ISSN 0000-0000
9 770000 000003

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ज्ञानेन शीलम्

Vol. 2 No. 1

ARIIBAS

January - 2014

Quest

Editorial

India is at the verge of a new transition, the common man replaced those who seemed to be unbeatable and “untouchable” for more than a decade. It looks like this is the era of revolution. People are expecting a colossal change and a fight against the monsters of corruption. There are hopes that the hitched common man will be soon set free to live a better life. The can of worms in his hands will disappear. These hopes kindled into the majority about a year ago when the lokpal scenario came into limelight. The best part is, people are getting more secular and are thinking about development, irrespective of the cast and religion. The country now is turning into one united nation. But the question is, are these expectations worth? Or is it just a momentary furry? Well, we’ll get the answers in the coming time. All we can do is wait, watch, support and hope for the best.

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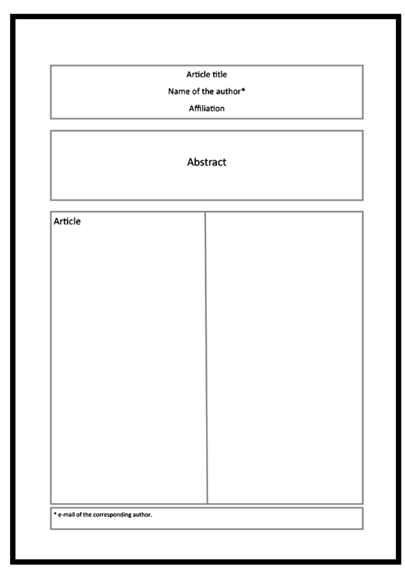
Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

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).



Jurassic park is possible and Biotechnologist can do it

A science project to bring back an extinct species of frog famous for giving birth out of its mouth has been named one of the best inventions of 2013. The Lazarus project, run by the University of Newcastle, Australia, revived the genomes of an extinct Australian frog using sophisticated cloning technology to implant a "dead" cell nucleus into a fresh egg from another frog species.

It was named as one of Time Magazine's 25 Best Inventions of the Year because it managed to resurrect the gastric-brooding frog, albeit for a short period of time. The Rheobatrachus silus, a species of frog which became extinct in 1983, was unique in that it swallowed its eggs and gave birth orally. Scientists in the team managed to extract cell nuclei from tissues recovered in the 1970s, which had been stored for 40 years in a conventional deep freezer. Their "de-extinction" project aimed to bring the frog back to life and results were published in March this year.

In repeated experiments over five years, the researchers used a laboratory technique known as somatic cell nuclear transfer. They took fresh donor eggs from the distantly related great barred frog, Mixophyes fasciolatus, inactivated the egg nuclei and replaced them with dead nuclei from the extinct frog. Some of the eggs began spontaneously dividing and growing to early embryo stage. None of the embryos survived beyond a few days but genetic tests confirmed the dividing cells contained genetic material from the extinct frog.

At the time, Professor Mike Archer, who led the Lazarus Project team said: "We are watching Lazarus arise from the dead, step by step.

"We've reactivated dead cells into living ones and revived the extinct frog's genome in the process. Now we have fresh cryo-preserved cells of the extinct frog to use in future cloning experiments," Professor Archer added.

"We're increasingly confident that the hurdles ahead are technological and not biological and that we will succeed. Importantly, we've demonstrated already the great promise this technology has as a conservation tool when hundreds of the world's amphibian species are in catastrophic decline."

The Six Big Biotech Stories of 2013

What were the most noteworthy happenings in biotech and biomedicine for 2013? It's always difficult to pick the most important developments since often their full impact isn't known or understand for years. With the caveat, though, here are the critical biotech/biomedical trends and events in 2013. In addition to their individual importance, each also have significant broader repercussions for biomedical research, healthcare, or the biotech industry:

The First Cloned Human Embryo

One of the major milestones in biomedical research this past year has to have been human cloning. Researchers at Oregon Health and Science cloned the first human embryo ever.

It's not really surprising the humans can also be cloned. The basic approach for cloning humans is not particularly different from cloning chimps, dogs, or even mice, all of which, and more, have been done. The group did over-come some notable technical challenges. However, the technical achievement isn't the compelling element. It's simply the fact that human cloning is a milestone for biomedicine. This cloning sets the groundwork for advances in stem cell and reproductive medicine.

Gene Patents Are a Thing of the Past—Sort Of

Of course, the biotech industry was paying close attention to the Supreme Court's review of Myriad's gene patents. In May, the Court ruled that human genes can't be patented, or did they?

Well, yes, the Court did actually rule that, since patents can't cover natural phenomena, genes themselves are not patentable. However, it said that processes to isolate genes, and DNA constructions made in the lab that include gene sequences are patentable. Unfortunately, though, the Court was ruling on process patented with 20 year old technology.

The processes used to isolate the genes and, to some extent, the DNA constructions used in the patents, aren't current state-of-the-art. The Myriad work predated the sequencing of the human genome by more than six year. Now, 10 years after all the DNA in humans, and many other animals, has been sequenced and cataloged, and technology has advanced to the point that large segments of DNA can be wholly made synthetically in the lab without ever going near an animal, the Court's ruling leaves open many patentability questions about the way genes and genetic elements are handled in modern biotech laboratories. Also, the loss of some key claims on many of their patents hasn't tempered Myriad's aggressive patent stance.

Since the Court ruling in May, Myriad has filed suit against Ambry, Gene-by-Gene, GeneDx, Quest, and InVitae to stop their marketing of the BRCA cancer gene tests. Ambry has countersued Myriad also with Gene-by-Gene, and so has InVitae. Well, it didn't take long for the whole process to start again.

Drawing a Bead on Alzheimer's, Parkinson's, and ALS

In October this year, researchers in the University of Leicester in the UK published a study where they were able to prevent the development of prion disease—a neurodegenerative disorder similar to Mad Cow disease—in mice using a single chemical compound. What's so special about that, there are lots of drugs tested in mice that don't work on humans, and prion disease is very rare in people? Well, it's not the drug so much as the underlying model for the disease that the drug provides evidence for.

Research is showing the prion disease, Alzheimer's, Pakinson's and related neurodegenerative diseases all seem to be caused by a similar process--a chain reaction of misfolded proteins that form clumps in the brain's nerve cells. The research group at Leicester in October targeted a protein that appeared to regulate this pathway and found that they could stop the process. In other words, they seem to have discovered something about the basic process of how this process occurs. Even though this drug may not pan out, there's a much clearer target now.

Anti-Aging Treatments Aren't Just a Cosmetic Fix Anymore

On the business front, biotechnology was booming for most of 2013. However, I think the most interesting business story in biomedicine may have been Google's founding of Calico. It's the not the fact that Google initiated a new venture that's so interesting. It's that they formed the venture to develop therapeutic solutions for the process of aging. The event marks a transition in aging research. This intriguing area of science is mature enough to move out of the research labs into the commercial sector.

While the Google story was the flashed all over the media, I know that it is not the only anti-aging venture coming to the market. A few others are flying under the radar. Although I am sure it will still be many years before we can pop some pills and feel like we're 30 again, the research in this field seems to clearly be at a point where researchers and investors believe they have some real-world practical strategies to address the complications of aging.

That view seems pretty consistent with recent developments in the science too. Work from leading researchers on aging, such as Linda Partridge and Judy Campesi, and David Sinclair. In other words, we may be on the verge of real change in the way medicine looks at and treats age-related illnesses. In fact, an experimental anti-aging drug may be moving into early trials as soon as next year.

How's Your World Feeling Today?

With regard to important biomedical things we didn't even realize we didn't know, we discovered that the wellbeing of each of is closely intertwined with the circle of microbial life that inhabits our bodies. In the last few years research has shown that we owe much more of our personal health to our microbiome—the icky critters living all over and in us—than we ever realized.

It seems our body's little inhabitants influence our weight, may be important in the development of diabetes have been implicated in heart disease and rheumatoid arthritis. The increasing realization of importance of this personal ecosystem on some many aspects of our health has prompted a real change in the way medicine views the swarm of bugs that call each of us home.

BA (6-Benzyladenine) V/S BAP (6-Benzylaminopurine)

Cytokinins are N^6-substituted adenine derivatives and cytokinins biosynthesis is through the biochemical modification of adenine. It is a class of plant hormones involved in plant growth and development (D'Agostino and Kieber 1999). The plant hormones cytokinins (CKs) comprise a class of growth regulators involved in the stress response, senescence, photosynthesis, nutrient assimilation and mobilization, as well as modulation of a plant tissue's ability to act as a sink or source of metabolites.

When I recently asked Professor Kalpesh Ishnava, Assistant Professor in Plant Biotechnology (ARIBAS), S P University, about why there are two names for the same (as I have personally found in various literature) cytokinin, BA (6-benzyladenine) and BAP (6-benzylaminopurine), the response I received was 3 things that differentiate this two component are (as he is not agreed that this both are same, yes he said that they are same but may differs at nuclear level),

Multiplication of shoot is high with BA

BAP not suitable with all plant species

Minor difference in molecular weight between both (5-10 gm/mol).

When I go through Plant Hormone-action and application (reference book), the response suggested that BAP is IUPAC name for BA.

There are other chemicals that can be named differently but are the same (glucose and dextrose come to mind). Although by understanding that they are the same cytokinin, the use appears to be culturally determined, or enforced by specific journals. While from PCTO-C's some says that they always personally prefer to use BA, based on plant biochemists' advice (Malito *et al.* 2004), but, for example, if another uses BAP in their text, would it be correct to refer to it as BA, either because of personal choice, or because the choice is imposed (by edi-tors or journals)?" These discrepancies within the world of plant science publishing can cause confusion, particularly among plant tissue culture scientists, and thus I decided to inves-tigate further.

For this professor Kalpesh Ishnava, Assistant Professor in Plant Biotechnology (ARIBAS), has demonstrated BA and BAP on ***Coccinia grandis (Lvy gourd)*** and he observe that by using BAP they get low induction and by using BA they get high induction. They also demonstrate BAP and BP both simultaneously then callus induction takes place and activity is also very high. Again they use BAP with kinetin than callus production takes place. While normally, 6-BA induces shoot elongation.

From my search in various analysis, 6-Benzylaminopurine, benzyl adenine or BAP is a first generation synthetic cytokinin that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division. BAP (benzyl adenine) is an inhibitor of respiratory kinase in plants and increases post-harvest life of green vegetables. BAP is synthetic cytokinin which together with auxins elicits plant growth and development responses. BAP is a widely used cytokinin supplement to plant growth media such as Murashinge and Skoog Medium , Gamborg's Medium and Chu's N6 Medium. It is difficult to identify the cytokinin nomenclature because of relatively complicated systematic names, which force plant physiologists to use semi-systematic as well as trivial names.

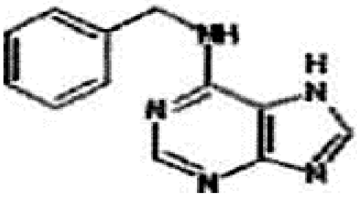
6-Benzylamino purine was first synthesized and tested in the laboratories of plant physiologist Folke K. Skoog. BA when applied to primary leaves of intact plants delayed the senescence of both the leaves and the entire shoot.

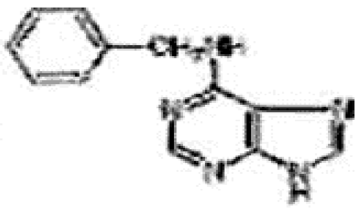
Comparison of Benzyl Adenine Metabolism in Two *Petunia hybrida* Lines Differing in Shoot Organogenesis- The uptake and metabolism of the cytokinin benzyl adenine (BA) was compared in two lines of *Petunia hybrida* Vilm, differing in their shoot organogenic response. Leaf transfer experiments using shoot induction medium containing 4.4 micromolar BA showed that leaf explants from petunia line St40 required a shoot induction period of 6 to 10 days for commitment to shoot organogenesis; whereas leaf explants from petunia TLV1 required 12 to 28 days. The short induction period of petunia St40 and the higher organogenic response was positively associated with a threefold higher absorption of BA from the medium.

Table 1 Definition of benzyladenine as per Wood (2012)

Benzyladenine	
IUPAC	N-benzyladenine or N-benzyl-7 H-purin-6-amine
CAS	N-(phenylmethyl)-1 H-purin-6-amine
Formula	$C_{12}H_{11}N_5$
Molecular Weight	230gm/mole
Melting point	230-233 °C
Appearance	White to off white powder

Structure





Benzyladenine (there is no entry for benzylaminopurine (Mol. Wt.225.25gm/mole))

[Note – practically melting point of BAP shows around 160, this is due to various reasons and they are like 1. Moisture increases, melting point decreases 2. As minor purity of product is decrease, its melting point decreases 3. As C (carbon) no. increases, melting point may decreases 4. May due to chemist variations.]

Table 2. Physiochemical Specification:

TEST	SPECIFICATION	RESULTS
Solubility	Miscible with water	Passes
pH (Neat)	For information only	11.14
Physical Appearance Color Texture	 Colorless Clear liquid	 Pale yellow Clear liquid
Soluble	Propylene Glycol & Water	Passes
Sterility by USP<71>	Sterile	Passes
Purity of Raw Material	Minimum 98.0%	98.7%

Table 3. Biological Testing:

TEST SPECIFICATION	PLANT CELL LINE	RESULTS
Supports and/or facilitates plant growth and/or shoot proliferation in two or more plant tissue cultured line with no morphological aberrations to plant	Tobacco callus, Dianthus, Achimenes, Hosta, African Violet	Passes

So most likely, scientists would select the cheaper option in their catalogue, BAP (about 30% cheaper than BA). While by analysis of various countries I get to know that some uses BA and some BAP. A country that uses BA more than BAP are like India, Germany, France, Italy, Australia , Poland, Brazil, Belgium, Argentina, Finland, Pakistan, Tunisia etc. While other using BAP more than BA are like USA, Japan, China, Canada, Spain, Mexico, UK, Netherlands, Switzerland, Israel, South Africa, Norway, New Zealand, Russia, Portugal, Nigeria, Hungary, Egypt, Kenya, Colombia, Serbia, Croatia, Jamaica etc.

There is a strong country-by-country use of either term due to its different names (also may be by various companies name). I could not answer the question which one is better, although I think both are nearly same cytokinin.

ACKNOWLEDGEMENTS

I wish to thank Dr. Kalpesh Ishnava (Assistant Professor in Plant Biotechnology, Ashok & Rita Patel Institute of Integrated Study & Research In Biotechnology And Allied Sciences (ARIBAS), S P University), Mr. Shailesh Patel (M.J.EXPORTS (Manufacture of Organic Manures and Bio-Product)), Dr. Sushil.Kajal (lecturer in botany, Ashok & Rita Patel Institute of Integrated Study & Research In Biotechnology And Allied Sciences (ARIBAS), S P University) for input, feed-back and valuable discussion.

Contributed By Taral Patel IG-IBT 10th Sem

IONIC LIQUID: A DESIGNER SOLVENT

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Abstract: Ionic liquids, are known as a green solvent and recently getting tremendous importance in the field of organic synthesis, electrochemistry and in separation technologies along with a number of other areas. In last decades, a wide range of chemical reactions were reported with great potential using ionic liquids.

Introduction:

Till date, most of chemical reactions have been carried out in organic or aqueous solvents. Organic synthesis of any material on large scale required lots of effort in terms of energy, chemical compounds, catalyst, solvents, separation, purification and crystallization etc., which releases numerous amounts of hazardous chemicals or pollutants. Green chemistry is an alternative field for innovative research in the field of chemistry, where reactions are carried out using green solvent. Many researchers have reported ionic liquids as good solvent for organic transformation1, 2. The first ionic liquid was described in 1914, for instance $[EtNH_3]^{-}[NO_3]$, having melting point 12^{0}C.

Ionic liquids are made solely of cations and anions, and the properties of ionic liquids can be altered with the variation in ionic component. This means their properties can be adjusted according to the requirement of a particular process. Hence, ionic liquids are referred as *Designer solvents.* They have many fascinating properties, which make them of fundamental interest to all chemists, since both the thermodynamics and kinetics of reactions carried out in ionic liquids are different to those in conventional molecular solvents, than the chemistry is different and unpredictable at our current state of knowledge. Therefore, looking to the need of clean and healthy environment, present article includes a brief introduction and importance of ionic liquids.

General properties of ionic liquids:

Interest in ionic liquids has been increased, because ionic liquids have several advantages over conventional organic solvents, which make them environmentally compatible. They have ability to dissolve many different organic, inorganic and organometallic materials. They are highly polar in nature and are liquid at room temperature. It consists of loosely co-coordinating bulky ions. They do not evaporate since they have very low vapor pressures. They are thermally stable, approximately up to 300^{0} C. Most of the ionic liquids are liquid below 200^{0}C which enables wide kinetic control. They have high thermal conductivity and large electrochemical properties. They have low melting points and are ease to recycle. They are immiscible with many organic solvents. They are non-aqueous polar alternatives for phase transfer processes. Their solvent properties can be tuned for a specific application by varying the anion/cation combination[3-11]. Solvent properties of ionic liquids depends on H-bond donor /acceptor ability of salt while physical properties of ionic liquids affected by charge distribution on the anions, H-bonding, polarity etc. Some basic properties of ionic liquids are mentioned below:

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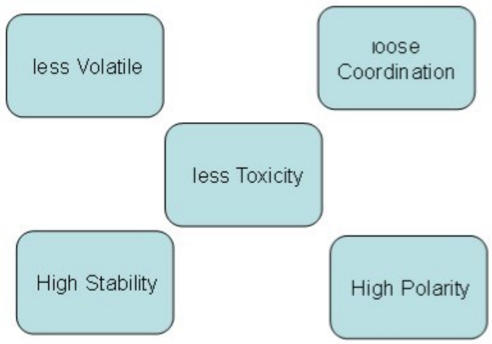

Figure 1: Basic properties of Ionic liquids.

General synthetic approach:

There are several possible combinations of cations and anions, resulting in to a variety of ionic liquids, which perform specific application or function as a potential benefit of these materials.

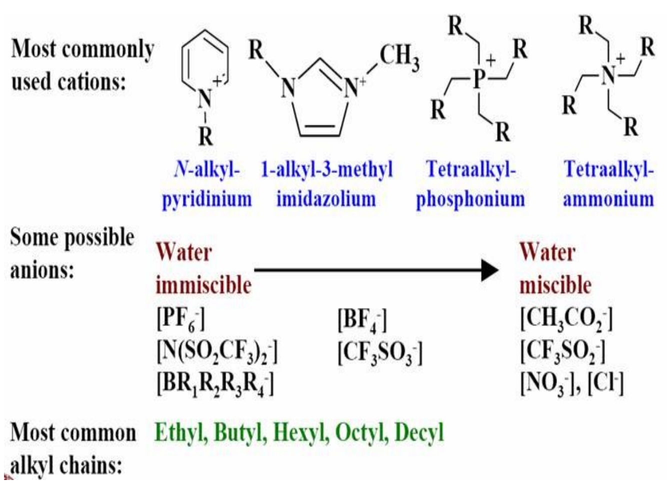

Figure 2: Most commonly used cation structures and possible anion types.[12]

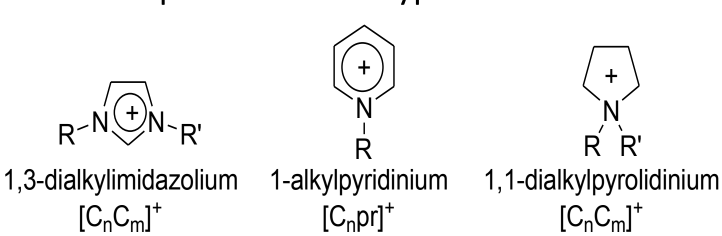

Figure 3: Some common cations used for ionic liquids.[13]

There are two basic methods for the preparation of ionic liquids: (i) metathesis of a halide salt with, first group metal ion or ammonium salt of the desired anion (ii) acid-base neutralization reactions.

About 1,018 ionic liquids are described in the literature, and approximately 300 are commercially available. The cation has a strong impact on the ionic liquid's properties and will often define its stability. Furthermore, choice of the anion controls the chemistry and functionality of the ionic liquid in general.

Applications of ionic liquids:

Many organic reactions have been successfully studied in ionic liquids includes Friedel-Crafts, Diels-Alder, Heck catalysis, chlorination, enzyme catalysis, polymerization, cracking, oxidation, hydrogenation, Sulfonation, Heck and Suzuki coupling, nitration, halogenation, reduction, diazotisation, Chiral hydrogenation, Oligomerisation, N-alkylation and O-alkylation and Aldol condensation.

In addition to that ionic liquids have applications like electrolyte in batteries, lubricants, plasticizers, solvents and catalysis in synthesis, matrices for mass spectroscopy, solvents to manufacture nano-materials, extraction, gas absorption agents, etc.

Various literature reports have also mentioned the use of ionic liquids as given below:

As catalysts:

Ionic liquid can be used in catalysis, as a combination of solvent and catalyst[14]. Many researchers have carried out alkylation and electrophilic aromatic substitution reaction of benzene using various ionic liquids[15].

As co-catalysts:

Ionic liquid can be useful if one of the ions present in ionic liquid acts as a promoter, or co-catalyst for a reaction like olefin dimerization[16-17].

As ligand sources:

In some case ionic liquids are used as a ligand source[18-20]. During hydrogenation and hydroformylation reaction an ionic liquid $[Et_4N][SnCl_3]$ was first used as a solvent, which serve as a ligand source. It was proposed that in the ionic liquid $[Et_4N][SnCl_3]$, $PtCl_2$ formed $[Pt(SnCl_3)_5]^{3-}$ and $[HPt(SnCl_3)_4]^{3-}$ complex.

As solvent for reactions:

The role played by an ionic liquid in a catalytic process is simply as the solvent for the reaction. In most of studies in this area ionic liquid being used for screening of reactions during some catalytic process without optimization or systematic investigation[21-24]

As solvents for electro catalysis:

As a conducting media, ionic liquids clearly have potential as solvents for electro catalysis synthesis[25-26]It is surprising that there has not been more research in this area, but it has great potential for the future.

Conclusion:

In summary, the present article clearly shows how the ionic liquids play an important role in the chemical reactions and in many other fields. Ionic liquids are receiving attention every day both in academic research as well as in commercial applications. Here, attempts have been made to introduce readers the importance of newly promising material known as ionic liquid (green solvents), because these material proved a very successful replacement of volatile organic solvents

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Title: Extremophiles – A Biotechnological Perspective

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Abstract: Extreme environments include those with either high (55 to 121 °C) or low (–2 to 20 °C) temperatures, high salinity (2–5 M NaCl) and either high alkalinity (pH>8) or high acidity (pH<4). Various extremophiles can tolerate other extreme conditions including high pressure, high levels of radiation or toxic compounds, or conditions that we consider unusual, such as living in rocks deep below the surface of the earth or living in extremely dry areas with very low water and nutrient supply. These microorganisms provide a important source not only for exploitation in novel biotechnological processes but also as models for research on how biomolecules are stabilized when subjected to extreme conditions.

Introduction

The term extremophile was first used by MacElroy in 1974, four decades ago. The extremophiles thrive in some of the harshest conditions on the planet. Extremophiles are source of biocatalysts that are functional under extreme conditions of temperature, pH, and salinity. Most of the extremophiles that have been identified till now belong to the domain of the archaea. However, many extremophiles from the eubacterial and eukaryotic kingdoms have also been recently identified and characterized[1].

Why are extremophiles coveted?

Although more than 3000 different enzymes have been identified till date and many of these have found their way into biotechnological and industrial applications, the present collection of enzymes is still not sufficient to meet all requirements. This is the due to the fact that many available enzymes cannot withstand harsh industrial process conditions[2]. Consequently, the characterization of microorganisms that are able to thrive in extreme environments are in great demand: such extremophiles are a valuable source of novel enzymes.Despite major advances in last two decades complete knowledge about the physiology, metabolism, enzymology and genetics of this fascinating group of extremophilic microorganisms is still limited.Currently, only 1–2 % of the microorganisms on the earth have been commercially exploited and amongst these there are only a few examples of extremophiles. However, new developments in the cultivation and production of extremophiles and success in the cloning and expression of their genes in mesophilic hosts has increased the biocatalytic applications of extremozymes.

Thermophiles

Thermophiles can be generally classified into moderate thermophiles (growth optimum 50–60 °C), extreme thermophiles (growth optimum 60–80 °C) and hyperthermophiles (growth optimum 80–110°C). Extreme thermophiles, growing optimally at 60–80°C, are distributed among the genera *Bacillus, Clostridium, Thermus, Fervidobacterium* etc. and many archaea. Their increased stability with respect to mesophilic enzymes makes them more suitable for harsh industrial conditions. In addition their theromostability is generally associated with a higher resistance to chemical denaturants commonly used in many industrial reactions[3]. They are mainly used for

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starch degradation, protease production, hyperhtermophilic enzymes for molecular biology industry, paper and pulp industry to name a few.

Fig. 1 Lion Geyser Eruption

Psychrophiles

Psychrophilic (cold-loving) or psychrotolerant (cold- -adapted) micro-organisms are found inhabiting the low temperature environments of the Earth, where temperatures never exceed 5°C. A diverse range of psychrophilic microorganisms, belonging to bacteria (*e.g. Pseudoalteromonas)* archaea (*e.g. Methanogenium,),* yeast (*Candida* and *Cryptococcus*) and fungi (*Penicillium* and Cladosporium) have been isolated from these cold environments.

How Does Psychrophile Survive? Compared to proteins from mesophiles, psychrophilic proteins show decreased ionic interactions and hydrogen bonds, possess less hydrophobic groups and more charged groups on their surface and longer surface loops[5].

Fig 2. A Psychrophile

Due to these modifications, at low temperatures psychrophilic proteins lose their rigidity and gain increased structural flexibility for enhanced catalytic function. As the psychrophilic membranes contain a higher proportion of unsaturated fatty acids, their fluidity and ability to transport nutrients are maintained under very cold conditions.

Moreover, the ability to synthesize cold-shock or antifreeze proteins as the temperature drops, the more efficient enzyme activity due to alterations in enzyme kinetics and the stabilization of microtubules enable the psychrophiles to survive.

Biotechnological Applications

More recently, enzymes from psychrophiles have become interesting for industrial application, partly because of ongoing efforts to decrease energy consumption[4].For example, with such enzymes it becomes feasible to develop laundry applications that can be performed at lower temperatures. This would reduce the energy consumption and wear and tear of textile fibers. For such processes, psychrophilic proteases, amylases or lipases have great commercial potential.The pulp and paper industry is also interested in polymer-degrading enzymes that are active at lower temperatures. The industrial dehairing of hides and skins at low temperatures using psychrophilic proteases or keratinase would not only save energy but also reduce the impacts of toxic chemicals used in dehairing. Several food processing applications would also benefit from the availability of low temperature enzymes.

Halophiles

Halophilic microorganisms require very high salt (NaCl) concentration for growth. They are found in salterns and hypersaline lakes, such as the Great Salt Lake, the Dead Sea and solar lakes in Africa, Europe and the USA.

(a) (b)

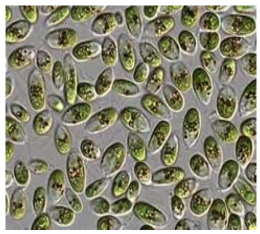

Fig 3 (a) *Dunaeilla,* a halophilic bacteria (b) Salt island, Dead Sea

Halophiles like *Halobacterium, Haloferax, Natronobacterium and Natronococcus* belong to the archaea while *Salinibacter ruber* is a bacterium.

Fig 4 Halphiles Flat, Kenya

The halophilic enzymes find application in aqueous/organic and non-aqueous media. Recently, a *p*-nitrophenylphosphate phosphatise from *Halobacterium salinarum* was used in an organic medium at very low salt concentrations after entrapping the enzyme in reversed micelles. Exploitation of reversed micelles in combination with halophilic enzymes is likely to result in the development of novel applications for these enzymes.

Adaptation and Compatible Molecules

Halophilic proteins typically have an excess of acidic amino acids (*i.e. glutamate and aspartate*) on their surface. Negative charges on the halophilic proteins bind significant amounts of hydrated ions, thus reducing their surface hydrophobicity and decreasing the tendency to aggregate at high salt concentration[6].

Halophiles respond to increases in osmotic pressure in different ways. The extremely halophilic archaea, the *Halobacteriaceae,* accumulate K^+, while other bacteria accumulate compatible solutes (*e.g. glycine, betaine, sugars,* polyols, amino acids and ectoines), which help them to maintain an environment isotonic with the growth medium[7]. These substances also help to protect cells against stresses like high temperature, desiccation and freezing.

Alkalithermophiles/Alkaliphiles

Alkalithermophilic microorganisms grow optimally under two extreme conditions: at pH values of 8/above and at high temperatures (50–85°C). On the other hand, microorganisms simply classified as alkaliphiles are mesophiles and consist of two main physiological groups: alkaliphiles and haloalkaliphiles. Alkaliphiles require an alkaline pH of 8 or more for their growth and have an optimal growth pH of around 10, whereas haloalkaliphiles require both an alkaline pH (pH>8) and high salinity (up to m/V (NaCl) = 33 %).

Fig.5 Nevada Hot Spring- Alkalithermophile

Allitermophiles as well as alkaliphiles have been from alkaline hot springs, the new alkaline hydrothermal vents[8].

Acidothermophiles/Acidophiles

Acidothermophiles thrive under conditions of low pH and high temperature. The acidothermophile *Sulfolobus solfataricus* grows at pH=3 and 80 °C. True acidophiles such as the archaea *Picrophilustorridus* and *P. oshimae* grow optimally at pH values *as* low as 0.7 and at 60 °C and produce starch-hydrolyzing enzymes (amylases, pullulanases, glucoamylases and glucosidases[8]. They can be used in the production of detergents.

(a) (b)

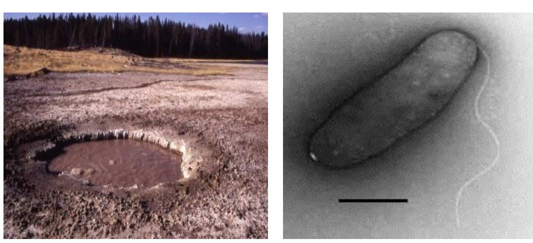

Fig. 6 (a) Acid mud pot; Yellow Stone Park, USA (b) *Moritellayayanosi* DB 21MT

Survival

Alkaliphiles have negatively charged cell-wall polymers in addition to peptidoglycan, which may reduce the charge density at the cell surface and help to stabilize the cell membrane. Cellular fatty acids in alkaliphilic bacterial strains contain predominantly saturated and mono-unsaturated straight-chain fatty acids.In order to withstand low pH, acidophiles employ a range of mechanisms such as: a positively charged membrane surface, a high internal buffer capacity, over-expression of H+ exporting enzymes and unique transport system[9].

Piezophiles

Microorganisms that like high-pressure conditions for growth are termed piezophiles. With an average pressure of 38 MPa, the world's oceans are home to piezophiles, including various thermophiles and hyper-thermophiles. Piezophilesare are distributed among the genera *Shewanella, Colwellia, Moritella, Methanococcus, Pyrococcus* and *Thermus. Moritellayayanosi* DB21MT bacterium was isolated from sediment in the deepest part of the world's ocean cannot grow at less than 50 MPa pressure[10]. Pressure-resistant proteins could be of use, in particular for food production, where high pressure is applied for processing and the sterilization of food materials[11]. Moreover, enzymes that can operate at increased pressure and temperature have great advantages in biotechnological applications. Enzymatic reactions that have a negative change in activation volume (V<0) are favored by increasing pressure, whereas reactions with a positive change (V>0) are not. The change in activation volume (V) can be used as a method to control reaction specificity[12]. However, it is not easy to cultivate piezophiles under high-pressure conditions using current technology.

Radiophiles

Microorganisms that are highly resistant to high levels of ionizing and ultraviolet radiation are called radiophiles. Examples are *Deinococcus radiodurans, Thermococcus marinus sp. nov. and Thermococcus radiotolerans.*

Fig. 7*Deinococcusradiodurans*- The world's toughest bacteria

*Deinococcus radiodurans*is contains a spectrum of genes that encode for multiple activities that repair DNA damage. The genes of three putative uracil-DNA glycosylases have been cloned and expressed to determine their biochemical function.

Why Do We Need Radiophiles?

The presence of toxic chemicals, heavy metals, halogenated solvents and radionuclides in many nuclear waste materials presents a challenging problem for separating different species and disposing of individual contaminants. *Deinococcus radiodurans*strains and other detoxifying microorganisms may be utilized to detoxify halogenated organics and toxic metals such as mercury & theoretically could be used to remove these classes of compounds selectively from mixed wastes under mild conditions[1].

Metallophiles

Microorganisms that can grow in the presence of high metal concentrations are called metallophiles. These organisms, including several members of the genus *Ralstonia,* colonize industrial sediments, soils or wastes with high contents of heavy metals. A typical feature of these metal-resistant is the presence of one or two large megaplasmids that contain genes for multiple resistances to heavy metals. These plasmids confer resistance to Zn, Cd, Co, Pb, Cu, Hg, Ni and Cr. Since pollution by heavy metals poses a threat to public health, fishery and wildlife, there has been an increased interest in developing systems that can remove or neutralize the toxic effects of heavy metals in soils, sediments and wastewaters[13].

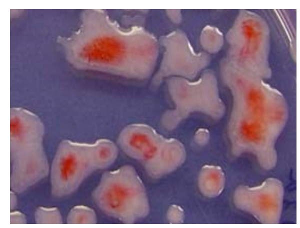

Fig. 8 Metallophilic *Ralstonia* sp.

Bacteria exhibit a number of enzymatic activities that transform certain metal species through oxidation, reduction, methylation and alkylation[14].

Limitations of Extremophiles

The major drawback of extremophilic biomass and enzyme production is the difficulty of cultivating them in laboratory and industrial environments. Some of conditions required for the growth of extremophiles, such as anaerobic, media of extreme pH, very high salinity even 5M salt may be required, which are incompatible with standard industrial fermentation and downstream processing equipment[8].

Future Belongs to Extremophiles

It will take a lot of research to turn extremozymes into industrial products. It is now possible to construct gene expression libraries from the most diverse sources. If such libraries are screened with fast and accurate detection technologies many new extremozymes will be discovered in the years to come. These extremozymes will be used in novel biocatalytic processes that are faster, more accurate, specific and environmentally friendly. Concurrent developments of protein engineering and directed evolution technologies will result in further tailoring and improving biocatalytic traits, which will increase the application of enzymes from extremophiles in industry.

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