Swarming Phenomena, Basis of a Method to Identify Motile Bacteria Densitometrically

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Swarming Phenomenon, Coarse Classification, Rapid-Diagnostics

The swarming phenomenon of 18 strains was tested with the intention to identify motile, swarming bacteria. Not all bacteria which are motile in liquid are able to swarm on semisolid nutrient broth, but all swarming bacteria show characteristic swarming developments, resulting in different swarming zones which could be scanned densitometrically. The densitograms are fully reproducible for the same strain and show similarities between strains of one species.

Introduction

Numerous efforts have already been undertaken for the rapid diagnosis of microorganisms. To identify enterobacteriaceae, staphylococci, streptococci, anaerobic bacteria, pseudomonads, and fungi an increasing number of commercial test kits have been developed in the last years. These test kits compile special reactions on prefabricated, standardized test files (*e.g.* API-System, Micur-Ident.). One problem at present is the rather limited spectrum of microorganisms suitable for a test kit identification, so that more techniques are being included in new investigations to give the rapid-diagnostics a wider range of applicability and a higher rank. Methods like mass spectrometry [1, 9], laser-induced fluorescence [2], gas chromatography [7], and swarming inhibition [6] are already successfully used.

In this study swarming phenomena were investigated for their potential use in the identification of motile bacteria. Swarming is a kind of surface translocation produced through the action of flagella but is different from swimming, which only takes place when the film of surface fluid is sufficiently thick [3]. The swarming movement is a continuous process and partly dependent on cell to cell interactions. Most cells are aggregated in bundles during the movement. The different bacteria of this study showed differences in swarming behavior and in the pattern of turbidity of their swarming zones. These characteristics allow a coarse classification of motile bacteria in a rapid and simple way. Another application of these swarming phenomena may be to proof the stability of the strains, *e.g.* when a culture has been incubated or stored for a long period of time.

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Materials and Methods

Strains

Azospirillum brasilense (DSM 2297), *Bacillus circulans* (ATCC 4513), *Enterobacter aerogenes* (DSM 30053), *Escherichia coli B* (Institut für Therapeutische Biochemie, Frankfurt), *Escherichia coli K* (DSM 498), *Proteus mirabilis* (ATCC 27035), *Proteus vulgaris* (DSM 30113), *Pseudomonas acidovorans* (ATCC 15005), Pseudomonas aeruginosa (ATCC 17933), *Pseudomonas aureofaciens* (Rüttgerswerke, Castrop-Rauxel), *Pseudomonas diminuta* (ATCC 8545), *Salmonella typhimurium LT2* (ATCC 15277), *Salmonella typhimurium 7-73* (Institut für Mikrobiologie, Universität Hohenheim), *Salmonella typhimurium TA 1535* and *TA 1538* were kindly provided by B. N. Ames, *Serratia marcescens* (Institut für Tierhygiene, Universität Hohenheim), *Serratia marcescens Sm1* and *Sm6* (mutants of *Serratia marcescens*) were received from the culture collection of our institute.

Media

In all cases nutrient broth (NB) medium (pH 7.0) was used: nutrient broth (Oxoid), 10.0 g; yeast extract, 5.0 g; NaCl, 5.0 g; distilled water, 1000 ml. For the swarming assay 2.5 g agar (Oxoid) were added to the broth. Petri dishes, 90 mm in diameter, contained 10 ml of this swarming medium.

Swarming assay

The strains were cultured at 30 °C in a liquid NB-medium up to an optical density of 0.7 (absorbance 400–600 nm) equivalent to 5×10^8 colony-forming units (cfu)/ml. Five microlitres of the cell suspension were placed on the agar surface of the swarming medium. The inoculated plates were stored at 25 °C. After the spreading zone reached 30–35 mm in diameter, depending on the strain after 8–48 h, it was scanned in a microdensitometer (Automatic Recording Microdensitometer Mk III CS, Joyce Loebl, Gateshead, England, 16-objective). The densito-

grams show the density pattern as a function of the diameters of the spreading zone.

Swarming development

The swarming behavior of 9 strains was observed for 20 h at 25 °C. Every other hour the diameter of the swarming zone was measured.

Results

Five of the investigated 18 motile strains, namely *Bacillus circulans, E. coli B, E. coli K12, Ps. aeruginosa,* and *S. typhimurium TA 1538,* showed no swarming. The other 13 strains varied greatly in their characteristics of swarming, so that great differences in the densitograms of their spreading zones are observed (Fig. 1a–m). Some strains, *e.g. Proteus mirabilis,* show extremely low turbidity over the whole swarming area, but others like the pseudomonads have swarming zones of high density. Moreover, there are characteristic differences in the number of circles formed by zones of different bacterial density, which in the densitograms appear as peaks. Furthermore the exterior edges of the swarming area are characteristic for the different bacteria: *e.g. Serratia marcescens* (Fig. 1a) with a circle of marked density, or *Azospirillum brasilense* (Fig. 1k) with a circle of very low density in the most exterior part of the swarming zone. At *Serratia marcescens mutant Sm6* (Fig. 1c) the prodigiosin synthesis has started before the swarming zones were scanned, so a higher density could be found.

The swarming behavior of 9 strains was observed during 20 h at 25 °C. Again characteristics in swarming could be seen for each strain (Fig. 2): With the exception of *Azospirillum brasilense* which started swarming not before 24 h, all strains started swarming 4–5 h after inoculation with a great variance of swarming rate: In this study velocities of 6 mm/h, 3 mm/h, or 1 mm/h could be measured.

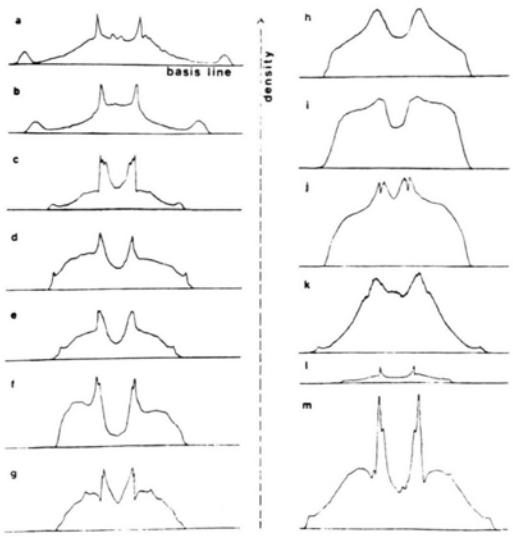

Fig. 1. Densitograms of the swarming zones of (a) *Serratia marcescens,* (b) and (c) its mutants *Sm1* and *Sm6,* (d) *Salmonella typhimurium LT2,* (e) and (f) its mutants *7-73* and *TA 1535,* (g) *Enterobacter aerogenes,* (h) *Pseudomonas acidovorans,* (i) *Pseudomonas aureofaciens,* (j) *Pseudomonas diminuta,* (k) *Azospirillum brasilense,* (l) *Proteus mirabilis,* (m) *Proteus vulgaris.* These densitograms were taken, when the diameters of the spreading zone amounted to about 30 mm. The basis line represents the density of the medium.

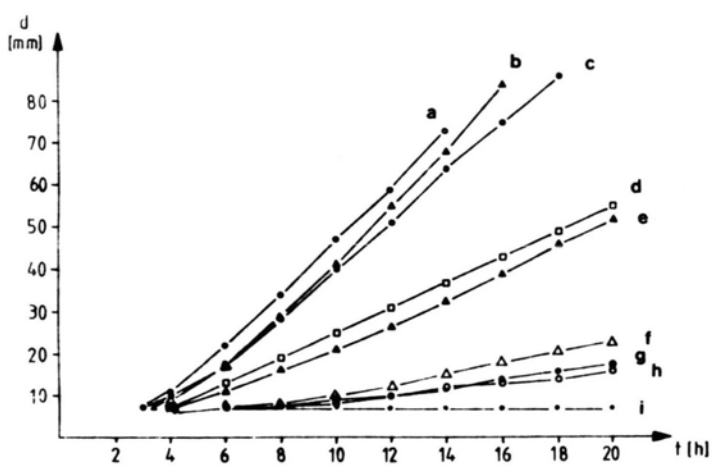

Fig. 2. Swarming rates of (a) *Serratia marcescens,* (b) its mutant *Sm1,* (c) *Salmonella typhimurium LT2,* (d) *Enterobacter aerogenes,* (e) *Salmonella typhimurium 7-73,* (f) *Proteus mirabilis,* (g) *Proteus vulgaris,* (h) *Serratia marcescens Sm6,* (i) *Azospirillum brasilense.*

The pattern of turbidity as well as the swarming velocity were temperature-dependent. Below 15 °C swarming is not really visible. With an increase of temperature up to 30–37 °C the swarming rate increased – depending on the strain used – and decreased at higher temperatures. The different swarming rates at different temperatures resulted in altered patterns of turbidity. At low temperatures a higher number of separate swarming circles could be seen. Medium, pH-value, and NaCl-concentration are other parameters affecting swarming activity [4, 8].

In order to use swarming behavior for identification of bacteria it is very important to work at strongly standardized conditions.

Discussion

The method described in this paper using densitograms for the determination of strain specific turbidity pattern is a simple, rapid, and fully reproducible test to differentiate between motile bacteria. Swarming velocity is an additional factor which can be used for pre-screening. Using defined conditions bacteria can be identified easily.

In general, strains of one species (*e.g.* strains of *Serratia marcescens* or *Salmonella typhimurium*) and strains of one genus (here: pseudomonads) show similarities in their densitograms.

Several Enterobacteriaceae were tested *(Salmonella, Proteus, Enterobacter)*, but no group characteristics could be found. The effect of mutations was investigated using *Serratia marcescens:* The mutants were selected for higher kojic acid sensitivity [5]. In this case the swarming attributes were changed simultaneously. Whereas mutant Sm1 resembled strongly its wild-type strain, mutant Sm6 differed greatly in its swarming behavior, even in its swarming velocity. Also the mutant 7-73 of *Salmonella typhimurium* showed modified swarming characteristics – *e.g.* slower swarming development – compared to its wild-type strain.

By densitograms of *Serratia marcescens* one more criterion has to be considered: 8 h after inoculation the synthesis of the bacterial dye, prodigiosin, started, and therefore higher density was measured.

Finally we can say, that the swarming characteristics offer a new reproducible and simple way for the identification or the identity verification of bacteria.

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