

percent saline. It must be mentioned at this point that saline is not specific and that equivalent osmotic quantities of other salts, such as potassium or even glucose, have a similar effect. It has been observed that juices from a leaf that has contained an insect for 48 hours will, if instilled in another leaf, cause spontaneous electrical activity and closure of the second leaf. Also, leaves which contain insects in the process of being digested show spontaneous electrical activity.

The loss of weight of the leaf when it is immersed in 3-percent saline may perhaps be explained by osmotic water loss. Or it may result from secretion of fluid and digestive enzymes, a process

initiated by the electrical activity associated with closure of the leaf. This remains to be determined. The mechanism of closure may or may not be associated with the loss of turgor.

N. MALCOLM BALOTIN

JOSEPH R. DIPALMA

Department of Pharmacology,
Hahnemann Medical College and
Hospital, Philadelphia, Pennsylvania

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Marine Bacteria with Antiyeast Activity

Abstract. Various marine substrates were examined quantitatively and qualitatively for marine bacteria. Of 132 isolates, 20 (six genera) showed some degree of inhibitory activity against 12 assay microorganisms. Inhibition was most frequent and most pronounced against terrestrial and marine-occurring yeasts.

Whereas studies have been made of the occurrence of antibacterial activity in marine microorganisms (1) little, if any, attention has been given to the existence of corresponding antiyeast (antifungal) activity in the ocean. The occurrence of an often abundant and diverse marine mycota, including yeasts and filamentous fungi, has been well demonstrated in recent years (2). In conjunction with our mycological investigations, a marine bacterium exhibiting marked specific antiyeast activity, both live and in cell-free filtrates, has been isolated (3). From these observations, recent studies were undertaken to establish the extent and distribution of such marine bacteria.

Bacteria were isolated from sea water, sediments, macro-algae, marine grass, and invertebrates, all collected from Biscayne Bay, Florida, between Key Biscayne and Soldiers Key, and off Bimini, the Bahamas. Samples were taken aseptically and homogenized in whole or part when necessary. Suitable dilutions were made with sterile sea water and spreadplates (4) were made on medium number three of Carlucci and Pramer (CP) (5) and incubated at 25°C for 48 to 72 hours. After the number of colonies had been counted, both predominant (in terms of numbers) and randomly selected colonies were transferred to agar slants of the sea water isolation medium used above.

Pure cultures of the bacterial isolates were tested for antimicrobial activity

against the following assay organisms: *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Rhodotorula (texensis) minuta* FY-75, *Candida albicans* UM-376, and a parent strain of *Cryptococcus neoformans* Y-2535.

Trypticase-soy agar (6) prepared in distilled water, to which 1.0 percent NaCl was added was used for examination of activity against the assay bacteria, while a sea-water medium, M-6 agar (2 percent glucose, 2.3 percent Bacto-Nutrient Agar, 0.1 percent yeast extract), was used in the testing of antiyeast activity. The two assay media supported growth of both the marine bacteria examined and the test organisms.

Activity was established initially by the cellulose disc method. The sterile disc was placed on an agar medium whose surface was inoculated with the test organism, and one to two drops of a 48-hour unfiltered broth culture of the tested marine bacterium were pipetted onto the disc. This technique allowed growth of the marine bacterium on the cellulose disc at the time of growth of the test organism. Antimicrobial activity was assessed by detecting growth inhibition or zones of clearing around the disc. Bacteria that showed inhibitory activity by this initial screening method were grown in CP broth for 48 hours; the cultures were shaken during this interval. Extracts

were obtained by centrifugation followed by filtration of the broth supernatant through membrane filters of 0.2 μ porosity (7). The cell-free filtrates were assayed by the cylinder (well) technique.

Quantitative data on the various marine materials are given in Table 1. A total of 132 strains were isolated and tested, and of these there were 20 strains of bacteria that exhibited antimicrobial properties, when tested by the disc technique. While none of the cell-free filtrates from the 20 isolates tested showed activity under our assay conditions, other studies of cell-free filtrates of marine bacteria with antimycotic activity have given positive results.

The 20 isolates have been tentatively identified, according to the method of Cleverdon, Leifson and Murchelano (8), as representatives of *Chromobacterium*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Flavobacterium*, and *Alcaligenes*. Of the 20 isolates, 15 (75 percent) were characterized by specific antiyeast activity, four inhibited only bacteria, and one showed inhibition of both bacteria and yeasts. Among the individual marine substrates examined, correlations were not noted between the total number of isolates and the number of inhibitory bacteria in each sample. However, it appeared that the isolates from algae were most frequently strains with antimycotic activity.

Table 1. The relation of the marine bacteria isolated to the number showing antimycotic activity. For the invertebrates more specific identification is given as follows: sponge, *Chondrilla* sp.; jelly fish, *Cassiopeia* sp.; sea cucumber, *Holothuria* sp.; starfish, *Asteroides* sp.

Source	No. of organisms per g or per ml	No. of cultures isolated	No. of inhibitory isolates
Seawater	120 $\times$ 10 ²	13	5
Sediment	140 $\times$ 10 ⁵	12	0
Grass			
<i>Thalassia</i> sp.	470 $\times$ 10 ²	10	1
Algae, Chlorophyta			
<i>Ulva</i> sp.	330 $\times$ 10 ³	4	1
<i>Rhizoclonium</i> sp.	140 $\times$ 10 ²	7	0
<i>Caulerpa</i> sp.	600 $\times$ 10 ⁵	10	5
<i>Batophora</i> sp.	200 $\times$ 10 ⁷	3	0
<i>Udotea</i> sp.	95 $\times$ 10 ²	7	0
<i>Enteromorpha</i> sp.	600	2	0
<i>Cladophoropsis</i> sp.	80 $\times$ 10 ²	4	0
Algae, Phaeophyta			
<i>Sargassum</i> sp.	530 $\times$ 10 ²	6	0
<i>Dictyota</i> sp.	410 $\times$ 10 ³	8	3
Algae, Rhodophyta			
<i>Hypnea</i> sp.	540 $\times$ 10 ³	3	0
<i>Gracilaria</i> sp.	250	1	0
<i>Dasya</i> sp.	410 $\times$ 10 ²	3	1
<i>Centroceras</i> sp.	800 $\times$ 10 ⁴	23	3
Invertebrates			
Sponge	20	2	1
Jellyfish	20 $\times$ 10 ²	3	0
Plankton suspension	100 $\times$ 10 ⁸	3	0
Sea cucumber (gut)	120 $\times$ 10 ³	6	0
Starfish (gut)	140 $\times$ 10 ⁶	2	0

Among the assay microorganisms, two species of yeasts, *S. cerevisiae* and *R. minuta*, were inhibited most often by 12 and 9 isolates, respectively. Against the other assay organisms the number of bacteria showing inhibition were as follows: *B. megaterium*, 2; *S. aureus*, 2; *E. coli*, 3; *P. aeruginosa*, 0; *C. albicans*, 7; and *C. neoformans*, 7. With the exception of *C. albicans*, the extent of inhibition of the assay yeasts was greater than that observed for the test bacteria, that is, <5 mm radial cleared zone for the latter organisms compared with radial zones of 5 to 10 mm, or more, for the assay yeasts. Approximately equal degrees of inhibition were observed for *S. cerevisiae*, *R. minuta*, and *C. neoformans*.

It is apparent from this preliminary work that bacteria with antiyeast (competitive?) properties may be encountered frequently in the marine environment. It is not known whether the inhibition observed is a result of competition for nutrients, physicochemical factors (pH, redox potential, etc.), or the actual elaboration of specific antagonistic substances, as has been shown in other investigations (3). Nevertheless, to assess adequately the diversity of marine microbial activity, investigations of antibiosis in the sea should incorporate appropriate species of yeasts into basic screening programs (9).

JOHN D. BUCK

SAMUEL P. MEYERS

KATHERINE M. KAMP

*Institute of Marine Science,
University of Miami, Miami, Florida*

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Moon Illusion: An Observation

Abstract. Size comparisons of the moon are made from different locations by direct viewing (as opposed to comparisons by instrumental techniques). Under the proper conditions, the illusion is seen while the moon's position remains essentially unaltered. By this means, evidence is adduced in favor of Ptolemy's apparent-distance hypothesis.

The illusion of change of lunar size yielded by direct observational comparison ordinarily requires a considerable waiting period during the moon's ascent from, or descent toward, the horizon. A simultaneous size comparison of the real or artificial horizon moon versus the elevated moon is made possible only by mirror arrangements, as in the extensive experiments of Holway and Boring (1) and of Kaufman and Rock (2).

Where the terrain is suitable, however, the observer's movement may substitute for the moon's movement. The illusion is then visible during certain months by direct observation of the moon at or near the full, at a relatively constant celestial elevation and with no significant time lapse.

A particular street in the Borough of Queens, New York City, slopes about 5°. With the slope behind him, the observer, at the summit of the slope, can view the moon before him against an expanse of open sky about 40 minutes after moonrise, at an elevation of about 10°. By this time the moon appears diminished in size compared to the horizon moon. From a point some 200 yards downslope the moon is seen with intervening landscape and with its lower rim touching the tops of trees and low buildings. By visual estimate, its diameter is now 1¼ to 1½ times as large as when seen from the summit. The observer can move back and forth along the slope several times and view these changes alternately for 10 or 15 minutes until the illusion disappears after the moon has risen too high. Since the apparent path of the moon through the sky exhibits periodic variations, the illusion is not visible every month. For example, on 21 May 1962 (moonrise 10:18 P.M., EDT; 2 days past the full) the foregoing observations were made near 11:00 P.M., EDT. The illusion was seen again in July and September; in October it was no longer visible. No observations were made in June and August.

The hypotheses concerning the moon illusion, or suggestions as to relevant

factors involved, may be summarized as follows:

1) The illusion depends, in some unestablished manner, upon the position of the eyes within the head. The horizon moon is seen with eyes level and the elevated moon with eyes raised (Holway and Boring's angle of regard hypothesis; see 1-4).

2) It depends upon the brightness of the image on the retina. The horizon moon appears fainter and, therefore, larger than the moon in elevation (Bishop Berkeley's hypothesis; see 2, 4, 5; 6, p. 361).

3) It is due to differences in light refraction based upon differences in the angle of incidence to the earth's atmosphere (see 2; 6, p. 360).

4) Factors such as gravity, or the redder and therefore larger appearance of the horizon moon, may be pertinent (2).

5) It is a consequence of the measure of great distance conveyed by the terrain in viewing the horizon moon (Ptolemy's apparent distance hypothesis; see 2; 6, pp. 290, 360; 7).

Under the described conditions of observation, the angle of regard, brightness, elevation, orientation with respect to gravity, and color are constant. Accordingly, this would appear to render untenable the first four considerations enumerated above, while lending support to the fifth.

In connection with Ptolemy's hypothesis, it may be of interest to note that the horizon is about 3 miles distant for a man standing on a level plain with unobstructed view; Kaufman and Rock, in one set of experiments, worked with a horizon about 700 yards distant; in the present description, the horizon is effectively only about 200 yards from the bottom of the slope. Clearly, the illusion can occur even when the intervening distance is much less than the usual horizon distance (8).

GILBERT GOLDSTEIN

*Department of Chemistry,
Mount Sinai Hospital, New York 29*

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