References and Notes

1. I am indebted to the Brush Beryllium Company for the contribution of their facilities; to Leonard F. Bunck, supervisor of the company's spectrographic and x-ray laboratory at Elmore, Ohio, who developed the methods and made the analyses; to Keith Wikle, manager of the metallurgy division, and William N. Ling, who undertook the metallographic studies and made the hardness and size measurements; and to Tom Davidson for the photograph.
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Spontaneous Electrical Activity of Dionaea muscipula

Abstract. Instillation of four to six drops of 3-percent saline in the leaf trap of *Dionaea muscipula* Ellis causes the appearance of a series of spontaneous action potentials. Mechanical stimulation of the sensitive leaf hair elicits only a single response. Immersion in 3-percent, but not in 1-percent, saline effects a loss of weight in *Dionaea* but not in the leaves of gardenia or geranium.

Dionaea muscipula Ellis, the Venus flytrap, contracts upon stimulation by mechanical, electrical, or chemical means (*1*). The action potential elicited is believed essential for the occurrence of this phenomenon and has been studied in detail (*2–4*). The mechanism of closure has been linked to a loss of turgor in the leaf trap upon stimulation of the sensitive hairs or stimulation of nearby areas (*2*) and linked more specifically to a sudden reduction in the hydrostatic pressure of the cells of the inner epidermis through depolarization (*5*). The study reported here concerned the discovery that saline solution in relatively low concentrations causes spontaneous electrical activity and leaf closure. An attempt was made to relate these findings to permeability changes in the leaf.

Table 1. Induction of spontaneous action potentials in *Dionaea muscipula* by instillation of saline. Since great variability of spontaneous activity was observed, an arbitrary system of grading was used to assign a value to the response, as follows: 0, No electrical activity elicited; 1, a single large action potential or a series of at least three action potentials; 2, a series of more than three action potentials of the magnitude of those represented in Fig. 1.

Leaves (*N*)	Mean activity	S.D.	S.E.	*p*
		Distilled water		
16	0.31	±0.58	±0.14	
		Saline, 1 percent		
45	.48	±.80	±.12	>.5
		Saline, 3 percent		
49	1.45	±1.11	±.16	<.01

The action potential was recorded by a technique previously reported (*4*). In brief, brush electrodes are applied to the outer surface of the leaf blades, and a direct-current amplifier and oscilloscope, with oscillograph, complete the circuit. Fresh adult leaves maintained in a terrarium under 24-hour fluorescent lighting were used. No spontaneous activity was observed in the resting leaves. Instillation of four drops (0.26 ml) of distilled water or of 1-percent saline into the trap effected an occasional response. Instillation of 3-percent saline in the same amount caused most of the leaves to demonstrate electrical activity and to close tightly. The electrical activity consisted of a series of action potentials, as previously described for activity elicited by mechanical touching of the sensitive leaf hair (*4*).

A typical record is shown in Fig. 1. The amplitude was 20 to 30 mvolt of 2- to 4-second duration. There was great variability in frequency and duration. Generally, the pulse frequency of spontaneous potentials was 2 to 4 pulses per minute, and the total duration was from 1 minute to several hours. There was a distinct tendency for the frequency to slow with time. Table 1 gives the system for grading the responses, together with data for a large number of leaves. The *p* values indicate that the effect of 1-percent saline was not very different from that of distilled water but that 3-percent saline was decidedly stimulatory.

In another experiment 1-percent and 3-percent saline were demonstrated to have opposite effects on leaf weight. Fresh *Dionaea* leaves were weighed and immediately immersed in saline solution for periods of time varying from 1 to 60 minutes. After exposure the leaves were carefully blotted and reweighed. As may be seen in Fig. 2, the *Dionaea* leaf immersed in the 1-percent saline always gained weight, unless exposure was very long; on the other hand, the leaf immersed in 3-percent saline always lost weight. Different results were obtained in two varieties of common leaves, *Gardenia jasminodes* and *Pelargonium* (geranium), chosen because of their contrasting textures. Both of these leaves gained weight on exposure to 3-percent saline for periods up to 10 minutes. After 15 minutes, geranium leaves showed a loss in weight.

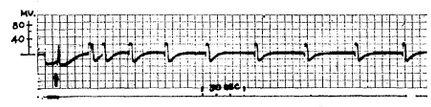

Fig. 1. Spontaneous activity induced in the leaf of *Dionaea muscipula* by the instillation of four to six drops of 3-percent saline.

The significance of the spontaneous electrical activity induced by exposure to 3-percent saline may be deduced from previous observations of the *Dionaea* leaf closure. In hundreds of experiments, no leaf ever closed without electrical activity. The more times the sensitive leaf hair is mechanically stimulated, the tighter the leaf closes. If no insect is in the trap, the leaf opens in 5 to 10 hours. By contrast, if there is an insect or a bit of meat in the trap, closure lasts for 10 or more days. When an insect is first caught, its struggles to escape produce mechanical stimulation of the sensitive hairs, but the tightening closure of the leaf soon causes the insect to die. Some mechanism must exist to keep the leaf closed beyond this initial period. It is suggested that the juices of the insect, which either are expressed by the compression of the leaf or exude during the struggle, have an action similar to that of 3-

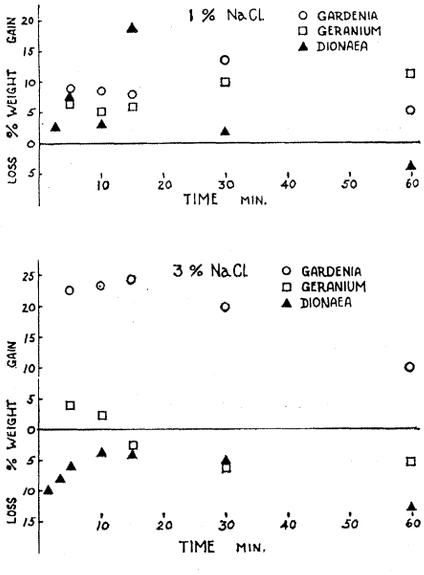

Fig. 2. Comparison of weight changes in leaves of *Dionaea*, gardenia, and geranium after immersion in 1-percent and in 3-percent saline for varying periods.

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.

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26 September 1962

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 cereviseae, 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		
	120×10^2	13	5
	Sediment		
	140×10^5	12	0
	Grass		
Thalassia sp.	470×10^2	10	1
	Algae, Chlorophyta		
Ulva sp.	330×10^3	4	1
Rhizoclonium sp.	140×10^2	7	0
Caulerpa sp.	600×10^5	10	5
Batophora sp.	200×10^7	3	0
Udotea sp.	95×10^2	7	0
Enteromorpha sp.	600	2	0
Cladophoropsis sp.	80×10^2	4	0
	Algae, Phaeophyta		
Sargassum sp.	530×10^2	6	0
Dictyota sp.	410×10^3	8	3
	Algae, Rhodophyta		
Hypnea sp.	540×10^3	3	0
Gracilaria sp.	250	1	0
Dasya sp.	410×10^2	3	1
Centroceras sp.	800×10^4	23	3
	Invertebrates		
Sponge	20	2	1
Jellyfish	20×10^2	3	0
Plankton suspension	100×10^8	3	0
Sea cucumber (gut)	120×10^3	6	0
Starfish (gut)	140×10^6	2	0