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Editorial

The Quest has always striven to report on the most exciting discoveries and trends across the life science spectrum, with the goal of offering researchers in academia an engaging glimpse of what's happening both in and outside their own disciplines. We would like report here the three-dimensional (3D) printing is one of the latest technologies of 21st century. Medical applications for 3D printing are expanding rapidly and are expected to revolutionize health care. It is used in the customization and personalization of medical products, drugs, and equipment; cost-effectiveness; increased productivity; the democratization of design and manufacturing; and enhanced collaboration. This technology is also important to address the intellectual property issues which arise in medicine industry. In this issue we also report that music plays an important role in the human behavior. Binaural beats are nothing but when two pure auditory signals of similar frequency are mixed together, the phase interference between their wave forms a typical beats which know as binaural beats. Binaural-beat is used in treatment of insomnia or stress as well as it helps in dreamless sleep, meditation, relaxation, anti depressant.

Also Animal's circadian clock is depended on the sunrise and sunset as well as the hormone melatonin which is synthesis by pineal gland. If the people take caffeine at night the bedtime induces 40 minute delay in internal clock.

The issue also focus that Fungus *Aspergillus tereus* is able of produce cellulase with good enzyme activity. The authors do the mutation in the fungus with the help of UV radiation, and get mutant veriouts of fungus which gives 15 to 18 % increase in enzyme activity when compared with parent strain of fungus.

Lipases, like all enzymes, help regulate chemical reactions. It is a group of fat-splitting enzymes found in the blood, gastric juices, pancreatic secretions, intestinal juices, and adipose tissues. Lipases hydrolyze triglycerides (fats) into their component fatty acid and glycerol molecules. Several researchers focused on improve microbial lipases production because of their wide applications such as synthesis of biopolymers and biodiesel, pharmaceuticals, agrochemicals, and flavor compounds. Present scenario emphasis on protein engineering for design desirable lipase properties for novel application of lipase enzyme.

We invite you to read this month's stories and contribute to these discussions.

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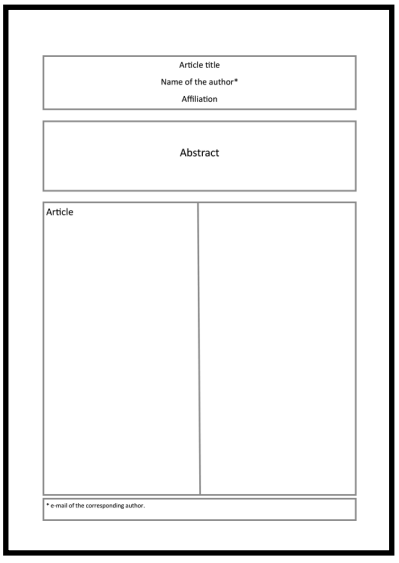
NEWS AND VIEWS:-

REVIEW ARTICLE:-

Notice to Authors

Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

Common for all: -
Font: Calibri
Font Size: 14
Columns: 2
Line Spacing: 1
Margin: Narrow
References: 1) In text citing, S No, Superscript.
2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).
3) Maximum number of references should not exceed than 25.



How 3-D Printing Is Revolutionizing Medicine

Medical researchers are increasingly turning to 3-D printing technology to make revolutionary advances in medicine. Video provided by Newsy.

The study found that with 3D printing in its infancy, there is no urgency to legislate at present as it is not a 'mass phenomenon' yet. However, the documents outlined that it is important to address the intellectual property (IP) issues arising in this area in order to create a climate better suited to tackling IP issues more successfully.

Phil Reeves of Econolyst Ltd emphasised the point by stating, "3D Printing and associated technologies like 3D scanning have great potential for businesses around the world, but particularly in high cost economies such as the UK. For industry to exploit 3D printing it is vital that the IP landscape is fully understood and respected."

Dinusha Mendis, Co-Director of the Centre for Intellectual Property Policy and Management at Bournemouth University and Principal Investigator of the project, said, "the 3D printing market for hardware, software and materials does not represent good value for money for the average user at present. Bearing this in mind, it can be concluded that the impact of the technology will not be felt among the general public for a few years to come.

"Although it is too early to tell when this will happen, our research concluded that there would really need to be evidence that 3D printing is an everyday reality before legislation is needed. Otherwise there is the danger that over-hasty legislation could stifle creativity and innovation.

The reports did make some important recommendations to government, the industry and intermediaries (online platforms) about how to regulate 3D printing without resorting to legislation.

Recommendations to government suggested the setting up of a working group to review the technology and the IP status particularly the position in relation to the software. Recommendation to industry focused on new business models and the traceability of spare parts.

Dinusha concluded, "It was a privilege to be able to look at this area on behalf of the government. Technologies such as 3D printing pose challenges for IP laws; however it is important to understand the extent and the impact of such challenges before looking to next-steps. Hopefully our research has helped navigate the murky waters of intellectual property to ensure that businesses and individuals are protected in the field of 3D printing."

Reference:-

Bournemouth University.

-Contributed by
Parth Patel
IGBT IV

A review of binaural beats on human behavioural

What is binaural beats?

When two pure auditory signals of similar frequency are mixed together, the phase interference between their wave forms produces complex signal with a frequency midway between the upper and lower frequencies. Likewise mixing of 114Hz and 124Hz of tones together gave effect of 20Hz. Similar thing occur when two distinct frequency played in right and left ear through headphones or earphones. Binaural auditory beats provide a mechanism for stimulating the auditory system at very low frequencies. Frequencies of binaural beats are less then 40Hz.

Types of binaural beats

Gamma waves: The frequency of this wave is greater than 40Hz. And occur in brain at when there is higher mental activity, Higher mental activity, including perception, problem solving, fear, and consciousness.

Beta waves: Frequency range of these waves is having range of 13-39Hz. These wave is particularly occur when subject mind is Active, busy or anxious thinking and active concentration, arousal, cognition.

Alpha waves: Range is between 7-13Hz. And it belongs to deep meditation/relaxation.

Delta waves: All the frequencies less then 4Hz are considered as Delta waves. It functions when subject is in deep dreamless sleep, loss of body awareness things.

EEG Spectral

Electrical impulses generated by nerve firings in brain can be measured by electrodes place on scalp. EEG activity is quite small signal, measured in microvolt (μV) with the main frequencies of interest up to approximately 30Hz. As per our physical and mental activity brain shows the response which can be shows a typical graph. These graphs are having mixture of all the waves i.e. is alpha, beta, delta etc.

Effect on human nervous system

When subject is targeted with any particular frequency the graph shows us the specific lines which are highly matched with this type of particular frequency. From that we can say that human brain is feel that type of feeling which is related to their particular frequencies. If binaural beat auditory stimulation can influence behaviour and mood, then such stimulation may have useful applications for the self-control of arousal, attention, and performance. Binaural-beat stimulation that decreases arousal may have applications in the treatment of insomnia or stress. It may also help in dreamless sleep, meditation, relaxation, anti depressant and etc.

Source: Binaural Auditory Beats Affect Vigilance Performance and Mood , Physiology & Behavior.

-Contributed by Shivam Patel
IGBT-IV

Caffeine at night delays human circadian clock

“Double espresso before bedtime induces 40-minute time delay in internal clock.”

For the first time, research shows that evening caffeine delays the internal circadian clock that tells us when to get ready for sleep and when to prepare to wake up.

A new study led by the University of Colorado Boulder and the Medical Research Council's Laboratory of Molecular Biology in Cambridge, England shows for the first time that evening caffeine delays the internal circadian clock that tells us when to get ready for sleep and when to prepare to wake up. The research team showed the amount of caffeine in a double espresso or its equivalent three hours before bedtime induced a 40-minute phase delay in the roughly 24-hour human biological clock.

The study also showed for the first time how caffeine affects "cellular timekeeping" in the human body, said CU-Boulder Professor Kenneth Wright, who co-led the study with John O'Neill of the Medical Research Council's Laboratory of Molecular Biology (LMB) in Cambridge. While it has been known that caffeine influences circadian clocks of even primitive creatures like algae and fruit flies, the new study shows that the internal clocks in human cells can be impacted by caffeine intake.

"This is the first study to show that caffeine, the mostly widely used psychoactive drug in the world, has an influence on the human circadian clock," said Wright, a professor in CU-Boulder's Department of Integrative Physiology. "It also provides new and exciting insights into the effects of caffeine on human physiology." It also provides new and exciting insights into the effects of caffeine on human physiology."

A paper on the subject led by Wright and O'Neill is being published online in the Sept 16 issue of *Science Translational Medicine*.

For the study the team recruited five human subjects, three females and two males, who went though a double-blind, placebo-controlled 49-day protocol through CU-Boulder's Sleep and Chronobiology Laboratory, which is directed by Wright. The subjects were tested under four conditions: low light and a placebo pill; low light and the equivalent of a 200-milligram caffeine pill dependent on the subject's weight; bright light and a placebo pill; and bright light and the caffeine pill.

Saliva samples of each participant were tested periodically during the study for levels of the hormone melatonin, which is produced naturally by the pineal gland when directed to do so by the brain's "master clock." The master clock is re-set by exposure to light and coordinates cellular clocks throughout the human body. Melatonin levels in the blood increase to signal the onset of biological nighttime during each 24-hour period and decrease at the start of biological daytime, said Wright.

Those who took the caffeine pill under low-light conditions were found to have a roughly 40-minute delay in their nightly circadian rhythm compared to those who took the placebo pill under low light conditions, said

caffeine dose was about half that of the delay induced in test subjects by a three-hour exposure to bright, overhead light that began at each person's normal bedtime.

The study also showed that bright light alone and bright light combined with caffeine induced circadian phase delays in the test subjects of about 85 minutes and 105 minutes respectively. There were no significant differences between the dim light/caffeine combination and the bright light/placebo combination. Nor were there significant differences between the bright light/placebo and bright light/caffeine combinations. The results may indicate a "ceiling" was reached in the phase delay of the human circadian clock due to the external factors, Wright said.

In addition, researchers at O'Neill's lab at the LMB in Cambridge used "reporter" genes that made cells glow when the clock genes were expressed to measure changes caused by caffeine. O'Neill's group showed that caffeine can block cell receptors of the neurotransmitter adenosine, which normally promotes sleep and suppresses arousal. The results may help to explain why caffeine-drinking "night owls" go to bed later and wake up later and may have implications for the treatment of some circadian sleep-wake disorders, said Wright.

The new results could benefit traveler. Properly timed caffeine use could help shift the circadian clocks of those flying west over multiple time zones, said Wright.

In a 2013 study, Wright and his research team showed one week of camping in the Rocky Mountains with no artificial light, not even flashlights, synchronized the circadian clocks of the eight study subjects with the timing of sunrise and sunset.

Reference:

University of Colorado at Boulder.

-Contributed by Krishna Saraiya
IGBT-IV

Screening of better Cellulase producing UV mutant of *Aspergillus tereus* and its Media Optimization

Bhargav Patel , Sandeep Chovatiya and Snehal Ingale*

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Affliated to "Sardar Patel University"

Abstract: Cellulase are group of hydrolytic enzyme and they are capable of degrading all types of lignocellulose materials. Present work focuses on the mutation of *Aspergillus tereus* and its cellulase enzyme production ability. In this study, 30 mutants were obtained by Ultra Violet light irradiation of Spores of *Aspergillus tereus*; few mutants showed more cellulase activity over the control and optimisation of cellulase by these mutants were carried out using different environmental factors such as pH(3-6), spore inoculum ($1x10^6$-$1x10^9$ cells/ml), different substrate(rice husk,banana stem and bagasses), moisture concentration(1:1-1:5) and substrate size (10mm,16mm and 22mm) were also determined. 15-18% increase in Cellulase Unit activity was obtained for Mutants as compared to parent strain during Submerged fermentation and Solid state fermentation.Further investigation in media optimization by statistical tool would help to get better insight of production ability of mutant isolate over control and thus it would help in production of cellulase in large quan-

Introduction:

In the present techno- economic era, increased demand of energy is one of the major problems which humanity is facing. All the cellulosic waste is a source of food and is also a potential source of energy[1]. Cellulose present in lignocellulosic material is considered to be the most abundant organic substrate on earth as chemical feed stock which is renewable source[2].

Cellulose is a branched glucose polymer. The breakdown of cellulose into sugar can be achieved by acid hydrolysis as well as by enzymatic hydrolysis. Cellulase, a group of enzymes which catalyze the hydrolysis of cellulose is considered a potential tool for industrial saccharification of cellulosic biomass[3]

Strain improvement in Industry are mostly attributed to the extensive application of mutation and selection of microorganism. UV rays are effective mutagenic agents used for strain improvement and for enhanced cellulase production[4].

The enzyme production is good by most of the fungi like *Aspergillus*and *Trichoderma*Sp. Enzymolysis of native cellulose is carried out by three components of cellulase as:

a. Exo- β-1-4, glucanase: It acts on the non-reducing end of the cellulose chain and successively removes single glucose units.
b. Endo-β-1-4, glucanase: It randomly attacks the internal β-1-4, linkages.
c. β-glucosidases or Cellobiases: The cellulose system also contains cellobiase, which eventually breaks down cellobiose, the building unit of cellulose, to glucose.

The Objectives of present study are:

1. Mutation in <u>*Aspergillustereus*</u> using UV ra-

** Corresponding Author: snehalingale@aribas.edu.in*

successively removes single glucose units.
b. Endo-β-1-4, glucanase: It randomly attacks the internal β-1-4, linkages.
c. β-glucosidases or Cellobiases: The cellulose system also contains cellobiase, which eventually breaks down cellobiose, the building unit of cellulose, to glucose.

The Objectives of present study are:
1. Mutation in *Aspergillustereus* using UV radiation.
2. Comparision of mutant obtained for cellulase production using Submerged and Solid State Fermentation.
3. Optimization of cellulase producing mutant using lignocellulase waste through submerged and Solid State Fermentation.

Materials and Methods:
Microorganism: Spores of *Aspergillus tereus* were taken from preserved soil stocks and cultured on Potato dextrose agar(PDA) plates and spores arepreserved on PDA plates/ slants at 4˚C.

UV Mutagenesis of *Aspergillus terreus*:Spores form the PDA plates/slants were collected using 20ml sterile saline Tween 80 solution.Spore suspension was collected in a sterile testube and spore counting was done using heamocytometer[5]. This solution was diluted to obtain desired spore count i.e. $1x10^6$ cells/ml and 0.1ml from the above suspension was spread inoculated on sterile PDA plates andmutation of *Aspergillus terreus* spores were carried under Ultra violet light of short wavelenghts for different period exposure time i.e. 1 min – 10 mins and after UV irradiation plates were wrapped in back paper and incubated at 37^oC for 5 days.Similar protocol was also performed in case of germinated spores where 0.1 ml of the spore suspension was inoculated in potato dextrose broth and spores were incubated on rotary shaker for overnight at 100 rpm. After overnight incubation germination of spores was observed under light microscope and 0.1 ml germinated spore suspension was spread inoculated onto sterile PDA plates. Mutation was carried similar way as mentioned previously. After incubation, the number of colonies were counted to determine the survival rate, killing rates due to UV irradiation.
The growth of survivors after UV mutagenesis were transferred to PDA slants. All mutants were preserved on slants at 4^oC till further testing of mutants for cellulase ability. The cellulase production was done in 100 ml Webber and Mandel medium containing $1x10^7$ spores of muatnts and incubating the flask on rotatary shaker maintained at 120 rpm. Mycelial growth was observed on the second day

Enzyme assay:
Cellulase activity was measured as described previously by Ghosh[6].One unit (IU) of endoglucanase activity was defined as the amount of enzyme releasing 1 mmole of reducing sugar per min .

Optimization of Mutant having highest cellulase production: Rice husk were taken as a substrate, was washed twiced and grinded to 10 mesh size. Solid state fermentation was carried out using Mandel &Webermedia with pH 4.8^7.$1x10^7$ spores/ml were inocculated in every flask as starting inoculum size to start fermentation.

Five parameters were considered which are substrate size, media concentration, different substrate, ph and spore quantity.

1. Effect of Substrate size: Different sieves were taken depending on the particles to be used for the bioprocess i.e. 10mm, 16mm and 22mm mesh size. 25 ml of the Mandel & Weber media and 5g Lignocellulosic substrate of different mesh size were added in 100 ml flask.
2. Effect of moisture level: 5g of substrate was added to different volumes of Mandel & Weber media i.e. 5ml,10ml,15ml,20ml and 25ml in 100ml flask.
3. Effect of Different substrate: 5g of Rice husk, bagasses and banana stem were taken as substrate containing 25ml Mandel & Weber media.
4. Effect of pH: Mandel & weber Media was adjusted to pH of 3,4,5 and 6.
5. Effect Spore load on fermentation: $1x10^6$, $1x10^7$,$1x10^8$ and $1x10^9$spores/ml were inoculated to 5g of Lignocellulosic substrate containing Mandel & Weber media for the fermentation.

Result and disscusion:
The wild type of *Aspergillus terreus* when exposed to Ultra Violet radiation for varying time periods gave 30 mutants with different abilities to produce Cellulases. The lethalityrate of *Aspergillus terreus*spores crossed 99.9% when exposed to UV radiations (*Table 1*).

Total of 30 mutants were screened for CMCase and FPU production using Submerged and Solid State Fermenttion.Out of the 30 mutants 4 mutants(M16,M17,M18 and M24) showed greater activity than the control (*Fig*.1&2). The results revealed that the highest Cellulases production was obtained by M18 when exposed to UV dose which represents a 15-20 % improved enzyme activity (FPA & CMCase) than that of wild type. Abo-State et al. (2010) found enhanced productivity in CMCase by gamma-irradiation at dose 0.5 KGywith 21% increase as compared with un-irradiated control.

Table 1:Effect of UV radiation on % Killing on Aspergillus terreus

UV exposure (min)	CFU	%Survival	%Killing
0	$1.58x10^5$	100	0
2	50	0.0032	99.968
3	60	0.0038	99.996
4	10	0.0063	99.991
5	12	0.0076	99.992
10	3	0.0019	99.998

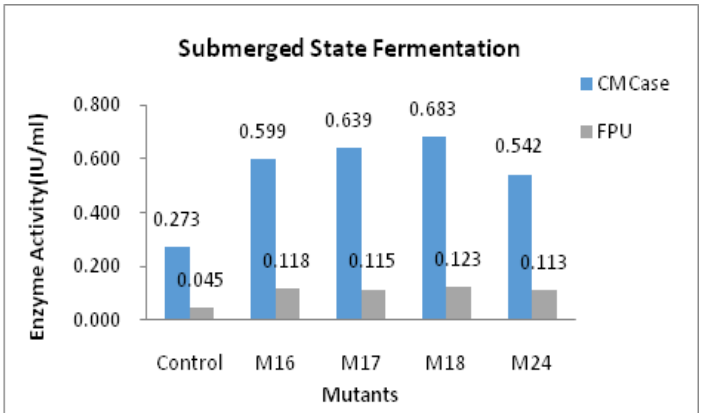

Figure 1 : Cellulase production by mutants using SmF

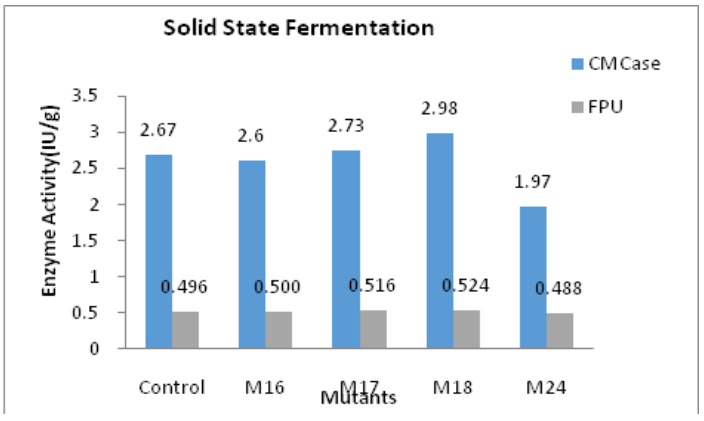

Figure 2 : Cellulase production by mutants using SSF

Optimization of Parameters for cellulase production using mutant M-18 :

1. Effect of particle size on cellulase production

The particle size of rice husk of size 0.78mm, 1.190mm and 2mm were. The fig.3 shows that the enzyme yield varied with rice husk size and optimum enzyme yield of 0.82 U/g was obtained for particle size of, whereas the enzyme yield was found to be reduced for smaller particles (1.19mm) and still smaller particles (0.78mm). Small particle size may lead to clumping of bran, resulting in reduced accessibility to nutrients anaerobic*cultural conditions with lower yield of the enzyme. Particle size is also responsible in making the substrate more accessible to the microorganism. In Fig. 3Endoglucanase activity was seen to be 3.384 U/g. Vyas et al showed that using groundnut of size 0.1mm as a substrate for SSF in gave activity of CMCase(0.41 U/g) and FPU (0.037 U/g). Moosavi suggested that keeping the particle size uniform gives the fungi a better surface area for growth. Keeping the size of sugar beet pulp of 0.25mm, FPU activity was 0.46 U/ml under SSF* [8].

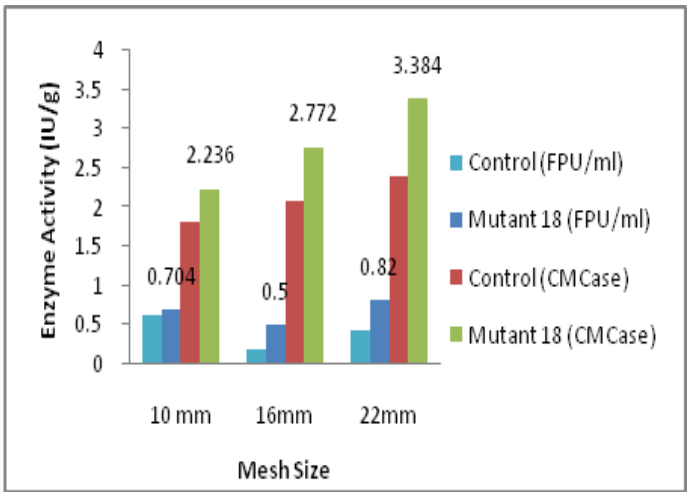

Figure3 : Effect of particle size on cellulase production

2. Effect of Moisture Concentration on Cellulase Production

In Solid State Fermentation, moisture level plays an important role in biosynthesis and secretion of many kinds of enzymes, especially cellulases. Very high moisture content in solid medium results in declined substrate porosity as well as reducing oxygen penetration between the substrate particles , but extremely low moisture levels in solid medium leads to poor microbial growth, reduced development and low accessibility to nutrients. Filter Paper Unit Activity was highest in 1:4 moisture ratio which was 0.56 U/g as compared to other moisture levels (*Fig*.4). Endoglucanase activity was 2.75 U/g in 1:4 moisture ratio.

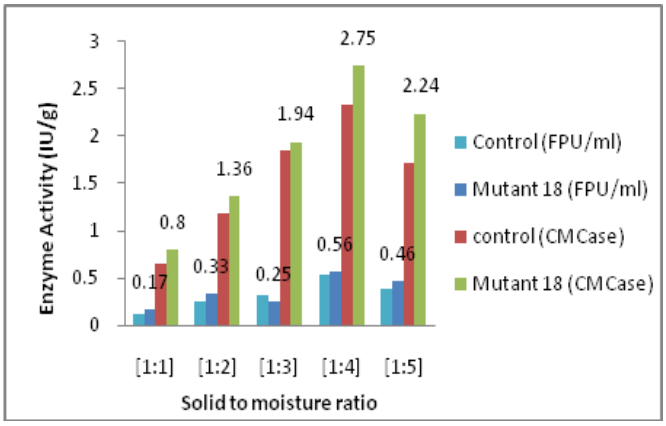

Figure4 : Effect of moisture concentration on cellulase production

Moisture content below or above were not suitable for higher enzyme activity. R. Singh and Mishra showed that taking the solid to moisture ratio of 1:1 in SSF using wheat straw gave enzyme activity of 152 U/g by *B. cereus* MTCC 1305[9]. M . Pensupa et al using wheat straw in SSF taking the solid to moisture ratio from 1:5, 1:6, 1:7, 1:8 and 1:9. Maximum activity of FPU was obtained in 1:7 ratio(5.50 U/g) [10].

3. Effect of different substrate on cellulase production

Rice husk, sugarcane bagasses and banana stem were used as different substrate. Among the three substrate tested (Fig. 5), highest FPU was observed in banana stem (0.8 U/g) then in rice husk (0.5 U/g) and lowest in sugarcane bagasses (0.3 U/g). J. Khan and S. Singh conducted similar type of experiment using A. niger and used four different substrate namely corncob, saw dust, wheat straw and newspaper. Corncob showed maximum activity of 0.027 U/g followed by wheat straw which gave

Endoglucanase activity was seen to be highest in banana stem rising to 5.892 U/g, next comes bagasses showing activity of 5.028 U/g and least by rice husk of 2.53 U/g. However, these rice husk & bagasses did not cause enzyme productions as high as banana stem. Therefore, banana stem has been found to be superior to other solid substrates for the synthesis of cellulase from *A. terreus* by Solid State Fermentation under these experimental conditions.

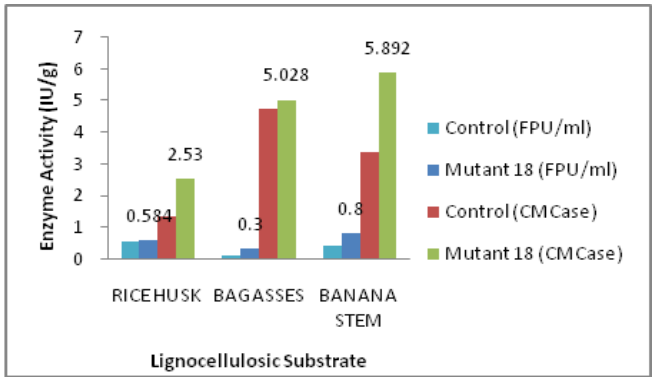

Figure 5 : Effect different substrate on cellulase production

4. Effect of pH on Cellulase Production

The optimal pH varies with different microorganisms and enzymes. Presently, the highest production of cellulase i.e. 1.02 U/g was observed at a pH of 5. The influence of pH on cellulase production highlighted the widely-known importance of pH for microbial growth and metabolic activities, and the sensitivity of the latter to pH change. The highest activity of endoglucanase was 2.96 U/g was also observed (*Fig*.6). Xing-Li observed maximum activity CMCase (0.181 U/g) of *T.viride* after exposing to UV radiation in SSF [4]. *A. terreus* DSM 826 used in study by Hasan was grown on modified Czapek-Dox's liquid medium containing rice husk was set at pH 5 and achieved 1.67 CMCase activity [12]. *A. terreus* GN1 used by John Wiley using modified czapek's medium where maximum CMCase activity (4.3 U/g) was expressed at pH 4.5, and that of maximum FPase activity (0.12 U/g) was noted at pH 5.5 [13].

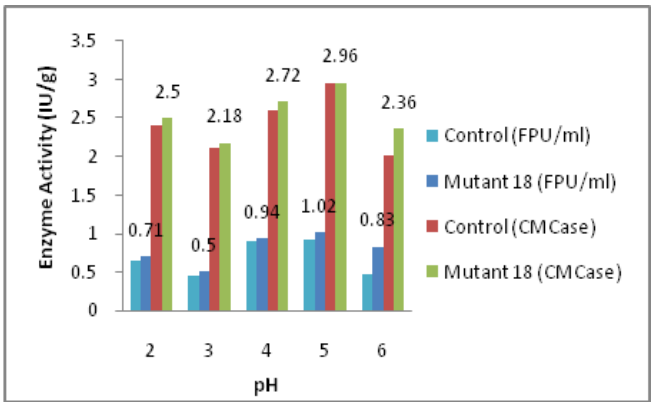

Figure 6 : Effect of pH on cellulase production

5. Effect of Spore Concentration on Cellulase Production

The number of spores at the beginning of fermetation added to the media does not

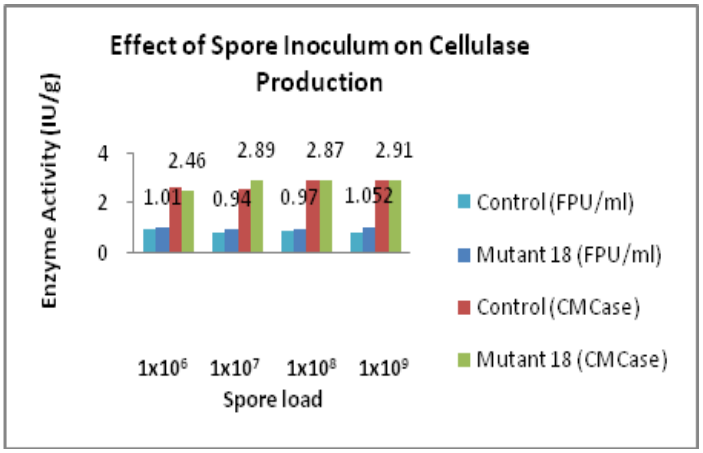

Figure 7 : Effect of spore load on cellulase production

gives any significantly variation in cellulase production. 1x 10^7 to 1 x 10^8 sporesinoculated in the production flask would be optimum for the cellulase production as seen in fig.7.

Conclusion :

1.UV Induced Mutation produced different mutants which gave higher cellulase production as compared to the parent.

2. Cellulase production was higher in Solid state fermentation (SSF)than the Submerged fermenation (Smf).SSF may be considered as cost effective means for large scale production of cellulase which probably would be several fold cheaper as compared to current commercial preparations.

3.Optimizing various parameters results in better cellulase enzyme production by mutant as compared to the parent strain.

4. Mutant isolate showed higher cellulase production(13.2%) in Banana pseudostem containing medium as compared to Rice husk.

Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization as biomass is abundant and cheap. Random mutagenesis will remain a choice method for strain improvement, especially for improving complexphenotypes or poorly characterized organisms.

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MICROBIAL LIPASES: PROPERTY AND APPLICATIONS

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Abstract: This review paper provides an overview regarding microbial lipase sources and substrate. And overview of Microbial lipase purification techniques, its prorerties and its application in various industries.

Introduction

Today, nearly 4000 enzymes are known, and of these, about 200 are in commercial use. The majority of the industrial enzymes are of microbial origin. Until the 1960s, the total sales of enzymes were only a few million dollars annually, but the market has since grown spectacularly[1].The major share of the industrial enzyme market is occupied by hydrolytic enzymes, such as lipases, proteases, amylases, amidases and esterases. Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the multibillion dollar underexploited lipid technology bio-industry and have been used in *in situ*lipid metabolism and *ex situ*multifaceted industrial applications[2] Lipases (triacylglycerol acyl hydrolase; EC 3.1.1.3) are water-soluble enzymes that catalyze the hydrolysis of triacylglycerol to release free fatty acids,mono or diacylglyceride and glycerol[3].

Source of lipase:

Lipase are produced by plant, animal and microbes. Present studies focus on lipase produced by microbes rather than by plants and animals. Because lipase production from plants and animals leads to high cost and very less yield[4].Many microorganisms are known as potential producers of extracellular lipases, including bacteria, yeast and fungi[5] which can produce lipases with different enzymological properties and substrate specificities[6] (table 1). Microbial lipases have gained special industrial attention due to their stability, selectivity and broad substrate specificity[7]. Extracellular lipase producing microorganism isolated from different oil contaminated soil sample like industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food , compost heaps, coal tips, and hot springs[6] and screened by various methods like rhodamine B agar plates[8], tributyrin agar plates[9] and Spirit Blue Agar[10].

Substrate for lipase:

Many factors influence in lipase production like type of carbon substrates and inducers[11]. Lipase is inducible enzyme. It required inducer in form of fatty acid, surfactant, fatty esters, lipid etc.Sardine oil, soy bean oil and triolein were effective inducers for lipase production[12].S. S. Kumar and Gupta, 2008 found that lipasefrom a newly isolated strain *T. asahii* MSR 54has been inducible enzymeand gave activity upto104 U/ml in presence of Tween-80, 80 U/ml in presence of corn oil, 48 U/ml in presence of kerosene. Hasan et al., 2006 found that lipase from Bacillus sp. FH5 was inducible and its yield affected by the type of carbon source used. Tween 80 came as the best inducer for lipase production[14].

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Grbavčić et al., 2007i nvestigated that higher lipase yield were obtained when medium supplemented with caprylic and oleic acids as inducers. Gupta et al., 2007 used olive oil/ oleic acid as the inducer for increase lipase production and suggested that lipase production catabolically repressed by glucose. Same thing reported byRapp, 1995 taht lipase production from *F. oxysporum f. sp. vasinfectum* increased in presence olive oil and oleic acid and repressed by glucose and glycerol.

Lipase Assay:

The fatty acids released by lipase-mediated hydrolysis can be determined using many methods like1. Titrimetry, 2.spectroscopy (photometry, fluorimetry, in- fra red), 3. Chromatography, 4.radioactivity, 5.interfacialtensiometry, 6. turbidimetry, 7. conductimetry, 8. immunochemistry, 9. Microscopy[17].

Purification process:

Industrial use of lipase need purification from crude enzyme for that many techniques use like prepurification by using ammonium sulphate or acetone [18] followed by chromatographic process that increase recovery yields and purification fold. Based on lipase nature purification techniques are used for purified lipase which gives high recovery yields and increase purification fold[19]. Other methods for lipase purification are:reversemicellar system (RMS) [20], Membrane processes, immunopurification and aqueous two-phase systems [21], aqueous two-phase flotation (ATPF), aqueous micellar two -phase system (AMTPS) [22].

Property of Lipase (table 1):

Lipases are serine hydrolases which act at the lipid water interface. The catalytic triad is composed of Ser-Asp/Glu-His and usually also a consensus sequence (Glyx-Ser-x- Gly) is found around the active site serine. The three-dimensional structures of lipases reveal the characteristic α/β-hydrolase fold [23]. There are two criteria to classify a lipolytic enzyme as a "true" lipase: (i) it should be activated by the presence of an interface, that is, its activity should increase as soon as the triglycerides form an emulsion. This phenomenon was termed as "interfacial activation"[24](ii) Interfacial activation has been related to the presence of a hydrophobic oligopeptide (the lid or flap) which enclosed active site and move away from active site when it contact with hydrophobic substrate [25] [26] . Often in the presence of organic solvents, the enzymes are effective catalysts for various inter-esterification and trans-esterification reactions such asacidolysis, alcoholysis and aminolysis [27]. Lipases are also known to show extreme versatility regarding fatty-acyl-chain length specificity, regiospecificity and chiral selectivity[28].

Application of lipase:

The most desired characteristics of the lipase for industrial use are its ability to utilize all mono-, di-, and tri-glycerides as well as the free fatty acids in transesterification reactions, low product inhibition, high activity/ yield in non-aqueous media, low reaction time, resistance to altered temperature, pH, alcohol and reusability of immobilized enzyme. Novel biotechnological applications of Lipase have been successfully established likethe synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agro-chemicals, and flavor compounds[30] (table2).

Table 1: Microorganisms cited in the literature as potential lipase producers with its properties

Source	Molecular weight	pH, temperature, stability	Substrate specificity
Actinetobacter-calcoaceticus	30.5 kDa	Stable at pH 8.0 and temperature 40^{o}C	Enzyme hydrolyzes tri,di,mono-acyglycerols
Actinetobacter sp. RAG-1	33 kDa	Active at temperatures up to 70^{o}C	Hydrolyzes wide range of pnp esters, but preference for medium-length acyl chains (C6,C8)
Alcaligenes sp.	---	65% residual activity at 60^{o}C after 10 min	Enzyme hydrolyzes natural fats and oils
Bacillus sp.	22 kDa	Stable over pH 5.0-11.5, stable at 65^{o}C for 30 min at pH 5.6	Tricaprylin, tricaprin, 1,3-regiospecific lipase
Bacillus sp.	45 kDa	Stable for 12h at 60^{o}C	Triolein hydrolyzed at all positions; broad fatty acid specificity
*Bacillus sp.*THL027	69 kDa	Stable over pH 6.0-8.0, 80% residual activity after 1h at 75^{o}C	Preference for C4-C12 fatty acid;1,3-regiospecific
B. subtillis 168	19kDa	Stable at pH 12;100% activity after 30 min at 40^{o}C	Preference for C3 fatty acid;1,3-regiospecifie
B. thrmo-oleovorans ID-1	34 kDa	Stable at pH 7.5, half life at 70^{o}C 30 min	Broad
P. cepacia DSM 50181	---	Stable over pH 2.0-12.0	---
P.fluorescens MC50	55kDa	Stable over pH 6.0-9.0	Trioxylglycerols
P.fluorescens AK 102	33kDa	PH 4.0-10.0 stable below 50^{o}C for 1h 100%	Broad

Table 2: Industrial applications of microbial lipases [23]

Industry	Action	Product of Application
Dairy food	Hydrolysis of milk, fat, cheese ripening, modification of butter fat	Development of flavouring agent in milk cheese and butter
Bakery food	Flavour improvement	Shelf life prolongation
Beverages	Improvement aroma	Alcoholic beverages e.g. sake wine
Food dressing	Quality improvement	Mayonnaise dressing and whippings.
Health food	Transesterification	Health food
Meat and fish	Flavour development	Meat and fish product fat removal
Laundry	Reducing biodegradable strains	Cleaning cloths
Cosmetics	Esterifiction	Skin and sun-tan cream, bath ioletc
Surfactants	Replaces phospholipase in production of lysophospholipids	Polyglycerol and carbohydrate fatty acid esters used as industrial detergent and as emulsifired in food formulation such as sauces and ice cream
Agrochemicals	Esterification	Herbicides such as phynoxypropionate
Pharmaceuitical	Hydrolysis of expolyesteralcohol	Produce various intermediates used in manufacture of medicine.
Fuel industries	Transesterification	Biodiesel production
Pollution control	Hydrolysis and transesterification of oils and grease	To remove hard strain, and hydrolyse oil and greases

Conclusion:

This review showed that many researchers worldwide focused on improve microbial lipases properties like low product inhibition, high activity/yield in non-aqueous media, low reaction time, resistance to altered temperature, pH, alcohol and reusability of immobilized enzyme for increase them applicability in industries. Now a days use of protein engineeringfor design desirable lipase properties which allow attainment of enzymes with new remarkable characteristics for a specific application.

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