SCREENING AND ISOLATION OF CELLULOLYTIC AND LIGNOLYTIC BACTERIA FROM GUT OF COMPOSTING EARTHWORM - AN AID IN PRETREATMENT OF BIOMASS FOR BIOFUEL PRODUCTION

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INTRODUCTION:
Earthworms are soil invertebrates playing an important role in recycling organic matters in soil. They are also known as “Ecocystem Engineers” (Satchell, 1967). The gut microflora plays an important role in digestion. Since earthworms are fed on waste organic matter during production of vermi-compost it is highly likely that its gut would be enriched with cellulolytic and lignolytic microbes. Such microbes will have high efficiency of conversion of cellulose to simple sugars. Bioethanol is considered as an important renewable fuel to partly replace fossil-derived fuels. Ethanol after burning, released carbon dioxide which is recycled back into plant material because plants use CO2 to synthesize cellulose during photosynthesis cycle (Wyman, 1999; Chandel et al., 2006a). Ethanol production process utilizes energy from renewable energy sources; no net carbon dioxide addition, making it an environmentally beneficial energy source.

OBJECTIVES:
1. To isolate the micro flora from the gut of composting earthworms (Eudrilus eugeniae).
2. Screen and characterize potential cellulase and lignin degrading bacteria.
3. To utilize the characterized strain for microbial pre-treatment of biomass along with existing pre-treatment methods.
4. Convert the pre-treated biomass source to ethanol using baker’s yeast.

WORK DONE
CULTIVATION OF EARTHWORM
Earthworms of the African species “Eudrilus Eugenia” were taken from biocentre Bangalore for cultivation in lab.

ISOLATION OF MICROORGANISM
Microorganisms isolated from the gut of the earthworm

Colony characterization
CMC Test
Gram’s staining
Biochemical characterization –
• Catalase test
• Fermentation test
• Manitol agar
• MR-VP
• Oxidative fermentative test
• Oxidase test
• Indole test
• EMB agar
• Mc Conkey Agar

WORK TO BE CARRIED OUT
After the microbial pretreatment methods the sample will be subjected for chemical pretreatment (acid). Determination of reducing non reducing and total sugars will be performed by DNS method. Estimation of protein content will be conducted by Folin Lowry method. The pre- treated raw material will be subjected to fermentation leading to bioethanol production. Ethanol yield will be estimated by titrimetric method using potassium dichromate, potassium iodide against sodium thiosulphate and using starch as indicator. The ethanol yield will also be calculated using specific gravity bottles as per AOAC charts.

RESULTS: After dilution the the micro organism was inoculated on the nutrient agar plate and 20 colonies were observed and it was characterized morphologically. After that the 20 strains were streaked on the CMC plate in which four strains showed zone of clearence i.e FN III, M2 III, HN III, H2 I. The gram reaction was performed in which two strains were +ve and the other two strains was –ve. The biochemical test performed in which the catalase test – all the strains were positive, fermentation test- a) glucose fermentation – all the strains were positive b) lactose fermentation-M2 III was positive and the three strains were negative, manitol agar test; -ve(FN III, H2 I), MR; -ve(H2 I), +ve(M2 III, HN III, FN III), VP; +ve (FN III, H2 I), -ve(HN III, M2 III), citrate test: +ve(FN III, H2 I), -

EXPECTED OUTCOMES AND APPLICATIONS

- Characterization of microflora isolated from gut of the earthworm.
- Screening for cellulolytic and lignolytic activity as well as isolation of these strains for biomass reduction and estimation of produced sugar (substrate for bioethanol production).
- Utilization of the pre-treated biomass for bioethanol production.
- Production of biofuel like ethanol utilizing invasive weed species as biomass source.
- Biodegradation of the invasive weed species using microbial isolates from earthworm gut.