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THE SWARMING OF PROTEUS ON SEMISYNTHETIC MEDIA

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PLATES I-IV

SWARMING by proteus on solid medium has been the subject of investigation since it was first described by Hauser (1885), as it is a phenomenon that does not occur to the same degree with other Gram-negative rods.

Lominski and Lendrum (1947) suggested that swarming is a reaction by the organisms to toxic products of their own metabolism, a view supported by the work of Hughes (1957), who demonstrated that the forms in a swarm are morphologically indistinguishable from those produced by the damaging action of penicillin. A relation exists between the ability of proteus to swarm on a solid medium and the composition of the medium. It is common knowledge that the presence of bile salts inhibits swarming and a variety of toxic substances as diverse as chloral hydrate (Krämer and Koch, 1931) and sulphonamides (Holman, 1957) inhibit swarming when added to media. Naylor (1960) devised a medium that did not promote swarming of proteus, by omitting sodium chloride from the conventional formula of nutrient agar. Jones and Park (1967) found that growth but not swarming occurred on a solid medium based on the minimal medium of Fildes (1938), but these workers also claimed that swarming on this medium was promoted by the addition of various amino compounds.

The present investigation is an attempt to define the components of solid media that are responsible for the promotion of swarming by proteus.

MATERIALS AND METHODS

Organisms

Proteus mirabilis NCTC5887 and 11 wild strains of *Proteus mirabilis* variously isolated from urinary infections, wounds, ulcers and rectal swabs were studied in the present investigation; the wild strains were identified as *Proteus mirabilis* by the criteria of Cowan and Steel (1965). The organisms were maintained in Robertson's cooked-meat broth at room temperature and were subcultured at monthly intervals. Before testing, the organisms were brought into the log phase of growth by inoculating them into 5 ml of nutrient broth and incubating for 5 hr at 37°C.

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Reagents

Demineralised water from an Elgastat B113 (Elga Products Ltd, Berks, England) was used in all experiments. All chemicals were of Analar quality. Biochemical reagents such as amino compounds and vitamins were obtained from BDH (British Drug House Ltd, Poole, England), from Hopkin and Williams (Essex, England) or from Koch-Light Laboratories Ltd (Colnbrook, England). Sephadex G-25 and G-50 was obtained from Pharmacia (Sweden).

Casein hydrolysate was obtained from Mead and Johnson International (Indiana, USA) and from Difco Laboratories (Detroit, USA). The Mead and Johnson preparation was made by digestion of casein with pancreatin and that of Difco was prepared by acid hydrolysis; both preparations gave identical results. Other preparations tested were made by acid hydrolysis and were obtained from Oxoid Ltd (London, England) and from BDH.

TABLE I

Composition of the minimal media from which the semisynthetic media were prepared

Medium	Component	Concentration (mm per litre)
Minimal medium I	Glucose	11
	Sodium citrate	1.7
	(NH ₄) ₂ SO ₄	7.6
	MgSO ₄	0.4
	K ₂ HPO ₄	40
	KH ₂ PO ₄	22
Minimal medium II	Glucose	11
	Sodium citrate	1.7
	(NH ₄) ₂ SO ₄	7.6
	MgSO ₄	0.4
	" TRIS "	100
	HCl	86

Yeast extract was obtained from Oxoid. This material is marketed both as a paste and a powder. Variation in activity was found between the various samples of yeast extract and three batches of paste (A, B and C) and two batches of powder (D and E) were tested.

Media

The source of Robertson's cooked-meat broth and nutrient broth was Oxoid. Ionagar no. 2 (Oxoid) was used at a 1 per cent. concentration to convert liquid media to solid media except in a few experiments in which it was replaced by Agarose (Seravac, Maidenhead, England). Two varieties of minimal medium were used (table I). Medium I was based on that of Davis and Mingioli (Cruickshank, Duguid and Swain, 1965) and in medium II the phosphate buffer was replaced by TRIS-HCl (trihydroxymethylmethyammine-hydrochloric acid) buffer. All the components with the exception of glucose were dissolved in water and the pH was measured with Jena dual micro-glass electrode 9259-81 in conjunction with a Vibret pH meter (Electronic Instruments Ltd, Surrey, England) and adjusted to 7.2 if necessary. Agar was added and dissolved by steaming the mixture for 45 min. after which the medium was dispensed into 100-ml screw-capped bottles and sterilised by autoclaving at 115°C for 10 min.

Before use, the medium was melted by steaming at 100°C for 20 min., cooled to 56°C and an aliquot of 50 per cent. (w/v) glucose solution, previously sterilised by autoclaving, was added. Solutions of vitamins, amino compounds and yeast fractions, previously sterilised by filtration through a Millipore filter DDA (Millipore UK, Wembley, Middlesex), were also added at this stage if required.

Minimal media were converted to semisynthetic media by the incorporation of casein hydrolysate or yeast extract or both. Weighed amounts of these substances were normally added to the minimal media with the agar and thus subjected to sterilisation by autoclaving (*vide supra*). In some experiments, solutions of casein hydrolysate and yeast extract previously sterilised by filtration were added to the minimal media at the same time as the glucose.

Column chromatography

Fractionation of yeast extract was carried out with Sephadex G-25 or G-50 in a 40×2.5 cm column. Five millilitres of a 10 per cent. solution of yeast extract in water was applied to the column and eluted with water supplied by a peristaltic pump at a rate of 36 ml per hr; 5-ml fractions were collected on a BTL Chromofrac fraction collector (Baird and Tatlock Ltd, Essex, England). The concentration of components in the fractions was measured at $\lambda = 280$ nm and $\lambda = 450$ nm with a Unicam SP 500 spectrophotometer (Unicam Instruments Ltd, Cambridge, England). The principal fractions were pooled and dried by lyophilisation. In some experiments, the batches of yeast extract of low activity (B and C) were eluted with 0.85 per cent. NaCl. These preparations were not lyophilised, but stored in the frozen state at -70°C and added directly to the medium after thawing.

Thin-layer chromatography

Ten milligrams of the glycopeptide fraction of yeast extract was hydrolysed for 6 hr with 5 ml of 6N-HCl for amino acid analysis or for 2 hr with N-HCl for carbohydrate analysis in sealed glass ampoules; the hydrolysates were prepared for chromatography by evaporating to dryness *in vacuo* over concentrated H_2SO_4 and solid NaOH. The residues were dissolved in 0.1-ml amounts of water.

Thin-layer chromatography was carried out on 20×20 cm glass plates. Amino acids were analysed on Whatman CC41 medium (H. Reeve Angel and Co. Ltd, London, England) of 0.25 mm thickness with a mixture of butan-1-ol:acetone:diethylamine:water (10:10:2:5) in one dimension and propan-2-ol:formic acid:water (40:2:10) or butan-3-ol:methyl ethyl ketone:dicyclohexylamine:water (10:10:2:5) in the other dimension (von Arx and Neher, 1963). Amino acid spots were located with a ninhydrin-cadmium stain (Easley, Zegers and de Vijlder, 1969).

Carbohydrates were analysed on silica gel S medium of 0.3 mm thickness (Hopkin and Williams) with a mixture of butan-1-ol:pyridine:water (8:4:3) for monosaccharides (Ovodov *et al.*, 1967) and ethanol:0.8 per cent. NH_4OH (85:15) for hexosamines (Moczar *et al.*, 1967). Monosaccharides were located with a naphthoresorcinolphosphoric acid stain and hexosamines with the Elson-Morgan reagent.

Testing for swarming

The ability of a medium to promote swarming was tested by touching the centre of a 9-cm plate with a straight wire previously dipped in a log-phase culture of one of the strains of proteus. Complete swarming and various degrees of partial swarming by the proteus strains occurred on the semisynthetic media and were assessed by both macroscopic (fig. 1) and microscopic (figs. 2-6) examination.

The plates were inspected after a 16-hr period of incubation at 37°C . Complete swarming was deemed to have occurred if at least 80 per cent. of the surface of the agar was covered by the organism (fig. 1*d*). If less than this proportion was covered, the medium was considered to promote only partial swarming. Star-shaped patterns (fig. 1*c*) or concentric zones in close apposition were commonly observed on the surface of media that promoted only partial swarming.

The edge of the colony was also observed under a phase-contrast microscope and this enabled further differentiation of the various degrees of partial swarming. The following categories were recognised. (1) *Absence of swarming* (fig. 2). The edge of the colony was smooth and made up of a convex margin of heaped-up organisms. Prolonged (greater than 40 hours) incubation sometimes gave rise to finger-like projections from this central colony

(fig. 3) but not to monolayers of swarming rods. The characteristic appearance of such a colony on an agar plate is shown in fig. 1*b*. (2) *Arrested swarming* (fig. 4). In this category, movement of the organisms in a monolayer had occurred on the surface of the medium, but cessation of progression and "heaping-up" of the swarm occurred during the 16-hr period of incubation with the result that the organisms covered less than 80 per cent. of the surface of the agar. Various patterns on the agar were observed and that shown in fig. 1*c* is characteristic of one of these. Translational movement of individual organisms and rafts of organisms were not seen. (3) *Slow swarming* (fig. 5). In some cases, arrest of swarming had occurred, but zones in the outer edge of the colony showed monolayers of organisms exhibiting active movement of individual organisms and rafts of organisms. Arrested swarming and this type of "slow swarming" characteristically occurred on media in which there was a threshold concentration of factors required to promote swarming and bizarre and irregular patterns were seen on the surface of the agar. (4) *Complete swarming* (fig. 6). This was characterised by the presence at the periphery of the swarm of monolayers of rafts of organisms exhibiting extremely active movement.

RESULTS

Promotion of swarming with yeast extract

Minimal media I and II (table I) just supported growth of all twelve strains of *Proteus mirabilis*. When 1 or 2 per cent. of casein hydrolysate was added to the minimal media, growth was encouraged, but there was no swarming (fig. 1*a* and 1*b*). In none of these cultures was the characteristic smell of proteus apparent. Swarming was promoted by the addition of 300 mg per cent. of BDH yeast-extract paste or powder to the semisynthetic casein hydrolysate medium (fig. 1*d*) and, furthermore, arrested swarming occurred when 300 mg yeast extract per 100 ml was added to the minimal media (fig. 1*c*). Four preparations of casein hydrolysate were tested to see whether or not they promoted swarming when incorporated in minimal media without the addition of yeast extract; only BDH casein hydrolysate, made by acid hydrolysis of casein, was found to promote swarming. Three batches of yeast-extract paste (A, B and C) and two batches of yeast-extract powder (D and E), all of which originated from Oxoid, were tested for their ability to promote swarming; two batches of the paste (B and C) failed to promote complete swarming at a concentration of 300 mg per 100 ml.

Concentrations of active yeast extract of 100 mg per 100 ml and less in semisynthetic media inconsistently promoted complete swarming and there was some evidence that the active components of yeast extract were slightly heat labile.

Influence of the composition of the medium

Various alterations were made in the composition of semisynthetic media, but none of these was able either to induce complete swarming by the test strain of proteus in the absence of yeast extract or to influence the promotion of swarming when yeast extract was added. Nicotinic acid (90 μ g per 100 ml; Fildes) was added to the minimal media in these experiments.

The presence or absence of the divalent cations Mg^{++} and Ca^{++} (2mm per litre) and of added sodium chloride (0.85 per cent.) neither caused swarming

by the test strains of *proteus* in the absence of yeast extract nor prevented the promotion of swarming when yeast extract was added. Similarly, the addition to minimal media of unheated casein hydrolysate, sterilised by filtration, or the addition to minimal media of a mixture of casein hydrolysate and glucose that had been autoclaved at 115°C or allowed to react for 12 hr at 56°C did not promote swarming in the absence of yeast extract. Replacement of Ionagar no. 2 with Agarose was without effect on the promotion of swarming by yeast extract and the substitution did not induce swarming in the absence of yeast extract. Jones and Park have claimed that seven amino compounds (alanine, asparagine, aspartic acid, glutamic acid, glutamine, proline and serine) when added either singly or in combination to a minimal medium containing nicotinic

TABLE II
Vitamins of the B group tested in semisynthetic media for their ability to promote complete swarming

Vitamin	Concentration in medium (μg per 100 ml)
Thiamine (B1)	60 (18-270)*
Riboflavin (B2)	18-36 (6-12)
Nicotinic acid	90-360 (90-300)
Pyridoxine (B6)	15 (11)
Ca pantothenate	40 (40-105)
<i>p</i> -Aminobenzoic acid	90-1000 (1.5-1.7)

* The figures in brackets indicate the likely concentration that would have been achieved in media to which 300 mg yeast extract was added per 100 ml.

acid promoted swarming by *proteus*. In neither of our minimal media was swarming induced when casein hydrolysate was replaced by this mixture of amino compounds.

Vitamins of the B group listed in table II were tested singly and in combination in semisynthetic media containing 1 per cent. of casein hydrolysate. Their concentrations were those likely to be achieved when 300 mg yeast extract per 100 ml was added to the medium. *p*-Aminobenzoic acid was tested at higher concentrations than those expected in yeast extract in a series of experiments connected with a separate enquiry. The six common vitamins of the B group, either singly or in combination, were unable to promote swarming by the 12 test strains of *proteus* when added to semisynthetic media containing 1 per cent. of casein hydrolysate.

Promotion of swarming with a fraction of yeast extract

The results of gel filtration of yeast extract on Sephadex G-25 are shown in fig. 7. The extract was resolved into five fractions called F1-F5. The activity of the pooled lyophilised fractions (table III) was tested against five of the strains of *proteus*. When the fractions were mixed proportionately

to the weights recovered, complete swarming was promoted by the addition of 300 mg of the mixture per 100 ml to semisynthetic medium containing casein hydrolysate only when F1 was present. No swarming occurred when F1 was omitted from a mixture, but omission of any fraction other than F1 resulted in complete swarming when such a mixture was added to the medium. The addition of 200 mg of F1 per 100 ml to semisynthetic media promoted complete swarming by all the five test strains, but none of the other fractions showed this activity when tested singly.

When the samples of yeast extract (B and C) that did not promote complete swarming were fractionated on Sephadex G-25, an elution pattern similar to that shown in fig. 7 was obtained except that the size of the F1 fraction was reduced and the percentage yield of F1 was less than that in the active yeast extract samples. Complete swarming, however, was not promoted by the addition of 300 mg of these F1 fractions to 100 ml semisynthetic medium.

TABLE III
*Results of fractionating batch A of yeast extract on Sephadex G-25;
the fractions were eluted with water*

Fraction	Percentage of total weight recovered	Components identified	Swarm-promoting activity
F1	20	Glycopeptide	+
F2	35	Nil	—
F3	42	Amino acids	—
F4	1	Riboflavin	—
F5	2	Nil	—

Properties of the F1 fraction of yeast extract

Thin-layer chromatography of the acid hydrolysate of F1 showed the presence of moderate amounts of glycine, alanine, proline, glutamic acid, aspartic acid, serine, lysine and leucine, small amounts of arginine, histidine, threonine, glutamine, phenylalanine and tyrosine, and traces of methionine and valine. Partition chromatography showed that the carbohydrates liberated on acid hydrolysis with N-HCl were glucose, mannose and glucosamine. Acid hydrolysis of the F1 component of the yeast extract samples of low activity gave closely similar results.

Gel filtration of the F1 fraction of yeast extract on Sephadex G-50 showed that the fraction was not a homogeneous molecular species (fig. 8). The first component isolated (F1a) was inconstantly present in the various pooled lyophilised F1 fractions. The other two major components (F1b and F1c) were always present in the pooled F1 fractions and accounted for 80 per cent. of the complex. All fractions showed swarm-promoting activity comparable with that of their parent F1 fraction.

Effect of vitamins and NaCl on the swarm-promoting properties of glycopeptide fractions from batches of yeast extract of low activity

The addition of nicotinic acid (250 μg per 100 ml) to semisynthetic media enhanced the swarm-promoting capacity of the glycopeptide fractions from batches of yeast extract of low activity. For example, slow swarming was observed in a medium that showed only arrested swarming after 18 hr at 37°C. Addition of the mixture of B vitamins listed in table II was more effective than

FIG. 7.—Elution pattern obtained when a 10 per cent. solution of yeast extract batch A was fractionated on Sephadex G-25 with water as an eluent. The extinction (E) of the 5-ml fractions was measured at 280 nm and 450 nm and the numbered arrows 1–5 indicate the groups of tubes that were pooled together to constitute fractions F1–F5 respectively.

nicotinic acid alone in promoting swarming on media containing these glycopeptide fractions.

Solutions in water of glycopeptide fractions from batches of yeast extract of low activity were turbid in appearance; this turbidity disappeared on addition of 0.85 per cent. of NaCl. When 0.85 per cent. NaCl was incorporated into media containing glycopeptide fractions of low activity, a greater degree of swarming was observed than in the absence of the added salt. Likewise, semisynthetic media containing glycopeptide preparations eluted from Sephadex G-25 with 0.85 per cent. NaCl were also more active in promoting swarming than corresponding media containing glycopeptide preparations eluted with water. Salt and vitamin mixtures acted synergistically in the promotion of swarming when added to media containing glycopeptide fractions of low activity.

DISCUSSION

When strains of *Proteus mirabilis* are inoculated on to the surface of nutrient agar, swarming occurs in a rhythmical pattern of zones so that normally the whole of the agar surface of a 9-cm culture plate is covered by the organism during a 16-hr period of incubation. This phenomenon was described by Russ-Münzer (1935) and the pattern of zones was attributed to the depletion of nutrients in the medium. Lominski and Lendrum (1947) and Hughes (1957)

FIG. 8.—Elution pattern of the glycopeptide fraction of yeast extract when an aqueous solution was fractionated on Sephadex G-50 with water as an eluent. The extinction (E) of the 5-ml fractions was measured at 280 nm. The numbered arrows 1, 2 and 3 indicate the groups of tubes that composed fractions F1a, F1b and F1c respectively.

considered that swarming was caused by the liberation of toxic products, a view that receives support from the work of Proom and Woiwod (1951), who reported amine production in the genus *Proteus*.

In the present investigation, a semisynthetic medium was devised that enabled *Proteus mirabilis* to grow luxuriantly, but did not promote swarming. It is noteworthy that the characteristic smell was absent from cultures of proteus grown on this medium. The semisynthetic medium consisted of a minimal medium to which was added an amino acid mixture in the form of casein hydrolysate. Replacement of this amino acid mixture with the amino compounds, either singly or in combination, that Jones and Park (1967) claimed to promote swarming by proteus, consistently failed to induce swarming even when nicotinic acid in a concentration similar to that employed by Fildes (1938) was added to the medium. Amino acids react with hexoses and pentoses

SWARMING OF PROTEUS

FIG. 1.—Effect of the composition of the culture medium on the growth and swarming of *Proteus mirabilis*. A log-phase culture of the organism was inoculated at the centre of each plate, which was then incubated for 24 hr at 37°C. (a) Semisynthetic medium containing 2 per cent. of casein hydrolysate; no swarming. (b) Semisynthetic medium containing 1 per cent. of casein hydrolysate; no swarming. (c) Minimal medium containing 300 mg yeast extract per 100 ml; star-shaped pattern of “arrested” swarming. (d) Semisynthetic medium containing 300 mg yeast extract per 100 ml; complete swarming.

SWARMING OF PROTEUS

FIG. 2.—Appearance of the periphery of a colony of *Proteus mirabilis* after incubation for 24 hr at 37°C on a semisynthetic medium that supports growth but does not promote swarming. Phase contrast (PC). $\times 100$.

FIG. 3.—Appearance of finger-like projections at the periphery of a colony after incubation for 48 hr at 37°C on a semisynthetic medium that supports growth but does not promote swarming. PC. $\times 100$.

SWARMING OF PROTEUS

FIG. 4.—Periphery of a swarm of *Proteus mirabilis* showing “arrested” swarming after 16 hours’ incubation on a semisynthetic medium containing threshold concentrations of swarm-promoting factors. PC. $\times 200$.

FIG. 5.—Periphery of a swarm of *Proteus mirabilis* showing “slow” swarming after 16 hours’ incubation on a semisynthetic medium containing threshold concentrations of swarm-promoting factors. PC. $\times 200$.

SWARMING OF PROTEUS

FIG. 6.—Periphery of a swarm of *Proteus mirabilis* showing “complete” swarming after 6 hours’ incubation on a semisynthetic medium containing 300 mg of an active yeast extract per 100 ml. The absence of clear outlines to the bacteria was due to the very active motility. PC. $\times 200$.

to form complexes (Maillard, 1912). Neither heating casein hydrolysate with glucose in an autoclave nor allowing the two substances to interact at 56°C for 12 hr caused the mixture to promote swarming when added to the medium. Likewise, the addition of the divalent cations Mg^{++} and Ca^{++} to semisynthetic medium based on TRIS buffer was unable to cause complete swarming by the proteus strains. This finding is of particular interest as Dr M. Sussman tells us that the addition of EDTA to nutrient agar inhibits swarming by proteus.

The addition of yeast extract to semisynthetic medium usually promoted complete swarming, but variation in activity was found between the batches of extract tested. Jeffries and Rogers (1968) isolated a water-soluble substance from agar that promoted swarming. We found, however, that replacement of Ionagar no. 2 with Agarose, a highly purified polygalactose fraction of agar, had no effect on the ability of yeast extract to promote swarming. Replacement of the yeast extract with vitamins and growth factors, singly and in combination, also failed to promote complete swarming.

Fractionation of an active yeast extract on Sephadex G-25 split the substance into five fractions. The first fraction F1, which was eluted in void volume of the column, was the only fraction that showed swarm-promoting activity. Testing of mixtures of the five fractions showed that the omission of any fraction other than F1 was without effect on the ability of the reconstituted extract to promote swarming. Likewise, the only fraction able to promote swarming when added singly to semisynthetic medium was F1. This suggests that the factors in yeast extract that promote swarming are contained in the high-molecular-weight fraction. The result of thin-layer chromatography suggested that this high-molecular-weight fraction was glycopeptide in nature, and further fractionation on Sephadex G-50 showed that it was non-homogeneous, consisting of at least three molecular species all of which were active.

Glycopeptide fractions, isolated from batches of yeast extract of low activity, had correspondingly low capacities to promote swarming. The addition of nicotinic acid or mixtures of B vitamins to media containing such glycopeptide fractions increased the ability of the media to promote swarming, thus suggesting that these growth factors play a role in the phenomenon of swarming. It was observed that aqueous solutions of low-activity glycopeptide fractions were turbid and that this turbidity disappeared on addition of salt. It was also found that addition of salt to media containing such glycopeptides increased the ability of the medium to promote swarming. Likewise, glycopeptide fractions of yeast extracts of low activity that were eluted from Sephadex with saline showed a greater capacity to promote swarming than corresponding fractions eluted with water. The increase in the solubility of glycopeptides brought about by salt may therefore partly explain the observation of Naylor (1960), who found that omission of salt from nutrient agar leads to a medium that promotes the growth but not the swarming of proteus.

Our results suggest that active glycopeptides, amino acids and growth factors should be constituents of a medium for complete swarming by proteus. If glycopeptide complexes are absent, true swarming does not occur, although

colonial movement may be seen. If there is a paucity of amino acids, arrested swarming occurs as seen when yeast extract is added to minimal medium. If growth factors such as nicotinic acid are deficient, "slow" swarming may occur and give rise to bizarre patterns on the agar surface.

These interpretations support the original concept of Russ-Münzer that zoning in a proteus swarm is a phenomenon of medium depletion, except that the material used up is likely to be one of the swarm-promoting factors rather than a nutrient substance. This theory of medium depletion is also supported by the occurrence of multiple zones when there is a threshold concentration of one of the swarm-promoting factors. In arrested swarming, there is a tendency for "true" colonial formation to take place by the multiplying and heaping-up of organisms to form a multilayer, but slow swarming occurs frequently at the periphery of such colonies on prolonged incubation. The apparently central role played by the glycopeptide complex in the promotion of swarming is puzzling. This class of compound is believed to influence cell division (Bullough *et al.*, 1967) and possibly the long forms and rafts of long forms that are consistently present in monolayers are incompletely divided organisms that exhibit their characteristic helical swarming movement by virtue of their rich endowment with flagella. The alternative hypothesis (Lominski and Lendrum) that production of toxic metabolites promotes swarming would seem to implicate amino acids and vitamins as the limiting factors in the induction of swarming. The essential but nonetheless subsidiary role played by amino acids and vitamins together with the absence of the characteristic "amine" smell in proteus cultures grown on solid semisynthetic medium would seem to indicate that factors connected with both cell division and the production of certain metabolites may be responsible for this curious but familiar phenomenon.

SUMMARY

A semisynthetic medium has been devised that supports the growth of proteus but does not promote its swarming. Addition of a glycopeptide fraction of yeast extract to the medium promoted swarming. Batch-to-batch variation in activity of the glycopeptide fractions was found between samples of yeast extract, but the swarm-promoting capacity of a preparation of low activity was increased by raising the solubility of the complex with sodium chloride. The ability to promote swarming on a medium containing a low-activity glycopeptide fraction was increased by the addition of vitamins of the B group. It is suggested that glycopeptides, vitamins of the B group and amino acids are necessary factors in the promotion of swarming by proteus on solid media.

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