Methods Of Isolation Of Indigenous Saprophytic Fungi And Screening Of Their Cellulolytic Activity – A Review

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ABSTRACT
The cellulose degradation is an important commercial process. As cellulose is complex organic polymer and need to be degraded in large scale so as to process further process it into commercial products. Cellulose degradation done successfully by soil microbes’ especially saprophytic fungi. Methods of isolation of indigenous fungi, identification based on invitro characterisations, and their cellulose activity assessment are discussed in this review.

KEY WORDS: indigenous saprophytic fungi, cellulolytic activity

INTRODUCTION
Cellulose is a most abundant organic polymer on earth (davidB.wilson, V.Makeshkumaret.al. P.Laksminarasimha.reddy et.al.Carlo.R.carere et.al.). It is main component of primary and secondary cell wall of the plants. Cellulose composed of thousands of D-glucose units linked with beta 1,4 linkage (Raj kumar et al., Nazamin nayebyazdi et.al.). As it is a complex structural element it has less direct applications (Carlo.R.care re et al.). But in nature saprophytic microorganisms present in the soil employed to degrade cellulose. So many fungal and bacterial spieces are reported with cellulolytic activity. But few fungal specious likeaspergillus(Nazamin nayebyazdi et.al., Laksminarasimhareddy,V.Makeshkumaret.al .,EdwardAbayeret.al.)Pencillium(Laksminarashimhareddy et.al.)and tricoderma(Raj kumar et.al.,Laksminarasimhareddy et.al.,Nazamin nayebyazdiet.al.,R.P.Tengerd yet.al.) are generally hyper active against cellulose degradation.

Cellulosomes are multi enzyme complexes. Generally present as an extracellular enzymes (Raj kumaret.al.) attached to the surface and for efficient degradation of the plant polysaccharides especially cellulose. Fungal cellulases have somany industrial applications they widely used in textile,
paper, and food processing industries. The production of biofuels from agricultural byproducts (Dominik antony et.al.), or plant biomass from forest wastage (Dominik antony et.al., V.V. Zeverlov et.al., Carlo R. Care et.al.) is emerging interest now a days. It reduces cost of raw material (Raj kumar et.al.) But the polymeric, complex nature of plant waste needs to be broken down into simple fermentable sugars to convert it into lipids through fermentation. This could be employed only by the large scale cellulose degradation. In the protoplast fusion techniques are employed using cellulase as wall degrading enzyme to isolate nascent protoplast. The review discussed about various methods to isolate the cellulolytic fungi from soil, methods to purify and screening for cellulolytic activity.

**Sources of fungi**

Saprophytic microorganisms show cosmopolitan spread. They could be isolated from agricultural fields or may be from the premises of the industrial regions like pulp (Nazamin Nayebyazdi et.al.) and paper industry waste (Carlo R. Care et.al.) disposing region also reported.

**Isolation of fungi**

The soil samples collected from the field possess verity of organisms. So the isolation of single strain suitable for the cellulose digestion is done. The soil samples are serially diluted up to 10 fold dilution and inoculated into basal medium using streak plate or sprinkle methods (Sivakumarsivaramn, Gomashe AV et.al. Lekhram et.al.). Basal medium containing potato dextrose agar which is supported for the fungal growth in initial culturing. To avoid contamination with bacteria PDA medium supplemented with antibiotics like chloramphenicol for initial culturing. The well grown colonies are taken for sub culturing using selection media for specific cellulolytic fungal isolation. Selective media have cellulose as sole carbon source, generally CMC media [carboxyl methyl cellulose] used for isolation purposes (Gomashe AV et.al. Lekhram et.al., Ibatsam et.al., Dale peculate et.al.). Only cellulolytic fungi can utilise the media and survive. Clear zones of cellulose degradation indicate the presence of cellulolytic fungi.

**Identification**

The identification of fungal colonies done by visual methods like morphological status on various media (Gomashe AV et.al. Ibatsamkhokhar et.al.), or also morphological identification also done with mounting fluid [lactophenol + cotton blue] (Lekhram et.al.) or may be done by DNA sequence homology analysis, molecular characterisation based on the isolated DNA, using DNA sequences analysis of two internal transcribed spacer regions of ribosomal DNA regions of ribosomal DNA that are ITS1 and ITS2 (Shona M. Duncan et.al.) or through colour of the colony, or through microscopic examinations like type of spore and the colour of it and growth pattern studies on basal medium (Gomashe et.al.). Sequence analysis with universal primer D2-LSU, metabolic characterisation of microorganisms done using BIOLOG™ system also reported for the fungal identification (Magdalena Fraq et.al.). Colony morphology and microscopic examinations of different reproductive structures and vegetative structures on pure cultures also been used as identification tools (P. Laksminarasimha reddy et.al.).

**Inducers**

cellobiose is common inducer of cellulose, sepharose, avicel are also reported in induction studies but bacteria like clostridium brought greater yield then the fungi in cellulose degradation (Siva bhat et.al.).

**Cellulose activity**

Cellulosome is a multi-component enzyme present as an extracellular protein. It is associated with cell surfaces and mediates cell attachment to insoluble substrates and degrades them into readily observable form. Fungal cellulosomes can act both on amorphous also crystalline forms. Generally they composed of three components exoglucanase, endo1, 4 betaglucanase and beta 1,4glycosidase (Rajkumar et.al.).

Several quantitative and qualitative assays for cellulase activity are described across world. IUPAC also recommended some standard methods like filter paper assay and plate clearing assay. Filter paper assay (Sivakumarsivaramnn, Dalepectiulyte) is widely used semi quantitative assay for determining cellulase activity. This is suitable for the liquid culture for assessing total cellulase activity and tri sodium dehydrate buffer is used. Plate clearing or plate screening assay (Ibatsamkhokhar et.al. Dale peciulyte) employed with CMC as sole carbon source along with mendal’s mineral salts and cango red as an indicator. Clear zone diameter/colony diameter will evolve index of relative enzyme activity (Ibatsamkhokhar et.al.). Tube test also designed for determining qualitative cellulase activity.
utilising dyed cellulose powder as substrates as substrate. Cellulose production results in release of dye which diffuses into the lower layer of the tube (R. Esmith, Magnelli PE et al.). Cango red is used dye, but several other dyes are also employed like grams iodine (Magalena frag), lacto phenol cotton blue for staining colony (V. Makesh kumar). The estimation of the activity quantitatively is done by measuring concentration of sugars (Nazamin) on spectroscopic studies at 540nm. Cellulolytic activity by kossem and nanni pieri reagent also reported (nazamin).

For the estimation of Individual effects of cellulosic enzymes different methods are reported. Beta–D-glycosidase activity assay reported by using cellulose or PNPG (Lekh ram et al.). Endo1, 4 beta glucanase assay using CMC by viscosity measurement. Bicinchonic acid also replaced with CMC is reported. Avicel used to assess’ exoglucosanase activity.

CONCLUSION

The enzymes with wide industrial applications are needed to be commercially produced in large scale to meet the present demand across world. In the review the standard methods for isolation of the fungus, identification and their enzyme activity assessments are reported. This may be help full in the basic isolation, identification, activity assessment, and also enlighten the future scope for development of methods like "the standardization of culture system, mass cellulose production by induction, enzyme purification in low cost, enzyme activity and stability, studies in immobilization for faith full recovery and high yield the present study will be helpful.

The bio fuel production from the cellulosic waste is emerging interest for current research. As the fossil fuels are exhaustible and first generation fuels are not being answer due to their high productive costs and high land usage. So the scope of future bio fuels has turned to most possibility of sustainable fuels produced from by-products of agriculture or paper pulp from the paper recycling units, municipal sewage waste etc. cellulose is the major component on all of the above raw material, but the complexity of cellulose made bio processing difficult. So cellulose is the major step in processing it into further downstream products. Cellulose degradation employed by bacteria fungi common in nature. Saprophytic fungi by having cosmopolitan distribution easily obtained nearly any area. And the research on cellulolytic organisms, cellulosome purifications, and optimal cellulose degradation is commercial hot core topic for scientific community.

REFERENCES


