

Biological Safety Cane Lab

Description of research in the lab:

Investigations in this lab involve the study of the mechanistic enzymology of natural product biosynthesis. As part of this research we clone and express genes encoding enzymes controlling one or more steps in natural product biosynthetic pathways. The resultant proteins are purified and studied mechanistically and structurally.

General experimental procedures:

Various non-pathogenic bacteria, fungi, or yeast are cultured by standard microbiological methods and used as sources of natural products, proteins, or DNA.

- *Natural product isolation.* Cultures of *Streptomyces*, fungi, or recombinant bacteria are grown under standard conditions (surface agar cultures, submerged shake cultures, 0.5 mL to 5 L scale), cells are harvested by centrifugation or filtration, and the supernatants or cells are extracted with organic solvents or aqueous buffers to obtain crude preparations of organic metabolites such as antibiotics, pigments, etc.

- *Protein isolation.* Cultures of *Streptomyces*, fungi, or recombinant bacteria are grown under standard conditions (surface agar cultures, submerged shake cultures, 0.5 mL to 5 L scale), cells are harvested by centrifugation or filtration, and the cells are disrupted by standard methods (sonication, French press, lysozyme treatment, freeze-thaw, grinding, etc). The cell extracts are harvested by centrifugation and the protein components are isolated and purified by standard techniques.

- *DNA isolation.* Microbial cultures are grown as above. The cells are disrupted by standard methods and the plasmid or genomic DNA is isolated by standard methods.

- *Recombinant DNA procedures.* Host cells (*E. coli*, *Streptomyces*) are grown by standard methods and transformed with standard cloning or protein expression vectors or cosmids (pET, pUC, cosmids)

12.d)

Exposure Control Plan

1.0. Project title:

Biosynthesis of Natural Products

2.0. Principle Investigator:

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David E. Cane, Ph.D.; Office Location: GeoChem 447; Lab Location: GeoChem 403, 405, 407, 404, 406, 412, 454A, 455.

3.0. Experimental protocol:

See attached description of research and general laboratory procedures.

4.0. The procedures utilized in this laboratory do not involve human, animal, or plant pathogens of any kind. No human tissues or blood products will be used. Cultures of *E. coli*, *Streptomyces*, and various non-pathogenic fungi will be used. Occupational exposure to such cells will occur during the process of initial culturing of cells in flasks and during subsequent harvesting and processing..

5.0. Positions with occupational exposure:

Postdoctoral fellows, graduate students, and undergraduate students.

6.0. Procedures

Although no pathogenic or infectious materials will be utilized, routine precautions will be taken for avoidance of cross contamination of samples and unnecessary exposure of lab personnel during microbial culture and fermentation procedures. Routine experiments with these cells after initial inoculation and culture can be carried out on the laboratory bench in the general lab area without need for further sterile technique or containment.

6.1. Laboratory Practices

- A laminar flow hood is employed for all work with *Streptomyces* requiring sterile technique, in order to avoid cross contamination by other bacteria..
- Needles and syringes (though rarely used in these protocols) will not be sheared, bent, broken, recapped or resheathed by hand. All syringes, needles, other sharps will be discarded in durable puncture resistant containers.
- Appropriate protective clothing will be worn at all times when microbial cultures. Open-toed shoes will not be allowed at any time in the laboratory.
- Food and drink will not be stored in refrigerators, freezers, or cabinets where hazardous or biological material may be found. No food or drink is to be consumed in the laboratory.

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- All materials placed in storage in any location will be properly labeled.

6.2. Personnel Practices

- Always wear the protective clothing required for the particular experiment.
- Sandals and open toed shoes shall not be worn in the laboratory.
- Mouth pipetting is strictly forbidden at all times.
- All culture media, cell suspensions, etc. shall be manipulated carefully to avoid production of aerosols and droplets.
- Eating, drinking, smoking, application of cosmetics or lip balm, handling of contact lenses are strictly prohibited in laboratory areas. An area for consumption of food and drink shall be provided in a space outside of the laboratory.
- Remove all personal protective equipment immediately upon leaving the laboratory or cell culture work area and place in an appropriately designated container for storage. Laboratory coats will be cleaned on a regular basis.
- Wash hands immediately or as soon as possible after removal of gloves or other personal equipment and after contact with potentially hazardous or infectious material.
- Do not pick up contaminated broken glassware with hands. Use as broom and dustpan.

6.3. Labeling

- Containers, refrigerators, and freezers in which bacterial or fungal cells are stored will be labeled.
- A biohazardous waste label will be attached to any potentially infectious waste and sharps containers.

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6.4. Waste Handling and Disposal

- Waste generated in research involving bacterial or fungal cells will be segregated from other forms of trash until autoclaved.
- Trash and disposable articles will be placed in an impervious autoclavable bag to prevent leakage.
- The bag will be sealed and labeled with a biohazardous warning label which will include the date and name of the principal investigator (David E. Cane).
- Waste will be autoclaved before disposal.
- All syringes, needles, and other sharps will be discarded in a durable puncture resistant container.

6.5. Housekeeping

- The laminar flow hood will be disinfected after each use by wiping down the surface with a solution of 70% ethanol. The UV light is to be turned on before and after use.
- Liquid waste routinely generated during bacterial or fungal culture (such as supernatants from spinning down cells) will be collected in a container. To prevent growth of potentially infectious agents, this solution will be disinfected by addition of bleach. This will then be disposed as hazardous waste or down the sanitary sewer.
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7.0. Laboratory Accidents

- Any spill occurring in the biosafety cabinet should be cleaned up immediately. The affected area should be wiped down with a 70% ethanol solution to disinfect.
- The same procedure should be used to control a spill in the general lab area.
- Accumulation of large volumes of potentially contaminated liquid waste materials should be avoided. However, in the event of a large spill, pour disinfectant solution (e.g. bleach solution) around spill to avoid producing

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airborne material and spreading the spill. Allow to stand for 20 min before cleaning up.

- A laboratory coat, gloves, and eye protection should be worn at all times. Shoe covers should be used for cleaning floor spills. Dispose clean up materials into biohazard bags.
- If broken glassware is involved, do not pick up broken pieces with hands. Use a broom and dustpan.
- Wash hands after removal of protective equipment.
- Notify principal investigator (David E. Cane) and Biosafety Officer of the spill. Give specific information as to the nature of the spill.