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BACTERIAL CULTIVATION USING SEPHADEX® AS A DIFFUSION MATRIX

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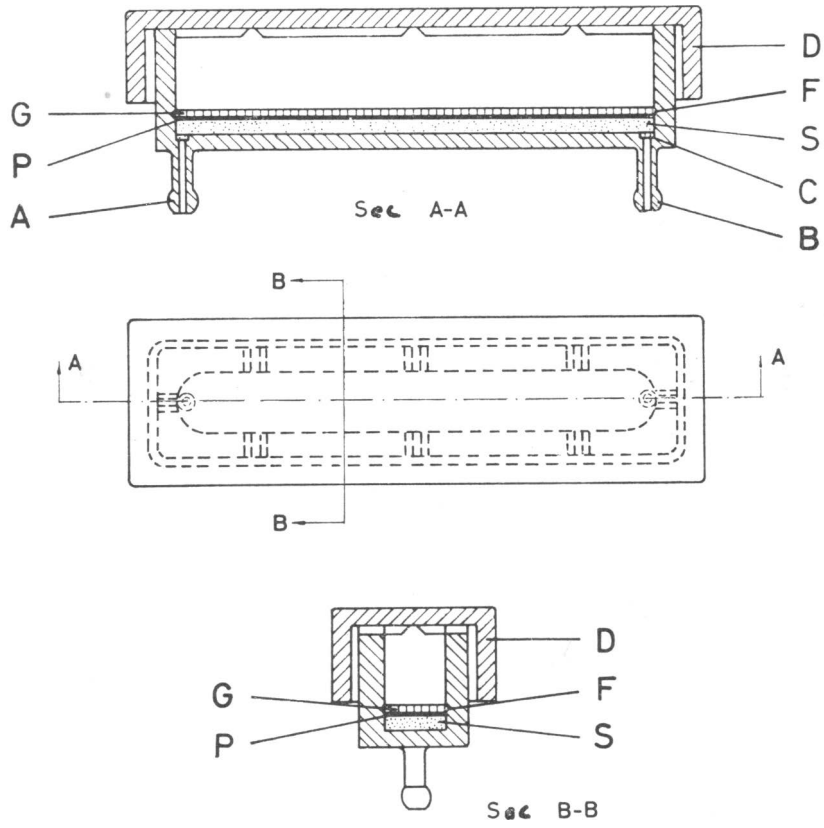
Sephadex (1) has been used earlier by the author in his two-phase culturing experiments (2). Here, a method is presented which permits control of the flow of liquid under a horizontal membrane surface the upper surface of which is used for cell cultivation. In the present case the Sephadex, following the pre-treatment described previously (2), has been used as a horizontal diffusion matrix through which it is possible to establish a controlled flow of liquid. The Sephadex bed carries a membrane layer on top of which cell cultivation is carried out. When used in connection with bacterial cultivation a 1.5 mm thick agar layer has been employed as membrane.

MATERIAL AND METHODS

The shape and design of the culture dish can be seen from Fig. 1. The dish is made of a plastic material that may be sterilized. The channel — 1.8 mm wide — through hose nipples A and B is slightly wider at the orifice in the dish bottom, and the dilated portion is plugged with a glass-wool packing (C) in each case. There is no airtight seal between the lid (D) of the culture dish and the edge of the dish wall.

When the culture dish is to be used a liquid trap is first connected to each hose nipple (A, B). The hose connections of the dish are filled with liquid nutrient medium. Finally, a carefully determined volume of Sephadex in liquid nutrient phase is added.



F i g u r e 1. (A) Hose nipple. (B) Hose nipple. (C) Glass wool. (D) Lid. (F) Groove. (G) Agar. (P) Rice paper. (S) Sephadex.

The liquid level in the dish is controlled by means of hose clips on the two liquid traps. The culture dish is then vibrated for a few seconds on a horizontal disk driven by a magnetic vibrator after which the Sephadex is allowed to sediment out on the dish. It is to advantage during the above-mentioned stages of operation to have the Sephadex material in the liquid nutrient phase at a temperature close to 100°C. Following sedimentation of the Sephadex the culture dish together with its contents is sterilized by rapid heating at 120°C in an autoclave.

One of the liquid traps is then replaced by a flask containing liquid nutrient medium and the other liquid trap is replaced by a pressure micropump that may be set to any capacity in the 0—300 ml/hr. range. The flask containing liquid nutrient medium

is fitted with a sterile air filter and the discharge tube at the micropump with a liquid trap. The pump is arranged to aspirate over hose nipple A — Sephadex layer (S) — hose nipple B. The free liquid surface in the liquid nutrient flask is adjusted so that the level within the flask is a few mm above that of the Sephadex layer. The liquid nutrient in the culture dish will then rise above the Sephadex surface, reaching as far as the lower margin of the groove (F). The Sephadex surface is covered by a sterilized rice paper (P) cut to adequate size. The flask containing the liquid nutrient medium is now lowered until the level of liquid in the culture dish has sunk to coincide with the upper surface of the Sephadex bed. This stage being reached a layer of agar (G) about 1.5 mm deep is carefully poured on top of the rice paper, which acts as a protection for the Sephadex bed against disturbances caused by the agar pouring procedure in this phase of the work. The agar layer will then fill up the groove (F) in the culture dish. Upon solidification of the agar the liquid level in the flask containing nutrient solution is adjusted to the same level as the upper surface of the Sephadex bed. It is then possible to establish a controlled flow of liquid through the culture dish by means of a micropump or by siphoning. The agar layer is inoculated with bacteria and the culture dish is incubated, care being taken that the evaporation of liquid is kept at the minimum.

DISCUSSION

The above described culturing technique as applied to the growing of bacteria on a membrane layer in conjunction with a controlled diffusion of liquid through an underlying layer of Sephadex gel may readily be adapted to other types of cell culturing. So, the culture dish can easily be designed to meet the requirements of particular culturing conditions or types of membrane.

REFERENCES

1) *Porath, J. & P. Flodin:* Gel filtration: A method for desalting and group separation. Nature (Lond.) 1959, *183*, 1657—1659.

2) *Bergrahm, B.:* Transmigration cultures in Sephadex medium. Life Sciences 1964, *3*, 499—502.

SUMMARY

A special culture dish has been devised for bacterial cultivation. The bottom of the dish is covered with a horizontal layer of Sephadex® on the top of which the type of membrane used in the individual experiment carries the cell cultures.

In the cultivation of bacteria a 1.5 mm deep agar layer has been used as the membrane on which the colonies have been grown. The flow of liquid through the underlying layer of Sephadex has been controlled by means of a micro-hose pump. The culture dish and the technique are both easy to adapt to other types of cell cultivation.

ZUSAMMENFASSUNG

Bakterienzüchtung unter Verwendung von Sephadex® als Diffusionsmatrix.

Eine besondere Kulturplatte ist für diese Kulturen konstruiert worden. Der Boden der Platte ist zunächst mit einer waagerechten Sephadex-Schicht gedeckt, die als Unterlage für die bei dem jeweiligen Versuch verwendete Membran dient, auf deren Oberfläche dann die Zellenzüchtung erfolgt.

Bei der Züchtung von Bakterien ist eine 1,5 mm starke Agarschicht als Membran, auf welcher sich die Kolonien entwickelt haben, benutzt worden. Die Strömung der Flüssigkeit durch die darunter befindliche Sephadex-Schicht ist mit Hilfe einer Mikro-Schlauchpumpe geregelt worden. Sowohl die Kulturplatte als auch das Verfahren lässt sich unschwer anderen Typen von Zellenzüchtung anpassen.

SAMMANFATTNING

Bakterieodling med Sephadex® som diffusionsmatrix.

För ändamålet har en speciell odlingsskål utformats. På bottnen av denna utbredes ett horisontellt Sephadexlager, och ovanpå detta appliceras den membrantyp, på vilken cellodlingen utföres.

Vid bakterieodling har ett 1,5 mm tjockt agarskikt fått utgöra den membran, på vilket kolonierna har utvecklat sig. Vätskeströmmen genom underliggande Sephadexskikt har reglerats med en mikropump. Såväl odlingsskålen som metodiken är lätta att anpassa till andra typer av cellodling.

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