Inhibition of *Proteus* Swarming by Nucleic Acid Products

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We noted that *Proteus* sp. failed to swarm on a medium designed to select for staphylococci which produced deoxyribonuclease. This effect was attributable to the DNA (sperm deoxyribonucleic acid, Lot no. 1701, Nutritional Biochemicals Corp., Cleveland, Ohio) which had been added to Tryptose Agar (Difco) because swarming developed freely on the unsupplemented Tryptose Agar. This observation added an exceptional compound to the numerous substances which are known to interfere with the ability of *Proteus* sp. to swarm on agar media. Agents such as ethyl alcohol, sodium chloride, aniline dyes, surface active agents, and sulfa drugs, which presumably act by modifying the availability of fluid in the medium or by selective bacteriostasis, have been used to inhibit swarming (1, 2, 4, 5). We investigated this unexpected effect on swarming manifested by DNA.

The DNA exerted no effect on swarming when incorporated into Brain Heart Infusion Agar (Difco) or Trypticase Soy Agar (BBL), both of which are maintained near neutrality by included buffer salts. However, we observed that the Tryptose Agar had a pH of about 6.0 whether containing DNA or not. When the DNA-Tryptose Agar was adjusted to pH 7.0, it failed to inhibit swarming. Therefore, in subsequent studies of the effect of DNA on swarming by *Proteus* sp. we employed a medium (BT agar) prepared by dissolving 1% tryptose (Difco), 0.1% glucose, and 1.0% agar in 0.05 M phosphate buffer of the desired pH. When additions were to be made, the volume of the medium was adjusted to accommodate any resulting dilution. The bacterial strains used for this study were primarily *P. mirabilis* (WP-1 and WP-22) and *P. vulgaris* (CDC 4440). Additional strains isolated from clinical materials, all of which swarmed readily on the control medium, also failed to swarm on the DNA-BT agar.

The pH of the medium was an important parameter in the inhibition of swarming by DNA. Swarming was inhibited on the DNA-BT agar only below pH 6.5, but from that pH upward swarming occurred even in the presence of DNA (Table 1). On the control BT agar, swarming developed over the range of pH 5.5 to 7.5, so that the inhibition of swarming was not simply a result of the decreased pH of the medium. The concentration of DNA was also important because at least 0.5% DNA was required to inhibit swarming. When 0.3% DNA was added to the medium, the effect was variable, and there was no effect with 0.1% DNA in the medium. Growth on the DNA-BT agar had no permanent effect on the ability to swarm because the *Proteus* strains again swarmed when returned to the control medium, even after 10 serial transfers on the DNA-BT agar.

A greater quantity (1%) of ribonucleic acid (RNA) was required in the BT agar to obtain irregular inhibition of swarming. The DNA which, in the later phases of this study, replaced the original sample was much less effective. To obtain inhibition of swarming with the new lot of DNA, we found it necessary to autoclave a stock solution of DNA. This solution was then added to BT agar which was sterilized by autoclaving. Thus, the hydrolysis products derived from the nucleic acid apparently act directly on the ability of *Proteus* sp. to swarm.

To evaluate further this aspect, DNA was fractionated by ultrafiltration or precipitation. The ultrafiltrate was obtained by placing an aqueous solution of DNA in a dialysis bag which was maintained at 4 C under slight negative pressure. The filtrate which accumulated in the flask was added to the medium to be the equivalent of 0.5% DNA; e.g., if a 5% solution of DNA had been placed in the bag, then filtrate equal to 0.1 of the total volume of medium was used. In all studies in which the ultrafiltrate was used, swarming was inhibited. The oligonucleotides were precipitated by adjusting an aqueous solution of DNA to pH 4.5; three volumes of ethyl alcohol were added and maintained at 4 C overnight. The precipitate, collected by centrifugation, was redissolved in water with a small amount of alkali if needed, dialyzed against flowing tap water overnight, and then against several changes of distilled water at 4 C. The product was steri-
lized by filtration (Millipore Corp., Bedford, Mass.), and the dry weight was determined. No inhibition of swarming was observed when this preparation was added to sterile BT agar to obtain 0.5% DNA. If, on the other hand, the polynucleotide solution was added to the BT agar and then sterilized by autoclaving, swarming was inhibited. This phenomenon and Table 1. Effect of pH on swarming by Proteus sp. in the presence of 0.5% DNA

<table>
<thead>
<tr>
<th>pH</th>
<th>DNA</th>
<th>Strain</th>
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<tr>
<td></td>
<td>WP-1</td>
<td>WP-22</td>
</tr>
<tr>
<td>5.5</td>
<td>Absent</td>
<td>++++</td>
</tr>
<tr>
<td>5.5</td>
<td>Present</td>
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</tr>
<tr>
<td>6.0</td>
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<td>6.0</td>
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<td>6.5</td>
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<tr>
<td>6.5</td>
<td>Present</td>
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</tr>
<tr>
<td>7.0</td>
<td>Absent</td>
<td>++++</td>
</tr>
<tr>
<td>7.0</td>
<td>Present</td>
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*Symbols: ++++, film of growth covers entire plate; +, a definite film of growth extends from the site of inoculation; —, growth only at the site of inoculation.*

the inhibition of swarming by the ultrafiltrate prepared from a solution of the DNA supported the conclusion that, in an acidic medium, hydrolysis products are liberated from the nucleic acid which interfere with the development of swarming.

With this indication that hydrolysis products of nucleic acid were responsible for the inhibitory effect, we determined the effect of purine and pyrimidine bases. In the BT agar, it was shown that the pyrimidines, thymine and cytosine, were without effect at 0.5%, but the purines, guanine and adenine, seemed to inhibit swarming. The evaluation of the effects of the purines was complicated because inhibitory levels of guanine, xanthine, hypoxanthine, and uric acid were difficult to attain because of the limited solubility of these compounds near neutrality. The defined medium (DM), previously described for studying swarming by Proteus sp. (3), was used because it proved more reliable than the BT agar for determination of the effects of purines and their derivatives. The minimal inhibitory concentration for adenine was 0.017 M (0.3%), and for aminophylline, a derivative of theophylline and ethylene-diamine, 0.004 M (0.18%). In this case, the pH of the medium exerted no effect on the ability of the bases to inhibit swarming. The deoxynucleotide of adenine effectively inhibited swarming at 0.04 M (1.0%), about twice th-

![Figure 1](http://aem.asm.org/)
amount necessary for the free base. Similarly, deoxyguanosine and deoxyguanylic acid inhibited swarming at 0.04 M (1.0%) and 0.04 M (1.39%), respectively.

The growth of *P. mirabilis* WP-1 in DM broth, determined by turbidimetry (Fig. 1), was inhibited by 0.025 M adenine and by 0.02 M aminophylline. In lower concentrations, the inhibitory effect of these two purine bases was less pronounced. A similar effect on the growth of *P. vulgaris* and other bacteria by purines had been observed by Simonetti and Terzani (6).

Inhibition of the growth of *Proteus* sp. by purine bases appears to account for the inhibition of swarming, which resulted from inclusion of 0.5% DNA in an acidic medium. It has been well established (7) that the purines of DNA are readily hydrolyzed from the sugar phosphate polymer to yield apurinic acid under conditions similar to those obtained in the preparation of the DNA-BT medium. The greater effectiveness of DNA compared with RNA also reflects the greater ease with which the DNA is hydrolyzed under acid conditions.

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**LITERATURE CITED**


