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Fishmeal extract agar – a medium to inhibit swarming of *Proteus* spp.

Swarming on appropriate solid medium is characteristic of *Proteus* spp. It is the result of migration of a group of cells (swarm cells) from the edge of the developing micro-colony to an uninoculated area of the medium producing thin films of concentric rings of growth[1]. The swarming of *Proteus* may make it difficult to isolate in pure culture other pathogens which may be present in clinical specimens. Several anti-swarming agents have been described. Many of these inhibit the growth of certain pathogenic bacteria[1]. Fishmeal-based media were used for growing *Entamoeba histolytica*[2] and for the isolation and antibiotic susceptibility testing of medically important bacteria[3,4]. We report here the use of fishmeal extract agar to inhibit swarming of *Proteus* spp., without affecting the growth of other pathogenic bacteria which may be present along with *Proteus* in clinical specimens.

Fishmeal extract agar (FMEA) was prepared as previously described[2]. Five grams of fishmeal was boiled in 100 ml of distilled water and filtered through Whatman no. 1 filter paper. The volume was made up to 100 ml and pH was adjusted to 7.4. Agar (Hi Media) was added to a concentration of 2.0%, the medium was sterilized by autoclaving at 121°C for 15 min and poured into sterile petri plates. The plates were dried at 37°C for 1 h before inoculation. FEMA with sodium chloride was prepared by incorporating 0.5% sodium chloride into the former.

Clinical isolates of 25 *P. mirabilis* and 15 *P. vulgaris*, together with 40 swarming strains of *Proteus* spp. were inoculated on FMEA and nutrient agar (NA). The latter served as control. The swarming strains of *Proteus* were also inoculated on FMEA incorporated with 0.5% sodium chloride. Smears were prepared from growth on FMEA, FMEA with 0.5% sodium chloride and NA after 2 and 4 h, stained by Gram's stain and microscopically examined.

All 40 strains of *Proteus* showed swarming on NA. None of these swarmed on FMEA and FMEA incorporated with 0.5% sodium chloride (Table 1). Microscopy of growth on FMEA and FMEA with 0.5% sodium chloride after 2 and 4 h did not reveal swarm cells. Similar examination of growth on NA showed swarm cells.

The swarming of *Proteus* may make it difficult to isolate in pure culture, other bacterial pathogens in clinical specimens from polymicrobial infections. Many ways of inhibiting swarming have been described. Restricting movement of *Proteus* cells by increasing agar concentration to 3–4%; preventing formation and interfering with structure and activity of flagella by incorporating into media polyvalent H antisera; ethanol, boric acid, bile salts, detergents or by retarding the growth rate by incorporating growth inhibitors such as sulphonamides, neomycin, chloral hydrate, barbiturates, sodium azides or purine bases. Many of the anti-swarming agents are toxic and prevent the growth of delicate pathogens. Others may interfere with the colonial morphology of the organisms or lyse red blood cells in the medium, making the recognition and detection of hemolytic organisms difficult[1].

Our study showed that *Proteus* spp. does not swarm on FMEA. It is known that the omission of sodium chloride from the medium prevents spreading of *Proteus* colonies[5]. Inhibition of swarming of *Proteus* spp. on FMEA was regardless of in-

Table 1. Anti-swarming nature of fishmeal extract agar (FMEA) compared with nutrient agar (NA)

Medium	No. of *Proteus* strains tested		No. of *Proteus* strains that showed swarming	
	P. mirabilis	*P. vulgaris*	*P. mirabilis*	*P. vulgaris*
NA	25	15	25	15
FMEA	25	15	Nil	Nil
FMEA with 0.5% sodium chloride	25	15	Nil	Nil

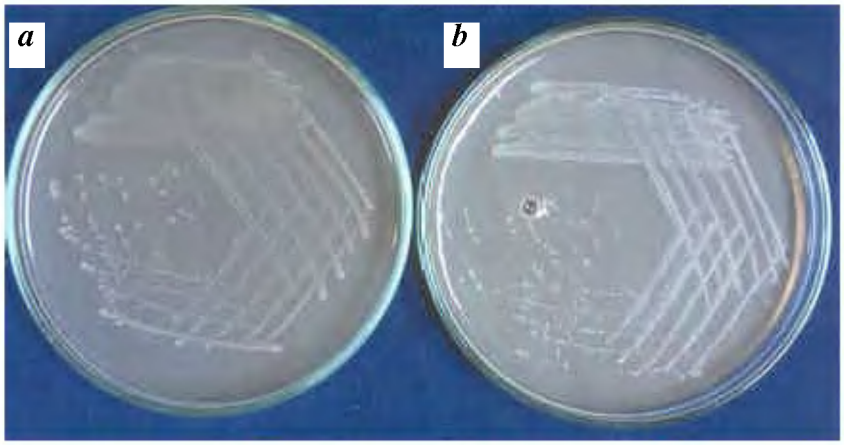

Figure 1. Growth of *Proteus mirabilis* on FMEA (***a***) and on FMEA with 0.5% sodium chloride (***b***) after 24 h of incubation at 37°C.

corporation of sodium chloride in the medium. However, growth was better on FMEA incorporated with 0.5% sodium chloride (Figure 1 *a*, *b*). After 24 h incubation at 37°C, colonies of both *P. mirabilis* and *P. vulgaris* on FMEA with sodium chloride were about 1–2 mm, circular, convex, smooth, translucent and non-swarming (Figure 1 *b*).

Formation of multinucleate, multiflagellate, nonseptate elongated cells, 20–80 µm in length, known as swarm cells is essential for the swarming activity of *Proteus*. Swarm cell were not formed by *Proteus* spp. on FMEA. Various factors such as glutathione, viscous environment or inhibition of flagellar rotation were reported to trigger swarm cell formation, resulting in swarming[1]. It appears that FMEA may be deficient in such factors which trigger swarm cell formation.

Fishmeal has already been used as a base in formulating medium for cultivation of *E. histolytica*[2], bacteria[3], and for antibiotic susceptibility testing[4]. Fishmeal extract obviates the need to incorporate yeast extract, beef extract and peptone when used to grow bacteria.

Fishmeal used in FMEA is the ground cake obtained after oil extraction from pooled sardines and used as fertilizer. Fishmeal is non-hygroscopic and cost-effective (Rs 16/kg) than peptone (Rs 1600/kg).

FMEA is therefore a cost-effective, simple medium that may be effectively used for isolation in pure culture of bacterial pathogens from clinical samples containing *Proteus* spp.

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