may be isolated and transferred into test tube culture media for making pure cultures. This technique is employed to estimate the viable bacterial count in a suspension. As we will see in another experiment.



هلء في شغلتين لازم انتبهلهم 1_اول اشي لما أدير ال agar solution لازم تكون حرارته بين 45و42 سليسيوس بحيث ما تكون عالية و تقتل ال microorganism
2_لما اجي بدي اعمل countللبكتيريا اللي مزروعة باستخدام ال pour methodd بدي انتبه انه البكتيريا ما رح تكون بس ع السطح رح يكون في كمان داخل ال agar plate .

اول اشي لازم نتعلمه كيف نعمل flaming لا neck تاهت ال bottle عشان أضمن انه ال mouthe tube ما عليه اي microorganism طيب كيف بعمل ؟
من خلال اني امرق ال neck فوق ال flame

Practical part

(i) Flam the neck of the bottles

This ensures that no microorganisms enter the mouth of the vessel to contaminate the culture or the medium. Passing the mouth of the bottle through a flame produces a convection current away from the opening, and helps to prevent contamination. The hot part of the flame is above the inner bright blue 'cone' and the vessel needs to be moved through the flame, not held in place.

لما نفتح الغطاي ما بصير نحطها على البينش

1. Loosen the cap of the bottle so that it can be removed easily.
2. Lift the bottle/ test tube with your left hand.
3. Remove the cap/ cotton wool plug of the bottle/ test tube with the little finger curled towards the palm of your right hand. (Turn the bottle, not the cap.)
4. Do not put down the cap/ cotton wool plug.
5. Flame the neck of the bottle/ test tube by passing the neck forwards and back through a hot Bunsen burner flame.
6. After carrying out the procedure required, for example, withdrawing culture, replace the cap/ cotton wool plug on the bottle/ test tube using your little finger. Take care! The bottle will be hot. (Turn the bottle, not the cap.)

(ii) Loop sterilization

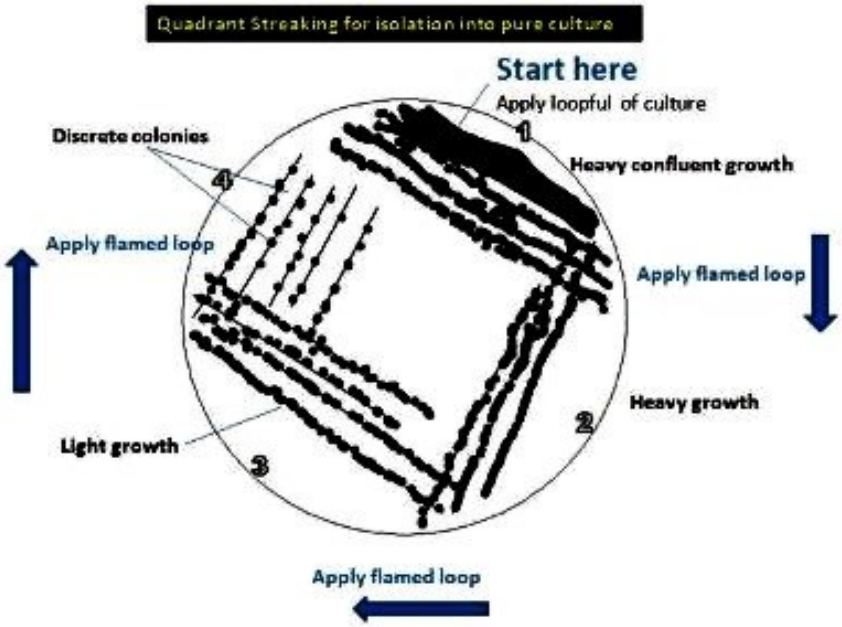
هون بجيب ال loop و بقربه على Bunsen burner ولازم يصير لونه احمر و هاي العملية بنسميها inserilation ولازم استنى 20ثانية مشان يبرد لأنه لو كان سخن حيقتل البكتيريا و بضل حاملته ف ايدي

1. The air-control of the Bunsen burner should be adjusted to give a flame with a short, blue, central cone
2. With the aluminum handle held as nearly vertical as possible without burning the fingers
3. The loop should be placed in the hottest part of the flame (just above the blue cone) until the whole length of the wire is heated to redness thus ensuring sterilization of the wire and the chuck of the handle
4. The loop should now be allowed to cool before use, or the bacteria that it touches will be killed (cooling takes approximately 20 seconds)

هلء هون البارت العملي صعب أكتب كل
إشي لأنه هو شرح و فهم فاحضروا هاد
البارت من الفيديو والله يوفقكو

(iii) The plating out procedure

1. Label a culture plate on its base with initials, sample and date
2. Sterilize the inoculating loop in the Bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.
3. Pick up the suspension provided in the left hand
4. Remove the plug with the little finger (with a twisting motion) of the right hand and flame the mouth of the tube
5. Dip the sterilized loop into the tube/culture bottle, withdraw a sample without touching the wall of the tube
6. Immediately streak the inoculating loop very gently over a quarter of the plate using a back-and-forth motion (see area 1 in the figure above).
7. Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2).
8. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3).
9. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate (area 4).
10. Flame your loop once more.
11. Replace plate in its lid
12. Incubate the plate inverted for 24 hours at 37 °C
13. Examine the colonies grown in the plate carefully. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate to obtain a pure culture.



يعني أنا بدي اعمل transfer of bacteria sample from pure culture to test tube يحتوي على ال broth والتيست تيوب اللي بحتوي على ال broth بنسميه destination container يعني التيست تيوب اللي بدي أزرع فيه

(iv) Subculturing from liquid culture to liquid broth شو يعني

Test 1:

1. Label a tube containing the sterile nutrient broth (destination container) be inoculated with the date, your name, and the name or mark of the test microorganism.
2. Place both test tubes (the sterile nutrient broth and that of containing the culture) in the palm of your hand in a V-like shape and stabilize them with your thumb. They should be held at an angle and thus not directly exposed to airborne laboratory contaminants.
3. Take the inoculating loop in the other hand and hold it like a pencil. Flame the inoculating loop along its full length over a Bunsen burner until the wire becomes red-hot.
4. Using the same hand that is holding the inoculating loop, remove the cap from the test tubes, hold it between your fingers, and briefly flame the neck of the tubes over a Bunsen burner by passing them through the flame to burn off any adhering dust and to create a temperature differential that temporarily prevents dust from falling into your tube. Do not put the caps onto the laboratory bench.
5. Insert the loop into the test tubes that containing the culture without touching the sides of the tube, and then remove it, carrying a loopful of bacterial cells.
6. Insert the loop containing the culture into the destination tube of sterile broth, swirl gently and remove.
7. Flame the necks of the tubes and close them with their caps.
8. Re-sterilize the loop before putting it down by inserting the loop into the flame *very slowly*! Doing this slowly allows any liquid remaining on the loop to evaporate rather than boil and avoid splattering live bacterial cells all over the bench and you.
9. Place the tubes and the inoculating loop into the rack.
10. Incubate at 37°C for one week.
11. Check the growth of the culture.

اللهم إني استودعتك ما قرأت و ما سمعت و ما فهمت و ما حفظت و ما وقعت عيني عليّ فرده الي عند حاجتي لا إله الا أنت سبحانك إني كنت من الظالمين 💛

Done by Nada Alhashlamoon

Artery Academy

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