hardened, a more permanent attachment to the carapace was made with Eastman 910 adhesive. An indifferent electrode was placed in the water.

When first placed in the test tank, the lobster's heart rate was always high (100–125 beats per minute, b/m). They were given 20 min to an hour to reach a steady state before testing was begun. Most testing was done with heart rates of 40 to 70 b/m although a few thresholds were determined using lower or higher rates. In 2 animals the audiograms were not completed, one because the rate became too slow and irregular and the other because a low ECG signal was masked by noise.

LARIMER[8], investigating heart rate responses in crayfish, showed that many external stimuli caused the animal to exhibit bradycardia (slowing of the heart rate). In my experiments the lobsters were shielded from most of these stimuli although occasional noises resulted in bradycardia. These were not common and within a short time (usually less than 30 sec) a more regular heart rate would be reached. Testing on any day was often terminated due to the heart rate becoming irregular.

Training was usually started with the conditional stimulus (CS) set at 37.5 Hz with pressure between 20 and 29 db. The CS lasted 10–11 sec. For the last 0.14 sec of the CS the unconditional stimulus (US) was applied. The US consisted of an AC current regulated by a variable transformer and administered through clips attached to the last pair of walking legs. Levels of US between 10 and 55 volts were used but 15–20 volts (about 0.15 to 0.2 amps) were found to be most effective.

3 of 7 animals showed bradycardia at the first CS presentation. One animal habituated to this response (no US given) in 8 trials. In all animals strong bradycardia or conditional response (CR) appeared within 4 reinforced trials. At high intensities the CR was as great as 20–30 b/m. However, this response was graded and near threshold the amount of change usually became less. Figure 1 is a polygraph record of 4 tests made near threshold and lasting about 7 min. 2 tests resulted in very distinct CR's. On some trials close to the threshold level tachycardia (increased rate) was observed. This was not considered as a CR, however if it was very strong (i.e. greater than about 7 b/m), the test level was repeated. The second trial in Figure 1 shows an example of this type of response.

Training and testing were similar to that used for fish[6]. The response threshold was determined using an up-and-down procedure with 2 db changes of the stimulus. At least 9 crossings or reversals of the CS amplitude were used for each threshold determination. Threshold values obtained early in training were always found to be higher than during later tests. Additional training, particularly at a second frequency, resulted in a lowered threshold (plateau effect). This was often observed during one testing session as a precipitous drop of about 10 db to a lower threshold level.

Frequencies tested were 10 Hz and at octave intervals from 18.7 to 150 Hz. Figures 2, a and b show the lowest thresholds for 5 animals relative to pressure and particle velocity respectively. Removal of the second antennae and chelipeds in one animal did not change its response level. There is reasonable agreement among 4 of the lobsters but the fifth one had a higher threshold in the more sensitive region for particle velocity. This animal was being retested when it molted. It may have had a higher threshold during premolt or it may have been less responsive to conditioning.

The 4 other animals showed the lowest threshold at 37.5 Hz for pressure (mean $-13$ db) and at 75 Hz for particle velocity (mean 19 db). It is not known which sound component acts as the stimulus, but it is believed that hair cells on the surface of fish are responsive to particle velocity or displacement (both vector quantities)[9].

Hair-fan organs, observed on various parts of the body and appendages, can probably respond to an adequate acoustic stimulus. Using a falling drop of water, LAVERACK[2] calculated the pressure to be 0.40 dyne/cm$^2$ (e.g. $-8$ db) at the physiological threshold of the hair-fan organ. This value is remarkably close to the threshold of pressure found in the present study (e.g. $-13$ db at 37.5 Hz). LAVERACK did not measure particle velocity.

COHEN[3], using tuning forks of 128–320 Hz, found that water-borne vibrations were ineffective. These frequencies are above the sensitive region found in this study and may account for the lack of response. The statocyst and other hair cells, as on the antenna, may also be responsive to acoustic stimuli. It may be that the perception of acoustic stimuli is not a simple process and multiple receptors may be the basis of the observed plateau effect as has been shown for fish[6].

*Résumé.* Pour le stimulant acoustique entre 10 et 150 Hz les seuils de réponses ont été obtenus sur 5 homards (*Homarus americanus*). La plus grande sensibilité était de 37,5 Hz pour une pression de $-13$ db re: 1 bar, ou bien de 75 Hz pour une rapidité de particules de 19 db re: 1 var, conditions classiques réalisées pour obtenir en bradycardie un si haut seuil tonal de stimulation.

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## Fungistatic Action of the Pigment Secreted by the Fungus *Epicoccum nigrum* Link

It was discovered by chance that when *Epicoccum purpurascens* and *Helminthosporum sativum* were grown side by side on agar media, the former fungus elaborated a diffusible substance which inhibited the hypal growth of the latter fungus. BAMFORD et al.[1] confirmed the production of an anti-fungal anti-biotic by *E. purpurascens* and by *E. andropogonis*.

The present investigation was carried out to determine whether the pigment secreted by *E. nigrum* (Strain 5-1-3) possessed any such anti-fungal effect.

*Materials and methods.* The microorganism used was Epicoccum nigrum (Strain 5-1-3)[2]. The inoculum consisted of uniform suspension of spores and mycelial fragments. Both Czapek-Dox glucose medium[3] and germination medium[4] were used for growing the fungus.

The fungus was grown as surface mat on shallow layers of liquid medium. Usually 1 ml of inoculum was used per 20 ml of a culture medium in a 200 ml conical flask. The flasks were incubated at 25 °C for 5 days exposed to 50 foot candles of light. The source of light was the daylight fluorescence electric tubes TL 80 W/55, 5′ suspended at a suitable distance above the culture vessels.

At the end of the incubation period, the mycelial felt was separated from the culture fluid by filtration or centrifugation, washed and dried to constant weight at 80 °C. Growth was expressed in terms of dry weight of mycelium.

Table I. Fungistatic action of the pigment

| Concentration of crude pigment | Growth of *Botrytis* in mg dry mycelium per flask | Inhibition (%) | Growth of *E. nigrum* in mg dry mycelium per flask | Inhibition (%) |
|---|---|---|---|---|
| 5 | 54 | 82 | 326 | 1 |
| 2.5 | 61 | 79 | 340 | 0.07 |
| 1.25 | 101 | 50 | 359 | 0.01 |
| 0.625 | 268 | 1 | 371 | −0.025 |
| 0.313 | 312 | −0.1 | 383 | −0.06 |
| 0 | 279 | 0 | 362 | 0 |

Table II. The effect of pH on the fungistatic action of the pigment (1.25% w/v) on *B. allii*

| Initial pH | Final pH | Growth in control flasks mg dry mycelium per flask | Growth in test flasks mg dry mycelium per flask | Inhibition of growth (%) |
|---|---|---|---|---|
| 2.8 | 3.22 | 87.4 | – | 90–100 |
| 3.4 | 3.83 | 131.8 | 71.5 | 66.0 |
| 4.0 | 4.46 | 137.0 | 63.2 | 54.0 |
| 5.0 | 4.95 | 245.6 | 203.4 | 17.2 |
| 6.2 | 6.0 | 256.3 | 245.1 | 4.4 |
| 7.2 | 6.24 | 161.5 | 256.2 | 2.0 |
| 8.0 | 6.87 | 101.2 | 102.4 | − 1.1 |
| 9.0 | 7.88 | 55.7 | 61.6 | −10.0 |

The pigment was extracted from the medium using diethylether after acidification with concentrated hydrochloric acid to pH 2.6. The ether extracts were collected together, and concentrated over a water-bath. The solvent was then filtered off and further concentrated and then allowed to cool. The pigment separated out as golden yellow crystals. The crystals were washed, dried, weighed, dissolved in water and tested for fungistatic action. The effect of a given amount of the pigment on mycelial yields by *Botrytis allii* and *E. nigrum* was determined. pH determination was made by both pH indicator papers and a pH meter (type 222).

*Results and discussion.* The results (Tables I and II) show the fungistatic action of the crude pigment. High concentrations of the pigment were highly inhibitory to growth of *Botrytis allii* and only slightly inhibitory to growth of *E. nigrum* (Table I). At sufficiently low concentrations the crude pigment tended to be slightly stimulatory to the growth of both organisms.

The fungistatic actions of the pigment was found to be pH dependent (Table II). There was a low anti-fungal activity at pH levels above 5.0. FOPPEN and GRIBANOVSKI-SASSU[5] reported that under their growth conditions no anti-fungal effect was detected in culture fluid of *E. nigrum*. The lack of anti-fungal activity might have been associated, at least in part, with the high pH (6.7–7.0) of their yeast extract.

The chemical nature of the pigment producing fungistatic action is under investigation. There are conflicting reports on the nature of the pigment produced by *Epicoccum* species. It has been designated flavipin[1], carotenoid[5], and humic acid-like substance[6].

*Résumé.* On a constaté que le pigment secrété par le mycète *Epicoccum nigrum* a une réaction fungistatique sur le *Boyrytis allii* utilisé comme un organisme d'essai. Cette réaction a été dépendante du pH et a été assez basse aux niveaux dépassant 5.0.

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## Normaler und aberranter Karyotyp von *Cepaea hortensis* (Müller) (Gastropoda)

Bei *Cepaea hortensis* fand RAINER[1] haploid 30 Chromosomen im Gegensatz zu zwei früheren Autoren (BALTZER[2], PERROT[3]), die für die gleiche Art haploid nur 22 Chromosomen beschrieben haben. Der Karyotyp war nicht bekannt. Unsere Untersuchungen bestätigten zwar die frühere Zahl $n = 22$, förderten jedoch eine Chromosomenaberration zu Tage.

Das untersuchte Material stammte von drei Fundplätzen im Kanton Zürich: 4 Tiere aus dem Klotener Ried, 3 Tiere vom linken Limmatufer gegenüber dem Kloster Fahr und 2 Tiere aus einer an den Friedhof in Uster grenzenden Wiese.

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