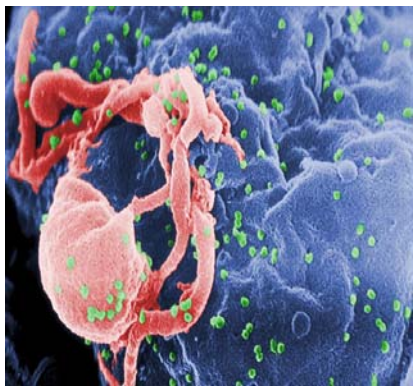

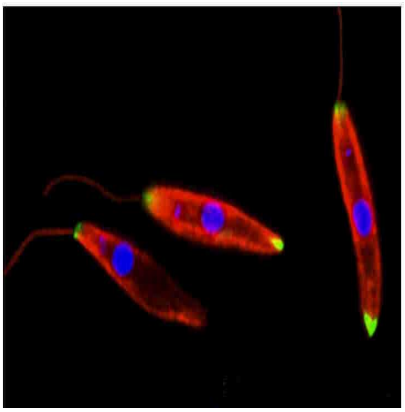

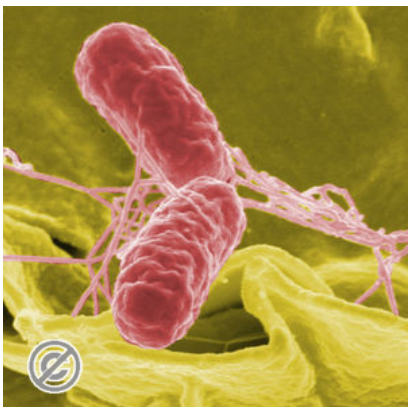

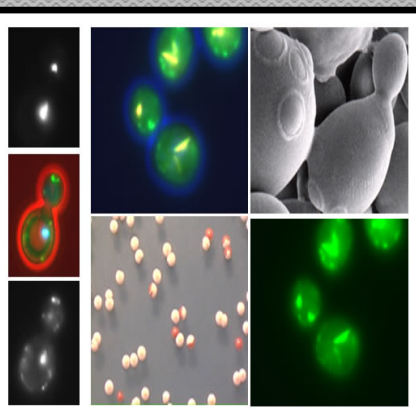

EMHGBN Newsletter

Vol. 2, Issue 8, August 28th, 2008

INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected centre of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

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HIV/AIDS in Pakistan: The battle begins

The article entitled " HIV/AIDS in Pakistan: The Battle Begins" aims to take the appropriate measures to curtail further spread of AIDS in patistan. Pakistan, the world's second most populous Muslim nation, has started to finally experience and confront the HIV/AIDS epidemic. The study was done by Dr. Mohammad A Rai, Haider J Warraich, Syed H Ali and Vivek R Nerurkar. Corresponding author of this paper, Dr. Mohammad Ali Rai is working in Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan and The paper was published in Retrovirology. 2007; 4: 22

HIV/AIDS in Pakistan: The Battle Begins

Pakistan, the world's second most populous Muslim nation, has started to finally experience and confront the HIV/AIDS epidemic. Largely portrayed as having free of this menace till now, this South-Asian republic seems to be following in suit with its HIV-havocked neighbour, India. With isolated outbreaks being reported all over the country, time already seems to be running out for the sixth most populous country in the world.

Dr. Mohammad A Rai

Aga Khan University, Karachi, Pakistan

Evolution

The first reports of HIV in Pakistan in 1987 implicate contaminated blood transfusions as one of the culprits. The other route alludes to expatriates or Pakistanis settled abroad. These seem to be the more important risk factor for acquisition of HIV, as demonstrated amply by the fact that around 70% of the total positive HIV cases from a sample of over 15,000 individuals over a period of six years (1986-1992) fell into this category. The bulk of the infected were deported workers from the Gulf States. Pakistan, as compared to its neighbours, has remained relatively safe from any indigenously acquired cases of HIV for about two decades. The situation however changed in 2004 when Pakistan experienced its first full-fledged HIV outbreak. In the remote desert town of Larkana, the HIV bubble-burst took place amongst the injection drug user (IDU) community. What this basically meant was that the virus had finally found a home-base, as evidenced later by outbreaks all over the nation.

High-Risk Populations

The HIV/AIDS epidemic in Pakistan is following along the same atypical lines as it has done so far in the rest of Asia. Starting from isolated high-risk population subgroups, the virus jumps the barrier to cross into the mainstream general populace. Once this barrier is crossed, little if at all anything can be done to prevent a complete HIV onslaught. Similar to its south-east Asian neighbours, the greatest risk for the spread of HIV in Pakistan stems from IDU. Currently estimated at over 180,000 in number, the ongoing strife in Afghanistan, the worlds largest poppy producing country, seems only to swell up this number even more in the future. IDU all over the country have started recording alarmingly high rates of HIV. According to the latest figures released by the National AIDS Control Program of Pakistan, HIV/AIDS prevalence among IDUs has jumped from 0.4% in December 2003 to 7.6% in 2004.

However, in Larkana, where Pakistan's first HIV outbreak among IDU was reported, the number approached an astounding, twenty-seven percent. After the Larkana episode, HIV has been documented among IDU all over Pakistan. Currently, IDU do not comprise the bulk of drug users in Pakistan. The number of IDU is bound to increase in the near future, and as this happens, the relative cases of HIV/AIDS will also rise. The first hurdle in the spread of HIV seems to be already traversed.
After the Larkana episode, HIV has been documented among IDU all over Pakistan. Currently, IDU do not comprise the bulk of drug users in Pakistan. The number of IDU is bound to increase in the near future, and as this happens, the relative cases of HIV/AIDS will also rise. The first hurdle in the spread of HIV seems to be already traversed.
Truck drivers are also a very important subgroup, primarily because of their role in fuelling the HIV epidemic in neighboring Madras, India. In a survey done in Lahore, Pakistan's central Hub for long-distance truckers, over 49% of them reported having sex with another man. The possibility of horizontal ellipsis across the border from India has also been raised. Once the high-risk populations have acquired the virus, it is only a matter of time before the general populace falls prey to it. IDU, commercial sex workers, truck drivers, etc., facilitate in bridging this gap. What is alarming is the fact that once the virus moves from the urban population to the rural population, the effect will be much more catastrophic, not only because the bulk of the Pakistani population resides here (only 34% lives in urban areas) but also due to almost non-existent healthcare-facilities.

Steps Underway

Decades of corruption and poor planning of resources have translated into a fight for Pakistan's very own continued existence. Keeping this in mind and the horde of other problems currently encountering Pakistan, any efforts directed towards prevention and control of HIV/AIDS are quite laudable.
The bulk of the credit in this regard goes to the private sector. Over 50 non-governmental organizations (NGO) are working to improve the HIV/AIDS status quo in Pakistan. Their work ranges from providing needle-exchange programs for IDU to spreading awareness about HIV/AIDS to the masses. Worth mentioning is the organization, 'AMAL,' which means 'action' in Pakistan's national language, Urdu. It has outreach HIV training programs focusing not only on IDU but also for the out-of-the-limelight population, female sex workers. On the other side, the current government policy falls under the auspices of the National HIV/AIDS Strategic Framework.

The program has four foci: improved HIV prevention, expanding interventions among vulnerable groups, preventing transfusion related infections and improving infrastructure. With over Rs. 2.9 billion (US $48 million) at its disposal, the program hopefully would chalk out a practical, concrete plan and then initiate work to implement it.

The Social Demographics

It may sound ludicrous but the fact remains that to properly combat any problem, the affected have to first accept it and then conquer over it. The society in Pakistan has as yet not accepted HIV/AIDS as having anything to do with them. Trends may be changing, but the age-old stigmas and taboos related to HIV still persist. HIV is considered extremely shameful, particularly in the rural setting. Even discussions on this topic are frowned upon. Awareness about HIV/AIDS in general is extremely limited. The severity of the situation could be deduced from a survey conducted among school teachers in the capital city, Islamabad. An outstanding sixty percent of the teachers responded by saying that 'they thought HIV was irrelevant in our cultural setting. This awareness and acceptance issue would indeed be a big challenge, because 'teachers' as well as 'children' will need to be taught.

UNAIDS latest figures estimate the number of cases in Pakistan bordering 85,000. Underreporting and limited surveillance means that the actual number of infected is much higher. Keeping in mind the poor healthcare facilities, the appallingly low literacy rate (in 2001, the illiteracy rate for Pakistani women over 15-year old was 72%) and a mushrooming population (growth rate of Pakistan lies at 2.5%); the stakes for a battle against HIV are indeed very low.

Conclusions

The situation concerning Pakistan and HIV is indeed very precarious. The country lies at a very crucial junction. HIV has as yet not exploded. Most of the populace remains safe, as for now. However, concentrated epidemics have emerged, which means that very little time is left before a steep rise in infections occurs. The battle against HIV/AIDS in Pakistan has to be fought on a number of fronts: not just the afflicted population, but also on changing peoples' perspectives and ushering in the proper government policies and response measures. Neighbouring China serves as a good example to follow as regards formulation of a national policy about HIV/AIDS. The Government has to come forward and face the truth about HIV in Pakistan. Embarking not only upon national-level mass awareness programs, practical steps including wide-spread screening for the high-risk populations has also to be instituted. Stigma and discrimination about HIV/AIDS in society could only be removed when prominent figures including politicians and sport stars start discussing about HIV/AIDS in public. As soon as this stigmatization barrier is overcome, a major chunk of the battle against HIV in Pakistan would be conquered. What has to be reiterated again is that the time to act is now. Timely steps taken at the present can go a long way in preventing a wide-spread HIV epidemic in Pakistan.

Role of *Mycobacterium vaccae* in the protection induced by first generation *Leishmania* vaccine against murine model of leishmaniasis

The article entitled " Role of Mycobacterium vaccae in the protection induced by first generation Leishmania vaccine against murine model of leishmaniasis" aims to indicate the role of Mycobacterium vaccae (M. vaccae) as an adjuvant mixed with either autoclaved Leishmania major (ALM) or freeze-thawed killed L. major (KLM) in increasing protection in susceptible and resistant mice. The study was done by Keshavarz Valian, Khoshabe Abdollah Kenedy, Nateghi Rostami, Miramin Mohammadi and Khamesipour. Dr. Ali Khamesipour is the corresponding author of the article who works in Center for Research and Training in Skin Diseases and Leprosy, Medical Sciences/University of Tehran, Tehran, Iran. The paper has been published in Parasitology Research, March 2008, Epub ahead of print.

Leishmaniasis represents a group of diseases caused by different species of *Leishmania* with a broad spectrum of clinical manifestations associated with the main forms of cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), visceral leishmaniasis (VL). Globally there is an estimation of 1.5-2 million new cases of leishmaniasis each year and leishmaniasis is associated with about 70 000 deaths per year. In leishmaniasis management, vector and reservoir control measures are not always effective. To date, despite of decades of efforts, there is no vaccine available against any form of human leishmaniasis. Various *Leishmania* antigens showed to induce protection when used with IL-12 as an adjuvant in animal model of leishmaniasis.

Limitations in using IL-12 justify searching for an appropriate adjuvant to accelerate induction of a Th1 type immune response and protection. *Mycobacterium bovis* BCG (Bacillus Calmette-Guérin) has long been used as an adjuvant in field efficacy trials of candidate vaccines against leishmaniasis. However, BCG requires strict cold-chain delivery and induces a lesion that leaves scar after cure. There are reports that *Mycobacterium bovis* BCG inoculation induced autoimmune reactions and might contribute in developing transient inflammatory arthritis.

Dr. Ali Khamesipour

Center for Research and Training in Skin Diseases and Leprosy, Medical Sciences/University of Tehran

Other mycobacterial preparations, such as killed *Mycobacterium vaccae* induces a Th1 response with less side effects compared to *Mycobacterium bovis* BCG. *M. vaccae* is a soil organism which was used in clinical trials as a potential immunotherapeutic agent for treatment of tuberculosis and some other diseases. In this study the role of *M. vaccae* as an adjuvant mixed with either autoclaved *Leishmania major* (ALM) or freeze-thawed killed *L. major* (KLM) in increasing protection in susceptible and resistant mice was studied.

Nineteen groups of BALB/c and nineteen groups of C57BL/6 mice, 10 mice per group were immunized 3 times in 45 days interval with either 2 doses of KLM ($1x10^8$ or $1x10^6$) or 3 doses of ALM ($1x10^8$, $1x10^7$, $1x10^6$) alone or mixed with either BCG (1×10^7 CFU) or 2 doses of *M. vaccae* (1×10^7 or 1×10^6) in a volume of 100 µL and a group of mice was injected with PBS as control of immunization. Immunized groups of mice were challenged with 2×10^6 promastigotes of L. Major, harvested at stationary phase at the base of the tail.
KLM was prepared by washing *L. major* with PBS, and then thimerosal was added to the pellet as a preservative in final concentration of 1:10,000 w/v. The final preparation was then freeze-thawed 5 times. ALM was prepared by washing of *L. major* 5 times and then the pellet was aliquoted and autoclaved for 15 min at 121 ˚C, 15 PSI.

Blood samples collected before and after challenge were used to titrate anti-*Leishmania* antibodies. The specific total IgG was measured using ELISA method. Briefly, 96-well micro titer plates were coated with *Leishmania* lysate in carbonate-bicarbonate buffer. Plates were blocked and dilutions of serum samples (1/1000, 1/100 and 1/10) were added to the plates. Finally, HRP-conjugated anti-mouse antibody was added and optical density was determined at 450 nm.

This study showed that in both susceptible BALB/c and resistant C57BL/6 strains of mice, combination of ALM or KLM with low dose of *M. vaccae* increased protection. Protection showed by development of a smaller ulcer size upon challenge by *L. major* in immunized mice compared with the PBS injected control group. The lower antibody titer was seen in group of mice with the smallest ulcer size. The present data showed that mice received low dose of *M. vaccae* mixed with ALM had a lower titer of serum IgG. In contrast, mice received a high dose of *M. vaccae* showed a higher level of IgG Ab titer. *M. vaccae* when administered at a suitable dose was shown to elicit a Th1 immune response. The primed Th1 response induced by injection of *M. vaccae* seems to be transient and it was shown that in human might return back to a Th2 type of immune response.

The mechanism(s) underlying the possible therapeutic effect of *M. vaccae* is not yet known. The time duration between vaccine injections and also the time interval between immunizations and challenge is a crucial factor for the generation of protection. The main reason for the selection of time between vaccination and booster injections and challenge in this study was to mimic *Leishmania* vaccine clinical trials. In this study administration of low dose of *M. vaccae* or BCG alone significantly prevent lesion development upon challenge with live virulent *L. major* in both susceptible BALB/c and resistant C57BL/6 mice. This is an indication that development of an effective vaccine against any form of leishmaniasis needs an appropriate *Leishmania* antigen(s) and more importantly identification of an effective adjuvant**.**

Medical Industry Overview

INDUSTRY SNAPSHOT

The global medical industry is one of the world's fastest growing industries, absorbing over 10% of gross domestic product of most developed nations. It constitutes of broad services offered by various hospitals, physicians, nursing homes, diagnostic laboratories, pharmacies and ably supported by drugs, pharmaceuticals, chemicals, medical equipment, manufacturers and suppliers.

The medical and health care industry provides enormous employment opportunities to choose from. Apart from using the services of medical professionals, this industry also utilizes the expert services of public policy workers, medical writers, clinical research lab workers, IT professionals, sales/marketing professionals and health insurance providers.

SIZE OF THE INDUSTRY

The United States of America has one of the largest medical and healthcare industries in the world, followed by Switzerland and Germany. The USA's medical industry comprises of more than 750,000 physicians and 5,200 hospitals. USA witnesses approximately 3.8 million inpatient visits and 20 million outpatients visit on a daily basis. Furthermore, the United States of America has the largest workforce i.e. one in every 11 US residents employed in the health care business.

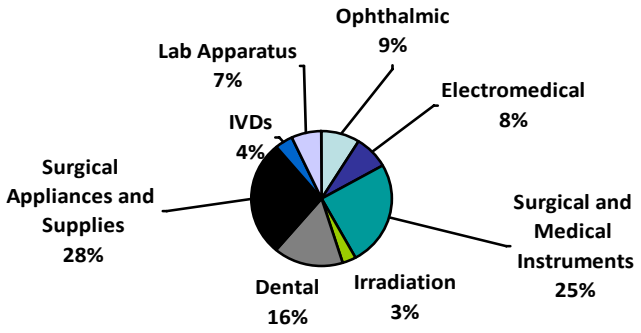
The Global prescription drug market was $550 billion in the year 2006. Also, the total health care expenditures across the world were $4.5 trillion last year. Of which, US solely account for $ 2.2 trillion, $ 2 trillion in OECD countries and remaining $ 0.3 in other countries of the world.

MAJOR SEGMENTS OF THE INDUSTRY

The global medical industry is highly fragmented, comprising of various ancillary sectors namely medical equipment and supplies, pharmaceutical, healthcare services, biotechnology, and alternative medicines sectors.

- **Medical Equipment and Supplies:** Consists of various establishments or units engaged in designing, manufacturing, selling and distributing of surgical and medical instruments, ophthalmic, lab apparatus, electro medical, dental, irradiation, surgical appliances and supplies.

Medical Equipments/Supplies



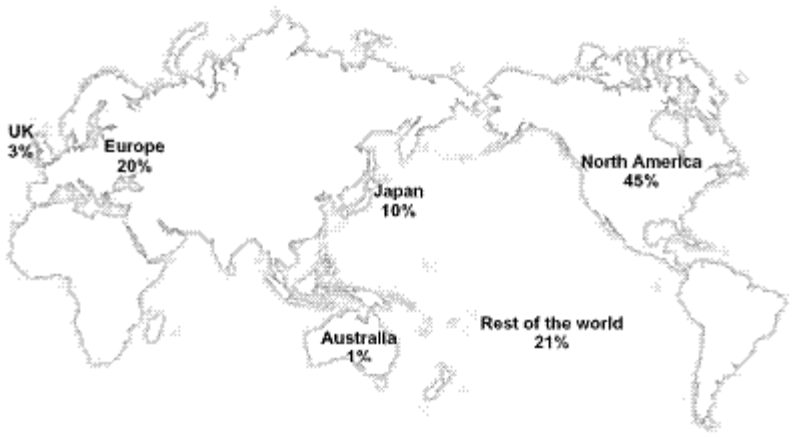
- **Healthcare Services Industry:** It includes various establishments dealing in different type of services like testing, outsourcing, transcription, quality assurance, validation, compliance, chemical analysis, and other types of services. The global market share of biotechnology services industry is worth US $ 50 billion, which is soon expected to witness a hike in coming years. Presently, pharmaceutical testing service industry values to US $ 5.9 billion, which is predicted to reach US $ 9.5 billion by the end of 2009. Microbiological testing service industry accounts for US $ 2.4 billion. Globally, the medical outsourcing services industry accounts for approximately US $ 200 billion.

- **Pharmaceutical Industry:** Comprises of several establishments involved in developing, researching, marketing and distributing drugs or medicines. Globally, the market share of pharmaceutical industry is US $340 billion. The global pharmaceutical sales account for US$ 602 billion, with an annual growth rate of 7%. In the year 2006, the global pharmaceutical exports totaled US $ 271.9 billion having an annual growth rate of 10%.

- **Biotechnology Industry:** It is one of the most research-intensive segments of the global healthcare industry. Biotechnology industry is composed of many establishments, which are engaged in making wide variety of biotech products. Biotechnology is primarily being used by the pharmaceutical industry but there are other industries like agriculture, mining, waste treatment industries as well, which are making continuous use of biotechnology. Biotechnology companies focus on developing methods or products used for preventing, diagnosing and treating dozens of life threatening and chronic diseases. The biotechnology industry has mushroomed since its inception and at present it is equivalent to US $ 50.7 billion. China, USA, India, Australia, and France are the market leaders of biotech products in the world.

- **Alternative Medication Industry:** It includes various groups involved in the promotion of different forms of alternative medications and therapies like ayurveda, homeopathy, aromatherapy, massage therapy etc. The total market size of alternative medicine is valued at US $2.7 billion while global market for traditional therapies accounts for US $60 billion.

Share of global market (sales)[5]



KEY GROWTH DRIVERS OF THIS INDUSTRY

There are various factors, which govern the growth of the medical and healthcare industry. Some of the key factors are:

- Continuous investments in research & development has resulted in increased productivity and better quality of drugs, medicines, medical instruments, hospital equipment, and other medical supplies used in medical industry.
- Provides employment to large chunk of human population. United States of America has the largest workforce i.e. one in every 11 US residents employed in the health care business.
- Increased costs in the medical treatment in the developed nations have driven patients to migrate to Asian countries.
- Rise in ailments among the ageing population especially in developed nations has led to the increase in demand of variety of drugs or medicines.
- Innovative techniques of drug discovery and drug development, new cures and treatments, gene testing for insurance, genetic predictions of disease and related issue, human cloning and reproductive technologies are the other key drivers of the medical industry.

FUTURE PERSPECTIVE OF THE INDUSTRY

The future perspective of medical industry seems to be immensely bright and encouraging for this industry in terms of the expected surge in global demand and upsurge in investments. Several trends such as globalization, continuous investments in research and development, newer techniques of drug development and discovery, product proliferation, mergers and acquisitions are the key drivers of this industry.

Increasing corporatization of Private Healthcare in the backdrop of a growing and affluent middle class is an emerging trend that has been pushing the growth of this industry. Health Insurance and Medical Tourism are the other significant trends, which are governing the global healthcare and medical industry. Most of the nations are now emphasizing on the accreditation of medical professionals so as to ensure legitimacy of the services provided by them. Robust advancement in the field of information technology will allow critical medical data to be processed and transferred quickly over larger distances, thereby saving time of both the patients and physicians in the speeding delivery of treatment.

[Source: The medica, Global Healthcare Marketplace, http://www.themedica.com]

Multiple Displacement Amplification (MDA)

In many situations there may not be sufficient DNA collected from patient or population cohorts to meet the requirements of genome-wide analysis of SNPs, genomic copy number polymorphisms, or acquired copy number alternations. When the amount of available DNA for genotype analysis is limited, high performance whole-genome amplification (WGA) represents a new development in genetic analysis. It is especially useful for analysis of DNA extracted from stored histology slides, tissue samples, buccal swabs, or blood stains collected on filter paper. The multiple displacement amplification (MDA) method, which relies on isothermal amplification using the DNA polymerase of the bacteriophage phi29, is a recently developed technique for high performance WGA. The main challenge of WGA methods is to obtain balanced and faithful replication of all chromosomal regions without the loss of or preferential amplification of any genomic loci or allele. In multiple comparisons to other WGA methods, MDA appears to be most reliable for genotyping, with the most favourable call rates, best genomic coverage, and lowest amplification bias.

Introduction

During recent years, numerous methods have been reported to be capable of amplifying from small numbers of cells or degraded DNA. A novel method of WGA, termed multiple displacement amplification (MDA) and using the φ29 enzyme that has very high fidelity, proof-reading activity and processivity, was first described in 2002. MDA can generate large quantities of genomic DNA product from small amounts of template. Using a hyperbranched replication mode, MDA has been reported to be capable of amplifying complete genomes with little loss of genetic information. MDA is dramatically changing the way that environmental studies can be conducted and is enabling new research strategies for microbial genetics, ecology, and infectious diseases. The availability of commercial MDA kits has simplified the process, with relative ease of preparation and amplification, generating microgram DNA amounts from nanogram starting templates.

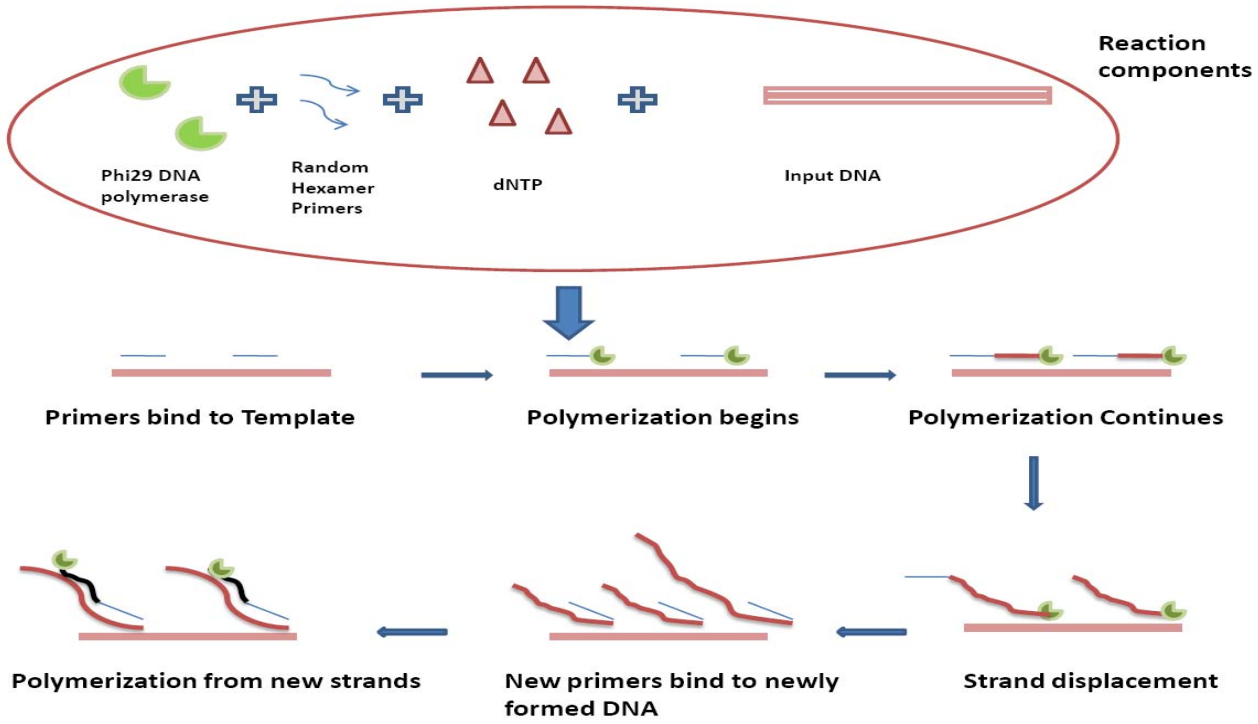
MDA process

MDA procedure amplifies genomic DNA at 30º C using bacteriophage Phi29 DNA polymerase within a few hours. Principally, DNA synthesis is initiated on denatured double-stranded DNA with modified random oligonucleotide primers, generating multiple replication forks. DNA synthesis proceeds by displacement of the DNA strands and in this way, secondary priming events occur on the displaced produced DNA strands. Hence, random priming allows synthesis of both stands, resulting in double-stranded products.

Advantages

This high fidelity DNA amplification method enriches each amplified DNA strand providing unlimited high molecular weight genomic DNA from clinical samples.

DNA sequencing and high density DNA array analysis has shown that MDA products are suitable for high resolution genetic analysis. Furthermore, MDA-DNA templates can be used in molecular analyses genome amplification based on MDA has been successfully used to amplify eukaryotic genomic DNA in molecular epidemiology and DNA sequencing template preparation.



Overview of the principle of multiple displacement amplification

MDA-generated DNA product is >10 kb, and its performance is demonstrated for a variety of applications, including single nucleotide polymorphism (SNP) analysis, restriction fragment length polymorphism (RFLP) and comparative genome hybridization. MDA was capable of accurate WGA from <10 human cells. This simple and robust method also uniformly amplified the human genome directly from whole blood without a requirement for DNA purification.
Another advantage of MDA-based single-cell genomics is that it is easily automated and can be combined with high throughput cell sorting of large numbers of microbes.

Limitations

The amplification efficiency of MDA is diminished as the molecular weight of the starting material decreases, which is problematic for amplification of formalin-fixed archival DNA.
The main limitations of using this method on single cells are the high allele drop-out (ADO) and preferential amplification (PA) rates compared with direct PCR on DNA from a single cell (without previous WGA). ADO is defined as the random non-amplification of one of the alleles present in a heterozygous sample. ADO decreases the accuracy of the genotyping of a sample and can lead to misdiagnosis in applications such as preimplantation and prenatal diagnosis. Two possible solutions to reduce the impact of ADO are to increase the number of loci that are studied and to analyze several replicates of the same sample. PA involves the relative over amplification of one of the alleles in comparison to the other. PA is random and affects small stretches of genomic DNA. Another consequence of PA is that, although MDA efficiently amplifies the genomic as well as the mitochondrial DNA, it should not be used for quantitative studies.

Applications

Generating high molecular weight genomic DNA from clinical specimens

Isothermal multiple strand displacement amplification (IMDA) of the whole human genome is a promising method for procuring abundant DNA from valuable and often limited clinical specimens. However, whether DNA generated by this method is of high quality and a faithful replication of the DNA in the original specimen, allowing for subsequent molecular diagnostic testing requires verification. Procuring sufficient DNA from clinical specimens to perform in-depth analysis at the molecular level is often hindered by the volume and cell count of a patient sample. For example, fine needle aspirates and small tissue biopsy specimens often yield inadequate amounts of DNA using Southern blot analysis. In addition, DNA obtained from small clinical specimens is frequently exhausted when multiple tests are performed, despite the use of techniques that require small amounts of DNA, such as the polymerase chain reaction (PCR). Hence, this method that representatively amplifies the entire genome with minimal bias would have a substantial impact on the capability to perform comprehensive molecular analysis using small patient specimens.

Environmental WGA amplification

The low biomass samples from nitrate and heavy metal contaminated soils yield DNA amounts which have limited use for direct, native analysis and screening. MDA can be used to amplify whole genomes from environmental, contaminated, subsurface sediments. By first amplifying the gDNA, biodiversity analysis and genomic DNA library construction of microbes found in contaminated soils will be possible.

Complex DNA libraries amplification

MDA can be used for amplifying plasmids and long strands of DNA in a cell-free system using phi29 polymerase. Such a system would be ideal for replacing the tedious solid-phase agar scraping steps used for the amplification of complex cloning-based libraries. The use of MDA would remove this bottleneck, as MDA is able to amplify complex mixtures with high accuracy and efficiency.

Uncultured organisms amplification

MDA can amplify the few femtograms of DNA in a bacterium up to micrograms to be used in sequencing. Previously, newly discovered microbes could only be sequenced if cultured isolates could be grown to sufficient quantities to provide the necessary DNA template. DNA template is obtained directly from individual cells without requiring development of culture methods. While amplification bias in the MDA reaction can result in under representation and loss of some sequences, the rewards are great often providing the first genomic sequences obtained from novel species.

Recent improvements to MDA methods

Reduction of the MDA reaction volume has recently been found to give greater specificity for a single copy DNA template. Specific amplification was enhanced by reducing amplification of contaminating DNA and nonspecific synthesis such as primer dimers, essentially by eliminating unnecessary reaction volume. Amplification specificity is improved using a 60 nl microfluidic reaction. Other research has led to a reduction in certain chimeric DNA rearrangements observed when sequencing from MDA reactions in which two segments of the genome are incorrectly joined together. The chimeras can be resolved during genome assembly by obtaining sufficient sequencing depth. However, reducing them before sequencing would simplify the process. Treatment of completed MDA reactions with S1 nuclease to remove single-stranded DNA forms resulted in an 80% reduction of chimeras. It has now been shown that MDA directly causes the rearrangements, and the reaction pathway leading to their formation has been solved. The branched DNA intermediates formed during MDA can result in some DNA strands being extended on an initial template and then being displaced and extended on a different template resulting in the chimeras.

The studies also clarified that the S1 nuclease treatment should reduce chimeric reads even for sequencing methods that do not use cloning steps since MDA generates the chimeras rather than subsequent library cloning steps. Insight into the enzymatic pathway of chimera formation also suggests MDA reaction modifications that are currently being tested to reduce the occurrence of chimeras. Identification of the specific types of rearrangements that occur might also lead to improved informatic methods to anticipate and resolve chimeras during the assembly process.

References

1) Simona Panelli , *et al*. Towards the analysis of the genomes of single cells: Further characterization of the multiple displacement amplification, *Gene*, 2006, 372: 1–7.

2) Rajyalakshmi Luthra and L. Jeffrey Medeiros. Isothermal Multiple Displacement Amplification *A Highly Reliable Approach for Generating Unlimited High Molecular Weight Genomic DNA from Clinical Specimens*. *J Mol Diagn*, 2004, 6:236-242.

3) Roger S Lasken. Single-cell genomic sequencing using Multiple Displacement Amplification. *Cur Opin Microbiol*, 2007, 10:510–516.

4) Melissa J, *et al*. The use of multiple displacement amplification to amplify complex DNA libraries. *Nucleic Acids Res*, 2008, 36:, No. 5 e32

5) http://www.biocompare.com/technicalarticle/754/GENETIC-SEQUENCING-AND-VARIATION-Representational-Amplification-Of-Genomic-DNA-from-GE-Healthcare-formerly-Amersham-Biosciences.html

National Institute of Genetic Engineering and Biotechnology

National Institute of Genetic Engineering and Biotechnology (Islamic Republic of Iran)

Introduction

The National Institute for Genetic Engineering and Biotechnology (NIGEB) was established in 1989 under the supervision of the Ministry of Science, Research and Technology of Iran. Since then, NIGEB has been given a mandate to undertake original, state-of the art research activities. It was established with dual purposes of promoting research in avant-garde areas of biological sciences and biotechnology and providing advanced training and education programs for scientists and students from other universities and academic institutions.

The center activities have been focused on five major areas, including, Medical Biotechnology, Plant Biotechnology, Animal and Marine Biotechnology, Industrial and Environmental Biotechnology and Basic Sciences. In each division a strong emphasis is placed on a highly innovative and pioneering investigation in the fields of both basic and applied biology.

Research Departments

- **Plant Biotechnology**
  - Plant Biotic Stress
  - Plant Abiotic Stress
  - Molecular Breeding
- **Medical Biotechnology**
  - Genetic Diseases

Research Areas

1. Detection of parental origin of chromosome 21 trisomy and its implication in study of Down syndrome etiology in Iran
2. Analysis of FMR1 mutations in fragile X syndrome and its relationship with FRAXAC1 and DXS548 markers haplotype
3. Study of Multiple Sclerosis (MS), Using New Mlecular Biology Methods
   - Cloning Autoantigen (S) involved in MS
   - Association of HLA with MS
   - Relationhsip between MS and infectious agents such as Bacteria and Viruses
4. Study of Genetic Diversity in Iranian Population
   Analysis of DNA samples from different populations using genetic markers
5. The Human Genome Diversity Project of Iran
   This Project (HGDPI) aims to collect biological samples from different population groups throughout Iran, with the aim of building up a representative database of human genetic diversity in Iranian populations.

6. The Human Genome Diversity Project of Iran
   This Project (HGDPI) aims to collect biological samples from different population groups throughout Iran, with the aim of building up a representative database of human genetic diversity in Iranian populations.
7. Detection of parental origin of chromosome 21 trisomy and its implication in study of Down syndrome aetiology in Iran
8. Analysis of FMR1 mutations in fragile X syndrome and its relationship with FRAXAC1 and DXS548 markers haplotype
9. Study of Multiple Sclerosis (MS), Using New Mlecular Biology Methods
   - Cloning Autoantigen (S) involved in MS
   - Association of HLA with MS
   - Relationship between MS and infectious agents such as Bacteria and viruses
10. Study of Genetic Diversity in Iranian Population
11. The Human Genome Diversity Project of Iran
    - This Project aims to collect biological samples from different population groups throughout Iran, with the aim of building up a representative database of human genetic diversity in Iranian populations
12. Investigation of LEBER point mutations in Iranian family
    - Leber's hereditary optic neuropathy (LHON) causes acute loss of vision in young adults, predominantly males. We are going to diagnosis those mutations and find the rate of mutations in Iran and find which one of these common point mutations in Iranian population.
13. Neurotrophins and their involvement in the nervous system
14. Phenylketonuria (PKU)
    - Studying the spectrum of mutations in the phenylalanine hydroxylase (PAH) gene which cause the metabolic disease phenylketonuria (PKU) and haplotypes associated with them in the Iranian population.

❖ Immunology

Research Areas

1. Immunobiology of Fetal Cells in Maternal Blood
2. Immunological Reactions Assay of Recombinant Drugs
3. Development of Novel Antibody-mediated Resistance against Plant Viruses
4. Construction of DNA Vaccines Vector for Hepatitis B and C

Aims

- To study the Immune response aginst HBs-Ag of Hepatitis B virus in Mice
- To study the Immune response against core antigen of Hepatitis C virus
- To compare immunity of DNA vaccine with recombinant protein vaccine against Hepatitis B
- To study the Applications of DNA vaccine technologies for prevention (against infectious disease) and immunotherapy

❖ Animal Biotechnology

Research Areas

1. Detection and Molecular Characterization of Infectious Bursal Disease Field Isolates by RT-

PCR and RFLP

2. Molecular Detection and Genotyping of Foot and Mouth Disease Viruses by RT-PCR and Nucleotide Sequencing
3. Detection of Infectious Bronchitis Virus and Differentiation of Serotypes by RT-PCR and Sequencing of S1 Gene

❖ Industrial and Environmental Biotechnology

Research Areas

1. Biotechnology Products
    - Production of recombinant human growth hormone
    - Productions of other therapeutic proteins such as GM-CFS
2. Industry and Environment (Environment Protection)
    - Characterization of microbial strains being able in desulfurization of petroleum and petrochemicals by genetic analysis of isolated Iranian strains.
    - Construction of recombinant strains capable of desufurization of petrochemicals without distracting the carbon skeleton of oil hydrocarbons.
3. Microbial Leaching (Bioprocess)
    - Microbial Leaching of Copper from Sulfide Ores is investigated with the cooperation of the chemical engineering department of Tarbiat Modarress University
    - Molecular identification of acidophile bacteria oxidizing iron and copper, their genetic modification and culture optimization for using in pilot scale and industry

❖ Basic Sciences

Research Areas

1. Over-expression of salt resistant gene in tobacco in order to obtain enhanced resistance to osmotic stress.
2. Hyper methylation of *APC* gene promoter and its application as a molecular marker in esophageal cancer patients in Iran.
3. Identification of molecular markers involved in salt tolerance in *Aleuropus Lagopoides* through applying Protemic methods.
4. Bioassay of recombinant protein.
5. Effects of NSAIDs on neuronal cells and Schwann cells in cultures.
6. Development of hippocampal cell culture.
7. Schwann cell culture from different sources without use of growth factors.
8. Separation and pure culture of glial cells including astrocytes, oligodendrocytes and microglia cells from CNS with minimum of time and media.
9. Study of the type one Insulin-like Growth Factor Receptor (*IGF-IR*) expression in human prostate cancer cells.
10. Production and purification of monoclonal and polyclonal antibodies to hGH.
11. Synthesis and expression of Hybrid protein GM-CSF and ST in *E.coli* and studying the immunogenicity of them.
12. Development and maintenance of various cell lines.
13. Studying the Intermediate protein in the induction of IFN-$Alpha_2$ Effect.
14. Expression and regulation of *IGF-1* gene in prostate cancer.
15. Immunogenicity of non structural Hybrid protein NSP-4 in Rotavirus infection.

New Yeast Trick for Eating Favorite Food

ScienceDaily (July 31, 2008) — It is well known that yeast, the humble ingredient that goes into our breads and beers, prefer to eat some sugars more than others. Glucose, their favorite food, provides more energy than any other sugar, and yeast has evolved a complex genetic network to ensure that they consume as much glucose as possible whenever it is available. UC San Diego bioengineers have recently identified a previously unknown mechanism that allows yeast to shut down the metabolism of another sugar, galactose, when they sense glucose in the environment. The findings will be published online by the journal Nature on 30 July 2008.

This research marks the first discovery of post-transcriptional gene regulation in a key model for gene regulation in higher organisms: the galactose genetic system in the yeast *Saccharomyces cerevisiae*.
Molecular biologists have long thought that the primary mechanism for regulating genes is through proteins called transcription factors, which can either increase or decrease the activity of a gene by binding directly to the DNA. However, a paradigm shift has occurred in recent years as researchers have shown that the control of genes frequently occurs at the intermediated stages between transcription and the formation of functional proteins. This "post-transcriptional" regulation provides cells with an additional level of control over phenotypic expression. The UCSD team demonstrated that the glucose network actively shuts down the galactose network by degrading messenger RNA that would otherwise go on to form the enzymes needed to metabolize galactose.
A better understanding of the yeast galactose network could lead to new insights in human cell behavior, human physiology and metabolic diseases such as diabetes. "The more we know about gene networks, the more we learn about how they can fail," said Bennett.

Feeding Yeast the Microfluidic Way

The work also highlights the kinds of important biological insights that scientists can gain by studying how gene networks operate in dynamic, life-like environments, rather than in steady-state environments. The bioengineers built yeast growth chambers in which food is delivered by microfluidic tubes. The design allowed for the raising and lowering of glucose levels with great control, while keeping galactose levels steady.
Bioengineering professor Jeff Hasty, the senior author on the Nature paper explained their new work demonstrating that the environment can be modified in a highly controlled way enable us to monitor single cells in order to see how specific gene networks respond to the environmental changes.
By controlling the exact growth conditions with microfluidic technology, the engineers determined that the canonical models for the yeast metabolic network underestimated how quickly and nimbly yeast can switch from galactose to glucose. When the glucose pulses started coming faster and faster, the model underestimates the ability of the yeast to react to the glucose pulse by shutting down the galactose metabolic network.
The experimental system was much better than the computational models predicted. Hasty who stressed the utility of their tried-and-true engineering approach said that the model started filtering out the glucose pulses too soon. They drove their system with a sine wave in typical engineering fashion, and sure enough, they learned something interesting. The undulating sine wave represents pulses of glucose delivered to the yeast cells while galactose levels remained constant.

This discrepancy between the experimental results and the model predictions got the bioengineers thinking about what could be happening that is not captured in the current model. A combination of computational modelling and experimental work led the researchers to a new post-transcriptional control mechanism in which jumps in glucose increase the degradation rate of messenger RNA that are crucial for the functioning of the galactose metabolic network.

Modified Salmonella Slows Tumor Growth

ScienceDaily (July 30, 2008) — Attenuated Salmonella bacteria engineered to express the Fas ligand (FasL) accumulate in tumors and reduce their growth, researchers report in the July 29 online issue of the *Journal of the National Cancer Institute*.

Salmonella typhimurium concentrates in tumors following intravenous injection in mice. Taking advantage of that observation, Markus Loeffler, M.D., and John Reed, M.D., Ph.D., of the Burnham Institute for Medical Research in La Jolla, Calif., engineered a genetically modified, less pathogenic strain of Salmonella to express FasL, a signalling protein that can attract neutrophils and can promote tumor cell killing by cytotoxic T cells. Although FasL is toxic when injected into the bloodstream, the authors hypothesized that Salmonella might be used to safely target this protein to tumors.
In the current study, Loeffler, Reed, and colleagues injected mice with tumors derived from mouse breast and colon cancers with attenuated FasL-expressing Salmonella.
Following the treatment, primary tumor growth was substantially inhibited in mice with either breast or colon tumors and lung metastases were reduced in the mice with breast cancer. The anti-cancer effect appeared dependent on the presence of inflammatory cells called neutrophils.
Although toxicology and other studies are needed before the approach can be tested in human clinical trials, "these results from murine cancer models suggest that FasL-expressing [Salmonella] could offer an acceptable strategy for employing FasL and possibly other toxic cytokines for cancer therapy," the authors conclude.

[Adapted from materials provided by Journal of the National Cancer Institute, via EurekAlert!, a service of AAAS.]

Announcement

ICHSTA-2008

International Conference on Healthcare Systems and Technology Assessment

The aim of the ICHSTA Conference is to provide an international forum for theoretical, practical and technical discussion among researchers, developers and users of healthcare systems from academia, business, government and industry.

Medical devices represent a central dimension that all healthcare systems and related issues are engaged with, linked to or affected by. ICHSTA 2008 will focus on research and development in theory and practical implementation of medical device development processes, usability, economics, logistics, technical assessment and procurement policy evaluation.

Multidisciplinary Assessment of Technology Centre for Healthcare

Conference: 21, 22 October, 2008
PhD Consortium: 22 October, 2008

Al Bustan Rotana Hotel, Dubai, UAE

Theme: "Medical Device Design, Utilisation, Evaluation and Procurement"

Announcement

Biotechnology and Society: Prospects and Challenges

November 11-13, 2008

Department of Biological Sciences & UNESCO Chair for Desert Studies

Yarmouk University

Irbid-Jordan

Scope and objectives

In the last decades, biotechnology has been witnessing tremendous developments. With the improvement of new approaches and modern technology, biotechnology industry and applications are acquiring new horizons enabling them to expand the quality of their products and applications.

Biotechnology has contributed towards the exploitations of organisms or biological processes through modern techniques which could be profitably used in medicine, agriculture, animal husbandry, and environmental applications.

Biotechnology has applications in several industrial areas including; health care, crop production and agriculture, non-food uses of crops (biodegradable plastics, biofuels, and vegetable oils ...etc) and environmental uses. Modern biotechnology finds promising applications in areas as pharmacogenomics, drug design and production, genetic testing and gene therapy, agriculture, bioengineering, bioremediation and biodegradation. Bioethics must be considered as cornerstone in the future of biotechnology and genetic engineering. This has to secure consistent and proper practice that should not be vulnerable to political whim.

Major topics

- **Agricultural Biotechnology**
- **Medical Biotechnology**
- **Industrial Biotechnology**
- **Nanobiotechnology**
- **Environmental and Alternative Energy**
- **Genomics and Proteomics**
- **Bio security and Bio ethics**

[Web site: http://www.yu.edu.jo/biotech2conf/index.html]

Title: Human Immunodeficiency Virus-1 (HIV-1)
Description: Scanning electron micrograph of HIV-1 (in green) budding from cultured lymphocyte. This image has been colored to highlight important features. Multiple round bumps on cell surface represent sites of assembly and budding of virions.
[Source: http://en.wikipedia.org/wiki]

Title: Leishmania major
Description: Leishmania is a parasite which causes human leishmaniasis. The genus Leishmania is unique among parasites in that its species are divided based not on the form and structure of the cells, but rather on the pathology and symptoms of the disease they cause. Leishmania may infect many vertebrates, but in human hosts the infection most frequently stems from the bites of sand fly vectors or, in the case of Leishmania major, from gerbils and other small rodent vectors. Leishmaniasis can take three forms: dermal cutaneous leishmaniasis, visceral leishmaniasis, and mucocutaneous leishmaniasis. Dermal Cutaneous leishmaniasis, generated by Leishmania major, causes sores on the human host's skin, which range in appearance and degree of discomfort. The sores are treatable with antibiotics and preventable with vaccination. Visceral leishmaniasis, produced by Leishmania donovani, is identified by an enlargement of the liver and spleen; symptoms include breathing difficulties, edema, diarrhea, and bleeding mucus membranes. If untreated for an extended period of time, visceral leishmaniasis may result in death. Mucocutaneous leishmaniasis, caused by Leishmania Brazilians, initially yields topical sores at the time of the vector bite, however secondary infections may lead to permanently disfiguring ulcerations in the mucus cavities of the mouth and nose
[Source: http://en.wikipedia.org/wiki]

Title: Salmonella
Description: *Salmonella* is a Gram-negative bacterium. It is found in many turtles and other reptiles. They cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel. On blood agar, they form moist colonies about 2 to 3 mm in diameter. When the cells are grown for a prolonged time at a range of 25—28°C, some strains produce a biofilm, which is a matrix of complex carbohydrates, cellulose and proteins. The ability to produce biofilm can be an indicator of dimorphism, which is the ability of a single genome to produce multiple phenotypes in response to environmental conditions. Salmonellae usually do not ferment lactose; most of them produce hydrogen sulfide which, in media containing ferric ammonium citrate, reacts to form a black spot in the centre of the creamy colonies. Color-enhanced scanning electron micrograph is showing *Salmonella typhimurium* (red) invading cultured human cells.
[Source: http://en.wikipedia.org/wiki]

Title: Sacharomyces cerevisiae cells in DIC microscopy
Description: Saccharomyces is a genus in the kingdom of fungi that includes many species of yeast. Saccharomyces is from Latin meaning sugar fungi. Many members of this genus are considered very important in food production. One example is Saccharomyces cerevisiae, which is used in making wine, bread, and beer. Other members of this genus include Saccharomyces bayanus, used in making wine, and Saccharomyces boulardii, used in medicine.
[Source: http://en.wikipedia.org/wiki]