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# POLYMORPHISM OF A DEEP MARINE BENTHIC BACTERIUM FROM THE GULF OF MEXICO

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## ABSTRACT

An interesting bacterium (#271) was isolated from the abyssal plain of the Sigsbee Deep of the Gulf of Mexico. The organism exhibits marked polymorphism (baciloid, coryneform and myceloid morphologies) in response to certain cultural conditions. The organism has been observed undergoing reproduction by transverse fission, fragmentation and arthrospore production. The presence of arthrospores indicates the bacterium is a member of the genus *Arthrobacter;* however, computed affinity coefficients do not confirm this genus. Until further studies have been completed on this isolate the authors are reluctant to place it in a generic group.

## INTRODUCTION

A particularly interesting bacterium (#271) was isolated during our investigations of the deep benthic microflora from the abyssal plain of the Gulf of Mexico. This organism exhibits marked polymorphism in response to certain cultural conditions. The isolate was initially considered to be an impure culture composed of long myceloid filaments (0.75$\mu$ by about 75$\mu$) and medium-length rods (0.75$\mu$ by 3.5$\mu$). Repeated attempts to separate and isolate these two forms consistently met with failure.

We finally became convinced that the two morphologies were different phenotypic expressions of the same species of bacterium only after the liberation of the medium-length rods from the long myceloid filaments was microscopically observed. Subsequent observations revealed that these long filaments were fragments of still longer myceloid growths which may be attached to a single hold-fast organelle at the proximal end. One to six filaments have been observed attached to the same hold-fast. The peculiarities of isolate #271 were so fascinating to us that we were led to perform the present study.

The authors wish to express their deepest thanks to Dr. Bill Bryant and Dr. Arnold Bouma of Texas A&M University for their aid in procuring the core sample used in this study and to Mr. Phelps Brown for his illustrations.

## MATERIAL AND METHODS

Marine bacteria taken directly from oceanic biotopes require sea water for growth on initial isolation plates and transfers (Pequegnat et al., 1967; McLeod and Onofrey, 1956, 1963; Pratt and Austin, 1963; Taylor et al., 1960). In view of this requirement, all test media and procedures used in this study were either designed to be compatable with sea water or they were altered in such a way that sea water could be utilized. Standard bacteriological tests were used with as little modification as possible to permit a high degree of comparability of the data and previous work by other researchers.

### *Core handling*

Isolate #271 was obtained from a bottom sedi-

ment sample from station 5E (Latitude, 23°50'; Longitude, 92°26') at an oceanic depth of 1,835 fathoms (3,356 meters PDR accuracy). Bottom topography in this location is almost flat with the Sigsbee Knolls located nearby to the east-northeast.

The sediment sample was taken with a Phleger gravity corer. The corer was raised to the surface, the core liner removed and immediately capped, labeled and placed in a freezer. Care was taken to maintain the core in an upright position during all on-board processing to prevent disturbing the water-sediment interface. The approximate temperature of core 5E was inferred by the introduction of a temperature probe into a discarded core (10.5 °C). The frozen core was transported to the laboratory and processed.

***Sample processing***

The core was permitted to equilibrate at 10°C for two hours and was then extruded onto sterile aluminum foil. Outer surface sediments which might have been contaminated by the core liner were removed with a sterile spatula. A 1 cc sample was taken at a distance of 1 cm from the *in situ* water-sediment interface using a small volumetric piston-corer designed for this purpose. One cc samples were then aseptically transferred to a sterile blender containing 99 cc of sterile sea water (at less than 10°C) and blended at high speed for 30 seconds. One-half cc of the 1/100 dilution suspension was pipetted into each of three culture dishes and spread over the surface of the medium with a sterile glass rod.

The culture plates were incubated at 20°C. When growth occurred, colony counts were made* and cultures were picked for the primary purity streaks. Isolations were made by a modified tri-streak method. Following the third consecutive purity streak, the cultures were transferred onto agar slants and held as stock cultures. Stock cultures were checked for purity using the criteria of the Gram stain, morphology and growth characteristics.

***Colony characteristics***

Colony characteristics were determined using basal sea-water medium in plates, slants and broths. Incubation temperature was 22°C. The medium was prepared as follows: Trypticase (BBL), 2.0 g; Yeast extract, 1.0 g; Vitamine $B_{12}$, 1.0 $\mu g$; Aged filtered sea water, 1.0 liter; and Agar (if desired), 16.0 g. The *p*H was adjusted to 7.0 prior to autoclaving (Pfister and Burkholder, 1965).

The battery of cytochemical and biochemical tests enumerated in Table 1 of "Results" was then performed to provide a comprehensive description of this organism. The results represent data from a growth temperature of 22°C (studies are now under way to determine the effects of different growth temperatures on these tests, especially those temperatures which induce cytomorphological changes).

***Cytomorphology***

In view of the striking polymorphism of isolate #271 and the extreme variations between these different morphologies, we have limited our statements to those descriptions for which we have photographic evidence. The photomicrography utilized phasecontrast illumination, a 100X objective and 10X oculars. An additional 1.7X factor was introduced by the optics of the camera producing about 1,700X magnifications. True dimensions were obtained using a calibrated micrometer-eyepiece. These dimensions were recorded on the photomicrographs at appropriate locations.

***Taxonomy***

Affinity coefficients were computed where indicated for the possible introduction of isolate #271

* These data not reported herein.

into certain genus groups. The following equation defines the affinity coefficients:

%A = 100 (NSP + NSN/NSP + NSN + ND)

where %A is the affinity coefficient,

NSP is the number of positive characteristics shared by both species,

NSN is the number of features negative to both species,

and ND is the number of features positive to one species and negative to the other (Sokal and Michner, 1956).

***Temperature growth optimum***

Temperature opitmum for isolate #271 was determined by culturing the organism in basal seawater medium for 12 and 24 hours at the following temperatures: 0, 5, 9, 15, 18, 25, 27, 30, and 36°C. Growth was determined through measuring the absorbance of the cultures on a B&L Spectronic 20 at a wave length of 525 m$\mu$. Total occlusion was used to calibrate the Spectronic 20 for 100% absorbance. A tube of basal medium treated with two drops of 40% formaldehyde was used to calibrate for zero absorbance. Absorbance was considered to be directly proportional to cell count. The highest absorbance (59%) was shown by plate counts to be equal to approximately 5 X $10^9$ cells/ml. All tests were run in triplicate and good agreement was obtained between all readings. The maximum deviation from the graphic representation was $\pm$ 1.6%.

## RESULTS

***Cytomorphology***

Isolate #271 is a Gram-negative, straight (or occasionally curved) rod in type growth and measures 0.75$\mu$ in width and 1.8 to 3.5$\mu$ in length. The temperature growth range is between O° and 36°C. When grown between O° and 25°C, the rods usually occur as singles or diploform pairs that separate soon after cell division. Occasional small Gram-positive cocci (arthrospores) are also

**Fig. 1.** Isolate #271 in type growth. An arthrospore is shown at "A" and normal transverse fission is shown at "B".

present (Fig. 1). The vegetative cells show rapid linear motility with a slight wiggle motion produced by unipolar monotrichous normal flagellation (Leifson, 1959). Myceloid morphologies do not exhibit motility but release motile rods. Growth above 25°C produces a tendency for the formation of long uniform chains. Growth at temperatures above 18°C appears to sponsor the formation of larger coccoid cells (cystites). Cystites are formed at the end of myceloid filaments which coexist with the long uniform chains (Fig. 3).

With patient searching, the observer may find an intact hold-fast organelle with up to six myceloid filaments attached (Fig. 2). The frequency of hold-fast aggregate sightings is greatly increased by the introduction of a sterile glass slide into a growing culture and incubating for about 6 hours. The slide is then removed; a cover slip is placed on one side to form a modified wet mount and the other side is cleaned. Phase-contrast observation produced very good results (Fig. 2).

The organism has a single optimum growth temperature near 27°C. The phenomenon of optimum growth temperature is considered under "Discussion". Temperatures above 27°C produce a rapid drop in growth rate until at 36°C no detectible growth occurs in 24 hours incubation

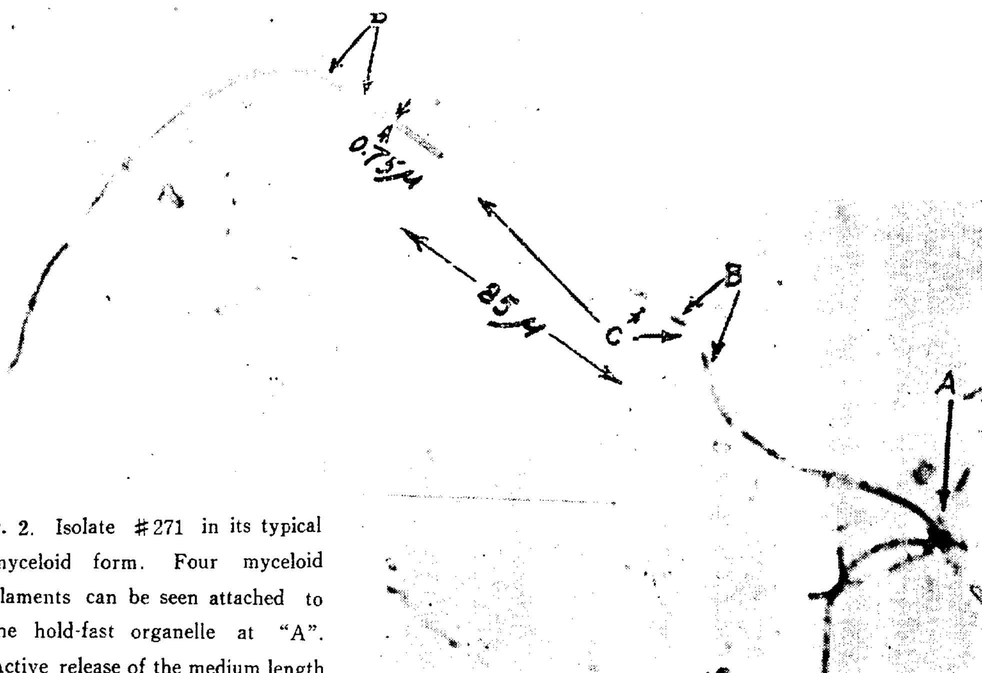


**Fig. 2.** Isolate #271 in its typical myceloid form. Four myceloid filaments can be seen attached to the hold-fast organelle at "A". Active release of the medium length rods from a filament is shown at "B" and a thin section of empty sheath is shown at "C". Septa can be seen at "D".

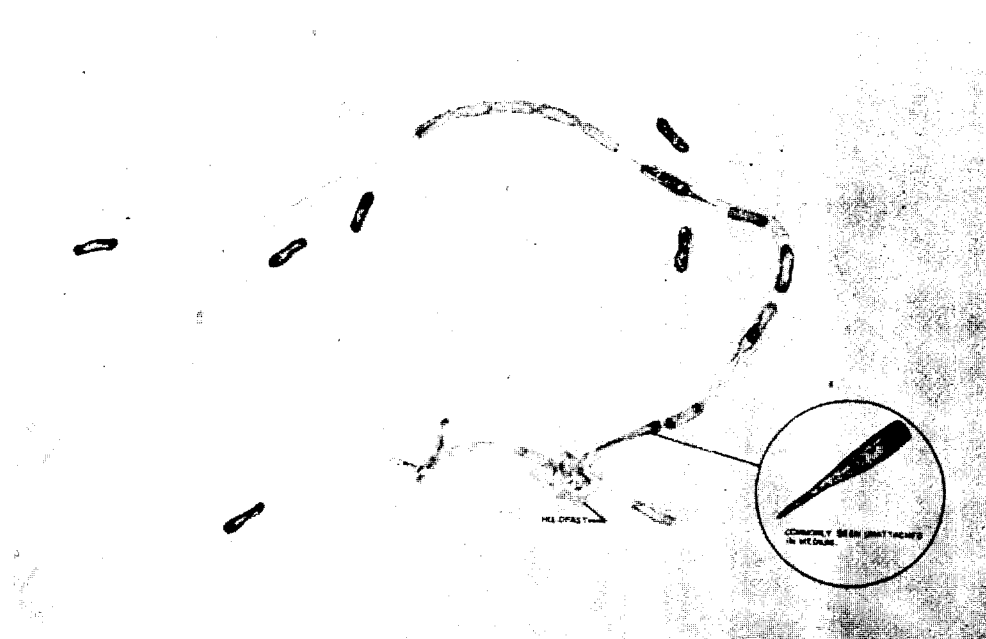

**Fig. 3.** An artist's conception of typical myceloid growth based on the microscopic field photographed in Fig. 2. This sketch clearly illustrates the process of cell release from the myceloid filaments as it has been observed microscopically. (Phelps Brown, artist)

time.

***Colony characteristics***

When grown on agar plates, isolate #271 produces medium-size colonies with a diameter of less than 1 mm. The colonies are circular with an entire edge and are slightly convex. Colonies are usually opaque and white. Agar slants produce a beaded growth pattern. Broth cultures become evenly turbid with the formation of a slight ring. Sea water is required for growth. Table 1 summarizes the cytochemical and biochemical properties of isolate #271.

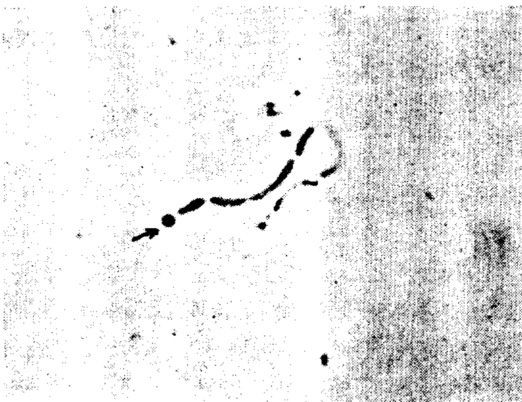

**Fig. 4.** The production of a cystite at the terminal end of a myceloid filament.

The results of the calculation of affinity coefficients for isolate #271 with selected bacteria from Bergey's Manual are presented in Table 2.

**TABLE 1.** Cytochemical and biochemical properties of isolate #271.

Staining reactions:
- Gram-negative rods
- Gram-positive arthrospores (after Hucker, 1922)
- Acid fast stain negative (Ziehl-Neelsen method)
- Lipid stain negative

Antibiotic sensitivities:
- Resistant to Bacitracin
- Resistant to Penicillin
- Resistant to Viomycin
- Sensitive to Novobiocin
- Sensitive to Oleandomycin
- Weakly sensitive to Tetracycline

Substrate utilization:
- Agar not digested
- Starch hydrolized (after Pfister and Burkholder, 1965)
- Gelatin liquefied (after Skerman, 1959)
- Citrate not utilized as carbon source (after Lyman and Fleming, 1940)
- Ammonium nitrate not reduced (after Pfister and Burkholder, 1965)
- Nitrate not reduced to nitrite (after *MMM*,* 1957)
- Monosaccharides not utilized aerobically or anaerobically (glucose, fructose, mannose and xylose tested after Skerman, 1959)
- Urea utilized
- $NH_3$ produced from basal agar
- Tributyrin and triolein not utilized (after Tendler and Burkholder, 1960)

Cytochemical reactions:
- Catalase negative to weakly positive
- Cytochrome oxidase positive (after Gaby and Free, 1958)
- Kovacs oxidase reaction positive (after Kovacs, 1956)
- Voges-Proskauer negative = no acetyl-methyl-carbinol produced (after Skerman, 1959)
- Indole negative (after Skerman, 1959)
- Methyl-red reaction negative (after *MMM*, 1957)

* Manual of Microbial Methods.

**TABLE 2.** Affinity coefficients between isolate #271 and other members of Arthrobacter and one species of Pseudomonas as listed in Bergey's Manual (1957).

| Bergey's Species Number | Genus and Species | Affinity Coefficient in %*A* |
|---|---|---|
| *Arthrobacter sp.* | | |
| 1 | *Arthrobacter globiformis* | 65.0 |
| 2 | *Arthrobacter pascens* | 60.0 |
| 3 | *Arthrobacter simplex* | 60.0 |
| 4 | *Arthrobacter oxydans* | 62.5 |
| 5 | *Arthrobacter aurescens* | 65.5 |
| 6 | *Arthrobacter ureafaciens* | 62.5 |
| 7 | *Arthrobacter tumescens* | 67.5 |
| 8 | *Arthrobacter citreus* | 72.5 |
| 9 | *Arthrobacter terregens* | 70.0 |
| *Pseudomonas sp.* | | |
| 53 | *Pseudomonas membranoformis* | 66.7 |

## DISCUSSION

Isolate #271 exhibits two distinct reproduction modes, the most common form being by transverse fission as seen in Fig. 1. The cells shown in this figure are not only produced by the transverse fission of the medium-length rods but have also

been microscopically observed emerging from the second morphological (myceloid) type.

The second reproductive mode entails the growth of long (85μ to 100μ), thin (0.75μ) myceloid filaments which may be attached to a hold-fast (Fig 2 and 3). Septa are formed within the strands at regular intervals of two to three microns (2μ to 3μ) beginning at the hold-fast and proceeding distally. The covering of the myceloid strand then splits longitudinally and the rapidly motile, medium-length rods are released (Fig 2 and 3). Active release of these cells is shown at "B" in the above figures. A thin portion of the empty myceloid sheath is shown at "C" and newly-formd septa are shown at "D".

Under certain growth-temperature conditions, different morphological expressions of the bacterium tend to be predominant in the culture. The medium-length cells become pleomorphic and produce arthrospores (small coccoid cells) by fragmentation at temperatures from 10° to 25°C. The larger coccoid cells (cystites) shown in Fig. 4 are produced from the myceloid cells incubated at temperatures above 25°C. Although the production of the myceloid morphology and hold-fast organelle have not yet been microscopically observed, the coexistence of the myceloid morphology, arthrospores and cystites in certain cultures introduces a strong possibility that the hold-fast and myceloid growths may arise as an outgrowth from the arthrospores or cystites. We have presented data that indicate that the cystites can be produced from the myceloid filaments (Fig. 4). Consequently we believe that the cystites are produced by the myceloid cells rather than giving rise to them. If this relationship is indeed the case, the implication is that there is a distinct cyclic nature to the growth of this culture. In this way the organism resembles the soil bacterium *Arthrobacter* described by Conn and Dimmick (1947).

The term *Arthrobacter* means a jointed rod. Conn and Dimmick (1947) place this group of organisms as intermediates between the true bacteria and true actinomycetes. The genus *Arthrobacter* has been used to designate all rod-shaped bacteria which produce "arthrospores".

*Arthrobacter* forms resemble actinomycetes only in their occasional production of short mycelia. In type growth, *Arthrobacters* are like ordinary bacteria. The members of the genus are generally nonmotile; however, isolate #271 exhibits rapid linear motility with a slight wiggle motion. Cells of a given culture of *Arthrobacter* may assume the following morphologies: 1) Gram-negative straight or curved rods, 2) Gram-negative to Gram-positive coccoid cells (arthrospores), 3) larger coccoid cells (cystites) or 4) short filaments with rudimentary budding (Bergey's Manual, 1957).

Dr. J. M. Sieburth (1964; personal communication, 4 January 1968) describes the marine *Arthrobacters* as Gram-negative pleomorphic organisms which, with certain nutrient additives or at certain growth temperatures, may form Gram-positive uniform rods which break up to produce Gram-positive cocci. Dr. Sieburth also comments that there are a number of characteristic bizarre forms.

If one accepts the hypothesis expressed by Conn and Dimmick (1947) and Bergey's Manual (1957) that the genus *Arthrobacter* applies to all bacteria which produce arthrospores, then our isolate #271 is indeed a member of this genus. However, when the affinity coefficients for isolate #271 and the other members of *Arthrobacter* listed in Bergey's Manual (1957) were computed, the range of similarity between #271 and the listed members of the genus ranged from 60.0% to 72.5%. This correlation is not high enough to justify the inclusion of isolate #271 in the genus *Arthrobacter*.

Isolate #271 exhibits a positive Kovacs oxidase test reaction. This fact led us to suspect that the genus *Pseudomonas* might be more appr-

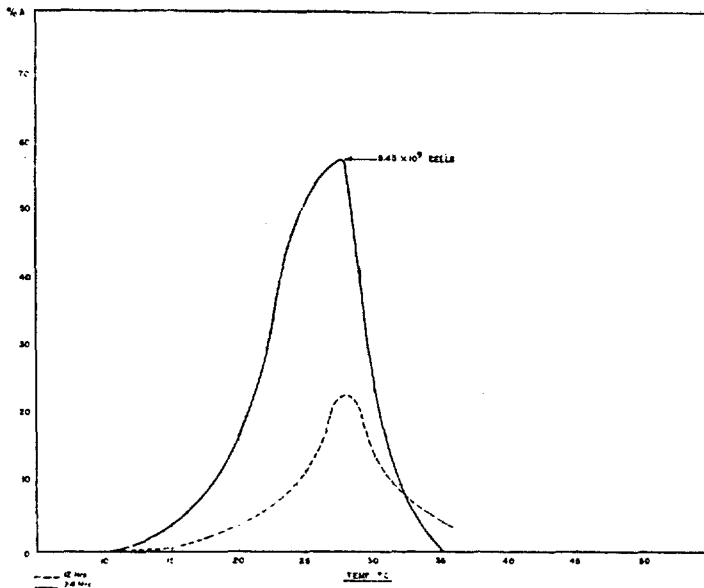

**Fig. 5.** Optimum growth temperature curve of isolate #271. Data from both 12 and 24 hour cultures indicate a single temperature growth optimum at 27°C.

opriate. The highest affinity coefficient to be found in this genus was 66.7% with *Pseudomonas membranoformis*. This low correlation between isolate #271 and members of the genus *Pseudomonas* can not justify its inclusion into the genus without excessive overweighting of the Kovacs reaction.

According to the studies of Drost-Hansen (1956), Oppenheimer and Drost-Hansen (1960), and Sieburth (1964) the properties of water at 0°, 9°, 18°, 27°, and 36°C do not favor growth optima. Indeed, results from other cultures in our laboratory have repeatedly confirmed their findings. We do not have an explanation for the peculiar optimum growth temperature for isolate #271 at 27°C; however, the fact that this culture fails to conform makes it a useful tool for the study of optimum growth temperatures. The optimum growth temperature curve is presented in Fig. 5.

At this writing we are undecided about where to place our isolate. The unusual peculiarities of this culture justify the extensive cytological and physiological investigation contained herein. It is hoped that future studies of this isolate will reveal some of the physiological changes which accompany polymorphism in response to different growth temperatures.

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