DISCOVERY AND DEVELOPMENT OF BIOLOGICAL CONTROL AGENTS

- Characteristics of a commercial biopesticide
  1. Economical to produce
  2. Have persistent storage stability
  3. Have adequate field persistence
  4. Be easy to handle, mix, and apply
  5. Provide consistently effective control of the target pest or group of pests
BASIC STEPS IN DISCOVERY OF BIOLOGICAL CONTROL AGENTS

- Isolate and identify microbes
  - Economically produced – ease of production
  - Aggressively suppressed a target population
  - Do not harm the crop or non target organisms

- Microbial cultivation and formulation
  - Minimize product cost
  - Maximized yield and quality
    - Pest control efficacy
    - Stability through drying and storage
    - Host compatibility
    - Adequate field persistence

- Application and registration
  - Successful commercialization hinges on:
    - Outcome of the development process
    - Limited by lack of knowledge of and experience
SELECTING VIABLE BIOCONTROL STRAINS

- Choice of patho-system
  - An exploitable pathogen etiology
  - Existence in an environment favoring bio agent colonization
  - Resistance to traditional pest control strategies
  - Capability to cause significant crop loss

- Example fusarium dry rot of potato tubers – *Fusarium sumbucinum*
A. RAPID ISOLATION

1. Should begin in areas where they naturally occur
2. Evaluate maximal number of putative biocontrol agents – this will increase chances of discovering an effective strain
3. Isolate from appropriate tissue and under appropriate environmental conditions to insure that the microbial antagonist isolated will be well adapted
Figure 2. Isolation of microbial antagonists effective in suppressing Fusarium dry rot of potatoes (Schisler & Slininger 1994, 1997).
B. REALISTIC BIOASSAY TO ASSESS COMMERCIAL POTENTIAL

- Bioassay that mimic commercial production application settings i.e selecting their performance under conditions stimulating key industrial challenges
  - Batch liquid cultivation
C. Batch Liquid Fermentation

Selection of strains based

1. on their ability to grow rapidly
2. high yield on a variety of liquid culture media
3. Strains with nutritional flexibility will then be challenge with glucose media ranging in richness from a minimal medium (with nitrogen supplied by urea) to a semi confined complete medium (with casamino acids and growth factors) to an undefined medium (with added yeast extract, peptone and tryptone)
4. Selected strains then are culture in shake flask provided with low oxygen transfer coefficient ($K_l a \sim 0.5 \text{ min}^{-1}$) and moderate temperature (25 °C)
5. Finally they are then bioassayed to assess efficacy.
For each bacterium, a relative performance index (RPI) calculated based on each kinetic parameter, such as cell yield and specific growth rate:

- \( F = \frac{(X - X_{\text{avg}})}{s} \) which may range from -2 to 2
  - \( X \) - single value observed per bacterium
  - \( X_{\text{avg}} \) and \( s \) are the average and standard deviation observed per bacterium
Using the formula

- \( RPI = (F+2) \times 100/4 \), data corresponding to each parameter types are translated to dimensionless indices, scale from 0 to 100, which reflect relative bacterial performances.

- For a given production trial, overall kinetic performance indices are calculated for each bacterium:
  - \( RPI_{\text{kinetic}} = (RPI_{\text{growth rate}} + RPI_{\text{cell yield}})/2 \)
  - Similarly a relative performance index based on biocontrol efficacy is calculated for each bacterium using log (disease rating) data:
    - \( RPI_{\text{efficacy}} = (2-F) \times 100/4; \quad (2-F) \) is used instead of \( (2 + F) \) because efficacy improves as disease rating decreases.
Figure 3. Two-dimensional liquid culture focusing method of objectively ranking the commercial development potentials of microbial strains based on relative performance indices (RPI) calculated from the strains’ growth kinetics and efficacy when produced in liquid culture. RPI’s are dimensionless indices with values between 0 and 100 (best performance) when data are normally distributed (Slininger et al. 1994, Schisler & Slininger 1994).
E. Multi-Dimensional Screens to Assess Commercial Potential

- These steps are necessary to preserve cells for convenient storage and handling in between production and application.
- The ability of the biocontrol agents to solve multiple pest control problems is another potential screening dimension.
F. Designing the Production Process

- For each strain of interest
  - Liquid culture production process must be designed to minimize cost and maximize product yield, product rate and quality
  - Quality factors include:
    - Biocontrol efficacy
    - Storage stability
    - Host compatibility
Factors often Neglected when Designing the production process

- Impact of liquid cultural conditions such
  - Carbon and nitrogen sources, carbon to nitrogen ratio, nutrients, temperature, pH, dissolved oxygen)
- Microbial physiology (growth state)
- Metabolites on the qualities of biocontrol products are little known but are fundamental to designing the production process