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|  | *T*his experiment was done in the hopes of acquiring a better understanding of how marine algae protect themselves from a hostile environment and predation by pathogens. We have selected eight representatives of Pacific coastline algae since fewer studies have been done on this area. The samples were collected various points along the coast from Half Moon Bay to Fort Ross. All were located in depths ranging from 10 to 20 feet and no more than thirty meters from the shoreline. Ulva, Macrocystis, Petrogophora, Egregia, and Gelidium were located on rocks while all others are long stalked plants. From this experiment we hope to learn the strength of the plants chemical defense mechanisms.  **Research information:**  **Background:** Plants have the ability to ward off attacks by pathogens. We can assume this to be the result of evolution through the interactions of plants and pathogens. Plant defenses can be mechanical and chemical, but for this experiment we focused upon the chemical aspects. (3)Plant diseases are caused by four classes or types of microorganisms: fungi, bacteria, viruses and nematodes. Recently, plant pathologists have found that some plant disorders are also caused by mycoplasma, spiroplasma, and other exotic agents.  **Fungus**- Most plant diseases are caused by fungi. Fungi are cannot make their own food from water, carbon dioxide, and the sun's energy. The fungi must obtain their food from organic matter of dead plants or from living plants. When the latter occurs, the symptoms of disease are produced on the host plant. Many fungi are mold-like organisms existing in the form of microscopic threads. Most are transmitted from plant to plant by tiny seed-like bodies called spores, which are carried by air currents, splashing water, or tools. Common examples of diseases caused by fungi include root rot of okra, bitter rot of apple, brown rot of peach, or any of the dozens of fungus diseases that annually cause problems. The first counter-reaction would come with a signal sent by the cells to activate the meristems into creating cork cells in order to wall of the infection. Other plant cells may send a signal to thicken their cell walls and trap the infection. The use of fungus is not suggested in this experiment because other factors make the experiment more complicated.  **Bacteria**- Bacteria are microscopic one-celled organisms. The forms causing plant diseases are rod-like or cylindrical in shape and reproduce by fission. That is, one bacterium divides, or splits transversely, to form two new cells. Millions of bacteria may be produced in a short period of time. Bacteria are carried around in the same manner as fungus. They usually enter through wounds or natural holes; stomata and lenticels.  **Viruses**- Viruses are submicroscopic, cylindrical or spherical bodies. They are not living organisms but rather particles composed of nucleic acids and other compounds that are similar to the chemical make-up of chromosomes of the host cells. These pathogens operate by entering through wounds of the plant or injecting RNA strands directly into the cells. The RNA causes the cell to produce more of the virus until lysing when the cell dies and the copies are released to infect more cells. The experiment requires a petri dish of the pathogen to place the solvents in. Since viruses are too small and do not congregate in such a fashion this particular pathogen will be set aside.  (3)**Nematodes**- Nematodes are microscopic thread-like round worms. Their mouth-parts contain a hypodermic or needle-like structure (stylet) used to penetrate the cells of roots. With it, they deliver a digestive juice that predigests the root cell materials, which are then sucked into the nematode. When nematode populations are high, their feeding can result in stunted plant growth. Some cause the plant to produce galls; other nematodes cause root lesions, which can be entry points for the fungi that cause root rot.  (4)**R**ecent study with plant resistance to pathogens has brought up tests with the enhancement of plant resistance to fungal pathogens such as Rhizoctonia solani. In this study they are attempting to provide more enzymes to the plant for degrading the cell walls of invading fungi. They accomplished this by modifying the timing of the host defense mechanism to make the plant fungal-resistant. A transgenic tobacco seedling was expressing a bean chitinase gene under the control of the cauliflower mosaic virus 35S promoter and found to be able to survive in soil infested with the fungus.  Normally the chitinase is found in low or basal levels of a plant and moved through the plant during a fungal invasion. The study showed that the promoter would only activate when the plant is under pathogenic attack.  Since the timing of the chitinase response is so important to the outcome of the attack they constructed a hybrid gene. It held the promoter region of the cauliflower mosaic virus transcript at the 5' regulatory region of a bean endochitinase gene. The new part helped the plant create a ready made toxin throughout the plant and helped it to grow stronger than the same plant under normal conditions.  This study shows that there is not always an available toxin to ward off the attack of pathogens. Even if there was some toxins will not be released until the plants defense network can reach the area they are located.  **Comparative Research:**  (1)Procedure: The template for our experiment came from the experimentation of Vlachos, Critchley, and von Holy, representatives of the University of Witwatersrand, Johannesburg, South Africa. In their experiment they chose six species of seaweed from southern Africa. They used a range of solvents (acetone, chloroform, diethyl ether, 80% ethanol and methanol ) and assumed two methods of extracting the plants anti-microbial agents.  The first procedure ground a ten gram sample of each seaweed and cleaned of epiphytes, sand, etc. After being ground they were extracted in 200ml of each solvent on an orbital shaker. Lastly they were separated from the algal material through a Whatman paper dried and redissolved in 5ml of their respective solvents.  The seconds procedure ground the seaweeds into a fine powder with the aid of a plant mill (Quartztech, U.K.). The ten gram samples were then boiled in 80% ethanol and seperated from the algal material through filtration using Whatman paper. Finally it was made up to the volume of 5ml by the addition of 80% ethanol.  (2)We used a variation of both of these methods. Some of the solvents were to dangerous to handle without a lab. We ground the seaweeds after cleaning them and strained them through the filter paper because the other methods of purification, boiling in ethanol and an orbital shaker, were either not possible or to unstable. We were also concerned that some of the anti-microbial agents could be influenced or altered by the additional heat in boiling so we limited the temperature range as much as possible.  The agar plates could only be created in one manner for our resources so we skipped Vlachos's preparation techniques. The Whatman discs were also created in a different manner. While Vlachos used two sizes we used the uniform .7mm disc. Vlachos had the discs sterilized and placed directly into the agar before the solution was soaked into the discs. We instead soaked the discs before placing them within the prepared petri dishes to be sure that the discs are not soaked in the agar solution.  Vlachos's results contradict our own which we can assume to be a result of the species of seaweed and bacteria used. In order of activity indices Vlachos found methanol as the best solvent followed by acetone (our number one), 80% ethanol, diethyl ether, and chloroform. Their second extraction procedure created very similar results as could be expected since the only controlled variable in the second procedure was whether the seaweed was ground or milled. Our results do compare in that Vlachos' strain of E. coli held the same resistance to the seaweed extracts as did ours.  If anyone else decides to attempt this experiment they may want to try using a well rather than a paper disc because Vlachos' results shows that this procedure was more effective for his extracts. Also, through experience and reading we have discovered that E coli strands are much to resistant to give very useful feedback and that for some reason gram-positive bacteria have been found to be more sensitive to the experiment.  Special thanks to Dr. Richard Fetter PhD, for his assistance and supervision during the experiment. |

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