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Review Article

Industrial Microbiology: Current Status of Research & Development in India

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Bio-Industrial Sector in India is still in its infancy, but growing steadily at CAGR of 11.2% accounting for total sales of USD 128 Millions. The multinational companies account for nearly 65% of the market, while the rest is met by the local industries. The Indian bio-industries are now reassessing and increasing their R&D capabilities and technical staff, establishing production units and developing an elaborate distribution system. The major industries employing microbial fermentation in India include alcoholic beverages and industrial ethanol, organic acids, enzymes and recombinant therapeutics. The upcoming lignocellulosics based biorefineries offers an exciting area of R&D and scale up at industrial level. However, there is urgent need to establish industries for fermentation of novel bio-molecules e. g. amino acids, novel antibiotics, etc. to be self reliant in face of vibrant geopolitical situation.

Keywords: Research & Development; Alcohols; Enzymes; Biochemicals

Introduction

Industrial microbiology forms the core of white biotechnology and thrives on the ability of selected microorganisms or genes derived thereof for producing important biomolecules/products/proteins. Man has been using the microorganisms since time immemorial. With the advances in the knowledge of genetics, metabolism and physiology, the spectrum of products derived from microorganisms have gone up steadily from the traditional procedures of making wine, leavening of dough for bread making and fermented milk to now advanced therapeutic biomolecules, amino acids, enzymes, solvents and chemicals. The global sale of fermentation-derived products was about $24.3 billion in 2015, which is expected to rise by 44% in the next five years to $35.1 billion that accounts for a compound annual growth rate (CAGR) of 7.7% (BCC research report FOD020C World Markets for Fermentation Ingredients).

Biochemicals synthesized through fermentation route are used in a variety of applications and serve as important feedstocks for developing commercially viable technologies. The use of these biochemicals by industries world overis primarily due to their being natural moieties that are available at affordable cost due touse of improved strains and process parameters. The major products produced through fermentation include enzymes, organic acids and alcohols that find use in a wide array of industrial processes like drugs and pharmaceuticals, foods and beverages, chemical, textile and rubber industries as prominent end users. The revenue generated through the use of these fermentation products was USD 41.568 billion in 2012, which is slated to reach USD 60.124 billion by 2019 at an annual growth of 5.4%.

Alcohols (potable liquor, solvents and blenders in fuels) were identified as the major contributor to the global fermentation based chemical market in 2012, with enzymes and organic acids being other important commodities. The revenues generated from sale of alcohol alone was USD 22.377 billion in 2012 which was more than half (53.8%) of the total consumption of fermentation derived molecules. The other major segments of fermentation products include aminoacids, enzymes, organic acids and antibiotics in that order, even vitamins and xanthan gum have contributed significantly to the sales (Fig. 1).

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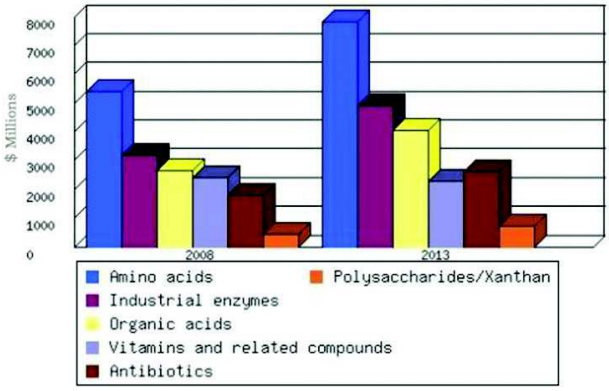
SUMMARY FIGURE
GLOBAL MARKET FOR FERMENTATION
PRODUCTS, 2008 AND 2013
($ MILLIONS)



Source: BBC Research

Fig. 1: Market share of major fermentation products in 2008 and 2013 (Source: www.transparencymarketresearch.com)

Geographically North America is the major and the most important market for the biochemical industry which is mainly due to steady rise in the pharmaceutical sector in U.S.A. In recent times Asia Pacific has emerged as the next important market for the fermentation based biochemicals. The North American and European markets are now reaching a plateau and future projections see Asia Pacific as strong bastion for growth. The consistent rise in food and beverage, pharmaceutical, healthcare and cosmetic industriesis expected tobethe major future driversof the demand for fermentation chemicals. The quest for green and ecofriendly technologies will further add tothe use of fermentation chemicals. The availability of raw materials, which contribute to around 55-60% of the product cost in a process, and the fluctuations in their prices are seen as negative factors in attaining smooth market growth. Some of the major companies involved in the production of fermentation chemicals are Ajinomoto Incorporation, Du Pont, Danisco A/S., Roche, BASF SE, Dow Chemical Company, Novozymes A/S and Cargill Incorporation.

Indian Scenario

Bio-Industrial Sector in India is still in its infancy, but growing steadily at CAGR of 11.2% accounting for total sales of USD 128 Millions. The multinational companies account for nearly 65% of the market, while the rest is met by the local industries (FICCI report, 2015). The Indian bio-industries are now reassessing and increasing their R&D capabilities and technical staff, establishing production units and developing an elaborate distribution system. The major industries based on fermentation in India are listed in Table 1.

The present review provides an insight into the production and R&D status of the fermentation related industries in India during the last 10 years.

Organic Acids Production

Lactic Acid

The demand for lactic acid in India was estimated at 2500 tonnes in the year 2015, which is partially met through imports. Lactochem India Ltd., Chennai, is main producer of lactic acid in India. The company produces natural L (+) Lactic Acid using cane molasses as carbon source. In recent times, another company Pratishta Industries, Secunderabad, have also ventured into lactic acid production through molasses fermentation. Efforts to replace molasses with cellulosic sugars derived from agro-residues such as sugarcane bagasse is now being tried at Godavari Biorefineries Ltd., using technology transferred from NCL Pune for the production of lactic acid at their demonstration scale bio-refinery facility in Karnataka. The researchers at NCL Pune (Adsul *et al.,* 2007) documented the production of L(+) lactic acid from cellulose derived from thermo-chemically treated sugarcane bagasse. The treated bagasse cellulose was subsequently hydrolysed using in-house cellulase produced by *Penicillium janthinellum* mutant EU1 employing *Lactobacillus delbrueckii* Uc-3 mutant strain that could metabolize cellobiose/cellotriose on addition to glucose in the hydrolysate to lactic acid. The strain produced high levels of lactic acid (67 g / L) from bagasse derived hydrolysate containing 80 g/ Lof sugars. The process registered high productivity of 0.93 g/L/h and yield ($Y_{p/s}$) of 0.83 g/g. Dumbrepatil *et al.* (2008) further reported that *L. delbrueckii* mutant Uc-3 strain can be used for producing high levels of lactic acid (166 g/l) from molasses achieving a high productivity (4.15 g/L/h). They claimed it to be one of the highest achieved lactic acid yields and productivity and advocated *L. delbrueckii* mutant Uc-3 as the potential strain for economical production of lactic acid from molasses.

Table 1: Industrial Microbiology related fermentation industries in India

Industry	Products	Application
Advanced Enzyme Technologies, Thane	Enzymes	Human, Healthcare, Textile, Leather, Food
Alembic, Vadodara	Pharma products, Erythromycin, Penicillin	R&D in Chemistry, Pharmaceuticals
Alltech Biotechnology, Bangalore	Natural yeast and Enzymes technology	Animal feed
Americos industry, Ahmedabad	Textile auxiliaries, Smart colorants	Textile
Anil Biochem, Ahmedabad	Enzymes, Microbes, Probiotics, Gluconates	Starch, Textile
Biocon India Limited, Bangalore	Lovastatin, Insulin, Insulin analogs, Bio-similar, MABs etc.	Biopharmaceuticals
Corncord Biotech, Ahmedabad	Enzymes, Statins	Food, Feed, Leather, Textile, Pharmaceuticals
Fermentor Biotech, Thane	Biocatalyst, Penicillin G amidase	Pharmaceuticals
Gujarat organics, Mumbai	Biochemicals	Pharma,Cosmetics, Agrochemical, Dyes
International Panacea, New Delhi	Biofertilizer, BiopesticidesNutraceuticals,	Recombinantproteins, Agriculture health care
Lactochem Ltd., Chennai	Lactic acid	Food, Pharmaceuticals
LumisBiotech, Mumbai	Industrial Enzymes	Textile, Feed, Food, Paper, Pharma
Lupin Pharmaceuticals, Tarapur	Rifampicin, lovastatin, other Statins	Healthcare
Maple Biotech, Pune	Cellulose, α-cellulose	Pharmaceuticals, Food
Maps (India), Ahmedabad	Industrial Enzymes	Food, Feed, Leather, Textile
Novozymes,India	Enzymes and biocatalysts	Food, Feed, Ethanol
Praj Industries, Pune	2G ethanol, Bio-diesel	Fuel
Prathista Industries Limited, Secunderabad	Multizyme, Lactic acid, gluconate, propionic acid	Feed, Food, Pharmaceuticals
Porya Chemicals, Mumbai	Amino acids	Nutraceuticals, Poultry, Veterinary
Proalgen Biotech, Chennai	β-carotene	Food, Healthcare
Resil Biotech, Bangalore	Enzymes	Food, Feed
RPGLife Sciences, Mumbai	Doxorubicin and Epirubicin, cyclosporin	Cancer and Life-saving drugs
Rozzari Biotech India, Mumbai	Enzymes	Bioscouring of wool and silk
Solaris Biochemicals, Ahmedabad	Citric acid	Food, Chemical
SPIC, Chennai	Fermentors, Fermentation ingredients for pharmaceuticals	Drug intermediates Nutraceuticals
Tex Biosciences, Chennai	Enzymes	Textile, Leather, Poultry
Zytex, Mumbai	Enzymes	Bakery, Food processing,

Dey *et al.* (2012) demonstrated a green approach for production of lactic acid using sugar cane juice by *Lactobacillus delbrueckii* in a process that involved membrane based non-neutralizing fermentation process.

Citric Acid

Citric acid is used by the food processing, cosmetics and detergent industries that was primarily produced (4,000 tons/annum) *by* Citurgia Biochemical Ltd, Surat) using molasses as substrate (Solomon, 2011). Later a commercial plant for citric acid fermentation was commissioned in 2003 by BILT (Solaris Biochemicals, Ahmedabad) using Vogelbusch proprietary citric acid production technology that uses *Aspergillus niger* spores as inoculum and low energy consuming air-lift fermenters. Citric acid production by *A. niger* LPB 21 strain that could utilize cassava bagasse as the substrate was demonstrated at laboratory and semi-pilot scale levels using solid-state fermentation (Vandenberghe *et al.,* 2004). Thermally treated cassava bagasse was found to be suitable for

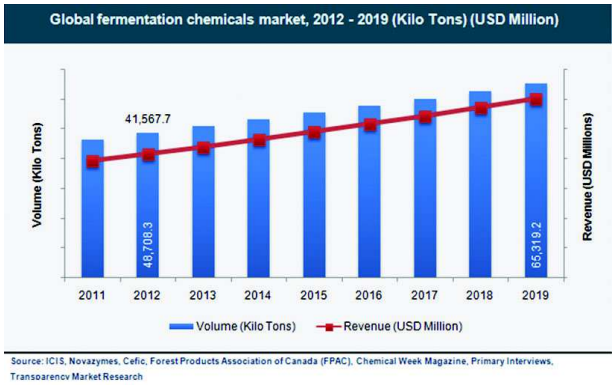


Fig. 2: Expected rise in volume and revenue fermentation products by 2019

achieving enhanced fermentation efficiencyresulting in 220 g citric acid/kg substrate. The process parameters like aeration rate (60 mL/min) and initial humidity (60%) were critical for achieving production of citric acid (265.7 g/kg of dry cassava bagasse) in horizontal drum bioreactor. Studies have also been carried out using apple pomace and red sea weed as substrates for the production of citric acid (Kumar *et al.,* 2010; Ramesh and Kalaiselvam, 2011).

Gluconic Acid

Gluconic acid is mainly produced through electrolysis of glucose. The first gluconic acid fermentation facility was set up by Prathista Industries Limited, Secunderabad in 2004 using aerobic fermentation and suitable down-stream processing based on the technology developed at CSIR (RRL, Jammu). Later, an Indian patent for gluconic acid production by a novel *Aspergillus* strain describing process for biosynthesis of gluconic acid was obtained (Puri *et al.,* 2007). Mutants of *A. oryzae* RP-NTG-12, capable of producing 72 (g/L) gluconic acid, have also been reported (Raksha *et al.,* 2012). There is no plant for itaconic acid production in India and hardly any research is being done on this aspect. Similarly, succinic acid fermentation has not yet been replacedby cost-effective chemical method although, succinic acid production by *Escherichia coli* (26.2 g/L) in 30 h has been reported at fermenter level (Agarwal *et al.,* 2007).

Amino Acids

Amino acids that are used in food, feed and pharmaceuticals industrieshavea major share of market to the tune of USD 5.4 billion in 2008 and is expected to rise steadily to USD 7.8 billion by 2013 at CAGR of 7.6%. There is no commercial fermentation plant for production of lysine and glutamic acid, therefore, India is totally dependent on imports. Theresearch institutes/industries have not shown interest on amino acid production as they are not very positive about competing with existing amino acid producing power houses (Japan, South Korea and China) for developing commercially viable process (TIFAC report). Some efforts at lab scale levels have been documented, where researchers have designed and evaluated two-stage fermentation strategy and obtained enhanced lysine productivity by ~1.5 times to that obtained using batch mode of fermentation. It was observed that initial dextrose concentration (72 g/L) in the fermentation medium was important for attaining high lysine productivity, while growth could be optimally sustained at dextrose concentration of 27g/L (Gayen and Venkatesh, 2007). The production of amino acid employing hydrolysates from rice straw and wheat bran was studied using recombinant pentose-utilizing *Corynebacterium glutamicum* with ethambutol as the medium supplement for enhanced production of amino acids, where 13.68 and 6.14 g/L of glutamate and L-lysine were synthesized respectively, thus establishing the possibility of utilizing acid hydrolysate from agro-residues as the alternative and cheap substrate for large-scale amino acid production (Gopinath *et al*., 2011). A comparison between free and immobilized cells of *C. glutamicum* MH 20-22 B for L-lysine production showed that immobilized *C. glutamicum* MH 20-22 B produced 31.58 (g/L) lysine, which was 20% higher as compared to free cells (Razak and Vishwanathan, 2015).

Exo-Polysaccharides

Exo-polysaccharides (xanthan, gelatine and pullulan) worth 2.25 billion dollars is sold annually. Pullulan alone had an annual demand of 10,000 tonnes (USD 250 million) in 2009 and is growing at 6.75% per year. An indigenous technology for pullulan production was developed at IMTECH (CSIR) Chandigarh using osmotolerant yeast *Aureobasidium pullulans* strain. The culture under optimized process conditions produced more than 70 (g/L) pullulan on corn steep liquor based medium (Sharma *et al.,* 2013). The research group showed de-oiled *Jatropha* oil seed

cake as suitable nitrogen source that can be considered as cheap alternative to costly yeast extract and peptone for attaining 83.98 (g/L) pullulan in 5 L laboratory fermenter (Roy Choudhary *et al.,* 2012). Besides pullulan, there are also reports of novel bacterial strain *Gluconacetobacter* sp. F6 capable of producing 4.5 (g/L) of cellulose under optimized culture conditions (Jahan *et al.,* 2012). Similarly a novel halo-tolerant marine bacterial isolate was shown to synthesise high levels of poly(3-hydroxy butyrate-o-3 hydroxyvalerate) (PHBV) (Moorkoth and Nampoothiri, 2016).

Antibiotics, Biomolecules and Therapeutic Proteins

The antibiotic market in India was worth USD 39.8 million in 2015. However, according to a report published in 2015 by Ministry of chemicals and fertilizers, Government of India, only rifamycin is being currently produced through fermentation route by two companies (Lupin and Sandoz). The fermentation of several antibiotics penicillin G, streptomycin, tertracylines, erythromycin, gentamycin and cephalosporinwas stopped during1990s, as these were not commercially viable any longer and are now imported. The search and discovery of novel biomolecules capable of combating cancer, inflammations, multi drug resistant pathogens, and life style and age related diseases (diabetes, hypercholestemia, alzheimer) are the key areas of research.

Rifamycin, a broad-spectrum antimicrobial drug effective against tuberculosis, AIDS-related mycobacterial infections and leprosy,is produced by submerged fermentation where the yields are quite low. Therefore, Nagavalli and co-workers (2015) subjected the producer strain *Amycolatopsis mediterranei* to sequential mutation, selection and optimization of culture conditions for developing an improved strain that produced 5.32 (g/L) of rifamycin SV. In an another recent study, the efficacy of the Rifamycin B was reportedly increased using molecular biology approach for the synthesis of better analogs of rifamycin, where, Nigam *et al.* (2015) carried out substitution of acyltransferase domain of rifamycin polyketide synthase module 6 with rapamycin polyketide synthase module 2. The resultant *A. mediterranei* S699 recombinant (*rif*AT6::*rap*AT2) synthesized novel rifamycin analogs, 24-desmethylrifamycin B and 24-desmethylrifamycin SV, with modified polyketide backbone and exhibited enhanced antibacterial activity against several rifampicin-resistant *M. tuberculosis* strains. *A. mediterranei* S699 is known to carry out very tightly regulated hierarchical amino acid utilization in defined medium that influences rifamycin B fermentation dynamics (Bapat *et al.,* 2006). The studies also showed that high production of novel glycopeptide antibiotic bahlimycin by *Amycolatopsis balhimycina* DSM 5908 was influenced by medium components as well as pellet morphology (Singh *et al.,* 2012). Reports on production and structural analysis of a lipopeptide from *Streptomyces amritsarensis* active against methicillin-resistant *Staphylococcus aureus* has also been documented (Sharma *et al*., 2014). Isolation of novel actinomycete, *Streptomyces sannanensis* strain SU118 isolated from *Phoomdi* in Loktak Lake of Manipur that exhibited antimicrobial activity against Gram-positive bacteria only was also reported (Singh *et al.,* 2014). Studies on isolation and production of efficient *Bacillus* strains for production of penicillin acylase used in the synthesis of semi-synthetic penicillins have also been reported (Vellore *et al.,* 2012; Rajendran *et al.,* 2013).

In search of novel biomolecules from diverse microbial strains of India, Department of Biotechnology, Ministry of Science and Technology sponsored a mega project "Screening for Bio-molecules from microbial diversity collected from different ecological niches" from 2007-2011.This involved nine different research institutes/universities (NEERI, Nagpur; NCCS, Pune, IGIB, New Delhi, ILS, Bhubhaneshwar, IBSD, Manipur, NIO Goa, GNDU, Amritsar, DU, Delhi and MSSRF, Chennai) for isolating diverse microorganisms from different ecological niches ranging from Himalayas, rivers, wetlands, effluents and marine samples. In three years period of project, 2, 47,000 microbial strains were isolated. The culture extracts from these were grown in three different media and methanolic extracts were subjected to robotic high throughput screening by the industrial partner in the project Piramal Life Sciences, Mumbai, for identifying microbes harbouring anti-cancer, anti-inflammatory, anti-diabetic and anti-microbial biomolecules. At the end of phase I of the project, 3643, 6676, 528 and 5443 three star hit cultures were identified as sources of anti-diabetic, anti-

inflammatory, anti cancer and anti-infective biomolecules, respectively. The selected promising and novel cultures were chosen for second phase of the project for structural elucidation, production and scale up studies. One of the cultures *Bacillus* sp., capable of producing thiopeptide which is effective in the treatment of *Clostridium difficile* associated infection was patented (Mahajan *et al.,* 2014). Piramal Life Sciences, however, discontinued its natural products division in 2015. The isolated cultures are now part of IDA approved culture repository at MCC, NCCS Pune. Presently, RPG Life Sciences, Mumbai is one of forerunner industry in India that produces life saving novel and unique products like daunorubicin, doxorubicin and epirubicin (anti-cancer drugs) in addition to cyclosporin a critical product used in organ transplant,is being produced by fermentation under stringent USP guidelines.

Statins,which are known to inhibit 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA), are produced commercially by BIOCON India Ltd., LUPIN and Krebs Biochem. Biocon India Ltd., is the world's largest producer of lovastatin based on a proprietary solid state fermentation technology (Plafractor). This novel bioreactor configuration has the advantage of both solid substrate and submerged fermentation, and poses fewer downstream processing problems during product extraction (Sreenivasan *et al.,* 2008). Some significant research papers in last few years have focussed on developing hyper-producing strains of *Aspergillus terreus* (Kaur *et al.,* 2009) and production by pellets and immobilized *A. terreus* mycelium cells in airlift reactor by continuous culture mode (Gupta *et al.,* 2009). Workers have also studied production of lovastatin by *Monascus purpureus* MTCC 369 in submerged and solid state fermentations (Sayyad *et al.,* 2007; Panda *et al.,* 2009). Studies reporting production of enzyme inhibitors i.e., α-glucosidase and α-amylase has been reported from endophytic fungi, actinobacteria like *Streptomyces, Rhodococcus, Arthrobacter* (Akshatha *et al*., 2012). These inhibitors find application in the management of diabetes, obesity andrelated disorders. The production of acetylcholinesterase (AChE) inhibitors used in treatment of neurodegenerative diseases (Alzheimer's disease, anti-cholinesterase poisoning, senile dementia, ataxia, Parkinson's diseases and myasthenia gravis) have also been reported from endophytic *Alternaria* sp. (Singh *et al.,* 2012; Bhagat *et al.,* 2016).

Enzymes

The global industrial enzyme market is expanding at a rapid rate. It was estimated at $3.8 billion in 2011 and is expected to rise to $ 5.9 billion by 2018 (Surrough *et al.,* 2012). Enzyme production market in India is rising steadily and the current estimated annual revenues are approximately Rs 2420 million (Binod *et al.,* 2013). Major industrial producers in India are Novozymes, Advanced Enzymes, Rossari Biotech, Zytex and Maps India, that cater to the textile, paper, leather, detergents, food, feed and pharmaceutical sectors (Singh *et al.,* 2016). These industries use an array of enzymes like amylases, cellulases, proteases, xylanases, phytases, asparaginases, uricases and several others. Binod and co-workers (2013) have exhaustively reviewed the research and development status of different enzymes in India.

Amylases

α-Amylase is critical and important enzyme for liquefaction of starch at high temperatures for its subsequent hydrolysis into dextrose and maltose by glucoamylases. *Bacillus* strains have been choicest source of α-amylases owing to their thermostability. The researchers have continued their quest for finding novel *Bacillus* strains and designing cheap culture media based on cassava waste and groundnut shells for amylase production (Paul *et al*., 2016; Selvam *et al*., 216). The recombinant *E. coli* and *Pichia pastoris* clones harbouring α-amylase gene from *Bacillus acidicola* TSAS1 were constructed for producing the enzyme in mixed fed batch high cell density cultivation (Prashar and Satyanarayana, 2016).

Cellulases/Hemicellulases

Cellulases, which comprise a complex of endoglucanases, cellobiohydrolases and β glucosidases, act synergistically for deconstruction of polymeric cellulose in lignocellulosics (LC) complex into fermentable sugars (Singhania *et al.,* 2010), therefore is an important area of R&D for evolving novel and catalytically efficient enzymes for bioconversions of LC into ethanol and other chemicals. In addition, cellulases enzyme components individually have specific role in different detergent, textile, food

industries (Badhan *et al.,* 2007). The recent researches have focussed on unravelling the entire lignocellulolytic secretome of *Talaromyces verruculosus* SGMNPf3 (Goyari *et al.,* 2015), *Termitomyces clypeatus* (Mukherjee and Khowala, 2015), *Malbrancheacinna momea* (Mahajan *et al.,* 2015), *Mycothermus thermophilus* (Basotra *et al.,* 2016), *Penicillium funiculosum* (Ogunmolu *et al.,* 2015), *Penicillium* sp. Dal5 (Rai *et al.,* 2016). Rapid purification and evaluation of catalytically distinct lignocellulolytic glycosyl hydrolases from thermo tolerant fungus *Acrophialophora* sp. (Rai *et al.,* 2016) and *Pyrenophora phaeocomes* S-1 have also been attempted for producing cellulose and hemicelluloses hydrolyzing enzyme cocktails (Rastogi *et al.,* 2016).

Proteases

Alkaline proteases are the most widely used enzymes which find application in detergent and leather industries. The researchers have studied the effect of amino acids on the repression of alkaline protease synthesis in halo-alkaliphilic *Nocardiopsis dassonvillei* (Sharma and Singh, 2016).A production technology including downstream processing andelucidation of structure-function relationship of solvent, detergent, thermo-alkali stable metallo-protease from psychrotrophic bacteria *Pseudomonas putida* SKG-1 was reported by Singh *et al*. (2013). Similarly, ametallo-protease stable at elevated temperatures from novel *Bacillus alkalitelluris* TWI3 isolated from tannery waste (Ananadhrajan *et al.,* 2016) and halo-alkaline protease from ascidian-associated *Virgibacillus halodenitrificans* RSK CAS1 capable of utilizing marine wastes (Sathishkumar *et al.,* 2015) have also been reported.

Phytases

Thermophilic moulds have attracted the attention of researchers as novel sources of phytases. Recently, *Humicola nigrescens* as novel and efficient source of thermostable phytase for improving nutritional quality of food was reported (Jain and Singh, 2017). Another thermophilic fungus *Sporotrichum thermophile* has been investigated for selecting a suitable fermentation method for improved phytase production (Kumari *et al.,* 2016). The phytase gene from *S. thermophile* has also been cloned and expressed in *P. pastoris* (Ranjan and Satyanarayna, 2016) and the structural and biochemical characteristics of recombinant phytase was reported by the same lab (Maurya *et al.,* 2017). The working group has also reported optimization of heterologous expression of the *Pichia anomala* phytase in *P. pastoris* and its applicability in fractionating allergenic glycinin from soy protein (Joshi and Satyanarayana, 2015).

Pectinases

Pectinases find application in fruit and fibre processing industries. The researchers have focused on producing high titers of alkaline, extracellular and thermo-tolerant pectinase by a newly isolated yeast *Pseudozyma* sp. SPJ (Sharma *et al*., 2014) and attained enhanced production of pectinase by *Saccharomyces cerevisiae* isolate using fruit and agro-industrial wastes employing response surface methodology (Poondala *et al*., 2016). The pectinase from *Aspergillus ibericus* was produced and immobilized onto functionalized nano-porous activated carbon for the treatment of pectin containing wastewater (Mahesh *et al*., 2016).

Pharmaceutically Important Enzymes/Proteins

L-Asparaginase is an important enzyme that has been established for its utility in the treatment of cancer. The enzyme prevents the proliferation of cancerous cells by decreasing the level of asparagine in the blood. *E. coli* is preferred for L-asparaginase production because of its higher specificity for asparagine with lower glutaminase activity. Production and characterization of asparaginase has been reported from a variety of microorganisms in recent past. Production, optimization, functional and molecular characterization of novel glutaminase free L-asparaginase from *Nocardiopsis alba* NIOT-VKMA08 was studied (Meena *et al.,* 2015). Asparaginase from *B. licheniformis* and *Pseudomonas otitidis* was shown to induce apoptosis in cancerous cell lines (Mahajan *et al.,* 2014; Husain *et al.,* 2016). Similarly, *Pseudomonas rhizohabitans* was shown to be a novel source of asparaginase that reduces acrylamide in processed potato chips besides exhibiting anti-cancer activity (Bhagat *et al.,* 2016). Asparaginase coding gene *ansB* from *E. coli* was cloned and expressed in new phenotypic protein expression system PichiaPink™ derived from the yeast *Pichia pastoris* (Sajitha *et al.,* 2015). Similarly,

uricase is being used as therapeutic enzyme for regulating the concentration of accumulated uric acid in gout disease. The researchers have reported new microbial strains of *Pseudomonas aeruginosa, Sphingobacterium thalpophilum* (VITPCB5) that are capable of producing high titres of catalytically efficient uricase (Khadke *et al.,* 2016; Ravichandran *et al.,* 2016).

Biomolecules Produced Using Recombinant-DNA

Hindustan Antibiotics Limited (HAL) Pune, established in 1950s as a public sector drug company through Government support later diversified into production of recombinant DNA products like rHU-erythropoietin (Hemax) in 1993. HAL is credited for introducing antiviral drugs for treatment of AIDS (Halpen, Haltax, Hexpan and Sati-HIV) in 2009. A technology for producing clot specific recombinant-DNA based streptokinase from *E. coli* was developed and transferred to Shasun Drugs and Chemicals, Chennai by the Institute of Microbial Technology, Chandigarh (Sahni *et al.,* 2007). This Clot Specific streptokinase was later developed and commercialized both in the developing countries and other worldwide markets by Nostrum Pharmaceuticals Inc., USA. In recent years, methylotrophic yeast *P. pastoris* has been used for heterologous cloning and production of different therapeutically important recombinant proteins including active streptokinase (Advitiya *et al.,* 2016), envelope protein E2 gene from different genotypes of hepatitis C virus (Perumal *et al.,* 2016) and human interferon gamma (hIFN-a) (Prabhu *et al.,* 2016). In addition, *P. pastoris* has also been reported for heterologous expression of lipase, nitrilase and â glucosidase (Vadhana *et al.,* 2013; Sohoni *et al.,* 2015; Ramani *et al.,* 2015).

Ethanol

The annual production of ethanol in India is 2.58 billion litres, which is primarily produced from 12.48 MT of cane molasses. The surplus ethanol (68 million litres) after its consumption in domestic (potable) and industrial sector (used as such as feedstock chemical) is now being used for blending petrol and is sold as E5 and E10 admixture in some states. The government plans to go for E20 by 2022 for which the existing molasses based ethanol will not be able to sustain the demand. Moreover, fluctuating molasses prices do not instil confidence in setting up new distilleries based on molasses. Therefore, sweet sorghum is being evaluated as source of ethanol for direct fermentation of sweet sorghum juice. The price of ethanol based on sweet sorghum has been estimated at Rs 13.60/L which is cost competitive. The existing molasses based distilleries suffer from low ethanol concentration in vats due to lower ethanol and temperature tolerance of distiller's yeast. The low ethanol content in the fermented mash means higher steam energy input for distillation of ethanol to rectified sprit (95 % (v/v). Researchers at IMTECH Chandigarh and VittalMallya Scientific Research Foundation, Bangalore jointly developed energy efficient alcohol production technology which was licensed to an Indian distillery company (UB group). The developed yeast strains produce10% (w/v) alcohol in the fermented mash in addition to being osmotolerantcapable of fermenting molasses at high sugar levels (25-30%) in 30 h. Due to higher ethanol in fermented wash,15-20% lesser steam for distillation was required. The current focus is now directed to develop 2G bio-ethanol conversion technologies for utilizing abundant lignocellulosics in the form of agro-residues. The government of India (Ministry of Petroleum) announced on World Biofuel day that it plans to invest Rs 5000 crores for setting up nine 2G ethanol plants based on lignocellulosics to meet the demand of E20 blended petrol by 2022. The first of the demonstration 2G ethanol plant based on bagasse has been set up at Kashipur (Uttarakhand, India) by Glycol India. The plant is based on continuous membrane based patented technology (Lali *et al.,* 2011) developed at DBT Bioenergy centre (ICT Mumbai). Similar 2G ethanol plants are also operational at PRAJ Industries, Pune and NIIST, Trivandrum. The technology required for lignocellulosics based ethanol platform involves various steps each with its own technical knowhow and thus leaves a wide scope for R&D to make it commercially viable. The first step of the process involves pre-treatment of lignocellulosics so as to disintegrate lignin from the complex separating cellulose component as fibres along with hetero-polymer xylose rich hemicellulosic fraction to certain extent. The pre-treated substrate with reduced lignin content is then hydrolysed enzymatically to monomeric sugars by cellulases, hemicellulases and other auxiliary enzymes (Singhania *et al.,* 2010; Mahajan *et al.,* 2016). The sugar-rich

hydrolysate that comes from saccharification primarily contains glucose and pentose sugars such as xylose/ arabinose/mannose/galactose (depending upon the composition of the LC substrate and pre-treatment process used). The fermentation of this mixed sugar hydrolysate that contains some inhibitors produced during pre-treatment of LC also poses challenge as the conventionally used yeast *S. cerevisiae* can only ferment hexoses but cannot utilize pentoses in addition to being sensitive to inhibitors. Dubey *et al.* (2016) evaluated the growth and fermentation efficiency of six natural yeast strains on a wide range of carbon sources, including rice and wheat straw hydrolysates, and further studied the factors that reduce the efficiency of the yeast in fermenting hydrolysates. The isolate *S. cerevisiae* MTCC4780 was found to be versatile and resulted in ethanol yields ($Y_{p/s}$) of 0.48 g/g, 0.42 g/g and 0.45 g/g, respectivelyduring fermentation of glucose, rice and wheat straw enzymatic hydrolysates in a bioreactor. The yeast isolates MTCC4781 and MTCC4796 were comparatively better producers of ethanol at elevated temperature and were able to tolerate theinhibitors. *Debaryomyce shansenii* (Menon *et al.,* 2010) and *Pichia* strain BY2 capable of fermenting xylose have been isolated from over-ripe banana (Hande *et al.,* 2013). The other important aspect in focus is the search for thermotolerant yeast that can be used for simultaneous saccharification and fermentation (SSF) of sugars in hydrolysates. The conventional yeast *S. cerevisiae*ferments actively at temperature between 28-33^{o}C, therefore, can only be inoculated after enzymatic hydrolysis step which is carried out at 50^{o}C, after adjusting the temperature. Therefore researchers have attempted simultaneous saccharification and fermentation (SSF) for bioconversion of alkali treated rice straw usingthermotolerant yeast *Pichia kudriavzevii* HOP-1, *Blastobotrysaden inivorans* RCKP 2012 strains (*Oberoi et al*., 2012; Antil *et al.,* 2015). Kumar and co-workers (2015) identified very efficient thermotolerant yeast *Kluyveromyces* sp. IIPE453 capable of fermenting both xylose and glucoserich bagasse hydrolysate obtained from the two-stage acid hydrolysis to 183 g xylitol and 165 g ethanol per kg bagasse. An oleaginous yeast strain *Cryptococcus vishniaccii* has also been reported recently for bioconversion of paper mill sludge into neutral lipids for biodiesel production (Deeba *et al.,* 2016).

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