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Posters

Igor L Katkov, J Biotechnol Biomater 2018, Volume 8
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Xeropreservation (drying without freezing) as the viable alternative to lyophilization (freeze-drying)

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Biostabilization (a.k.a. biopreservation) is a process that leads to cessation of the basic chemical and biological reactions so the bio samples can be pooled and stored (biobanked) for long time. There are 5 basics ways of achieving long-term storage, which all essentially lead to vitrification of cells. Three of them, namely slow freezing, equilibrium and kinetic vitrification are cryogenic, i.e., they require cryogenic (far below above 0°C) stable storage of the bio samples. The other two: freeze-drying lyophilization (LPh), and vacuum/air flow drying at temperatures above 0°C (xeropreservation, XP), don't require cryogenic storage if sufficiently high amount of water has been removed. In this presentation, we compare lyophilization vs. xeropreservation and show that XP is far more advantageous both from its far lesser damage, especially for cry sensitive items like mammalian cells, and from the practical point of view of its scalability and ability to stably store at substantially higher than 0°C temperatures, which is beneficial for many applications. We will also present the glass transition temperature (Tg) diagram and show that the Tg of the sample cannot be higher than the highest temperature of drying in the cycle to the contrary to very often reported otherwise. The sources of the error in the estimation Tg will be discussed. In regards to the scalability of XP, the three major approaches, namely drying in a thin layer, spraying and foaming will be compared. We will then show the advantages of foaming over the other two.

Recent Publications:

1. Katkov II and Levine F (2004) Prediction of the glass transition temperature of water solutions: comparison of different models. Cryobiology 49:62-82.
2. Katkov II et al. (2006) Low- and high- temperature vitrification as a new approach to biostabilization of reproductive and progenitor cells. International Journal of Refrigeration 29:346-357.
3. Katkov II (2014) Stopping biological clocks: The science and art of biopreservation. BioProcess International 12(4):42-52.

Biography

Igor L Katkov is a trained biophysicist with 30+ years of experience in cryobiology and cryogenic engineering. His last years of research have been focused on the fundamental aspects of kinetic vitrification (K-VF) as well on designing the practical system for K-VF KrioBlast™ (in cooperation with V F Bolyukh). Currently, the Head of the Laboratory of the Amorphous state at the Belgorod National Research University BelSU, Russia. He has recently accepted a Professor level position as the Head of the Laboratory of Cryobiology at the V I Kulakov Research Center of Obstetrics, Gynecology and Perinatology (RCGOP), Moscow, Russia and Chief Scientific Officer of Celltronix, San Diego, CA, USA.

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Kaloyan K Petrov et al., J Biotechnol Biomater 2018, Volume 8
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Novel *Bacillus* sp. isolates producing 2,3-butanediol have the potential to degrade lignocellulose

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2,3-Butanediol (2,3-BD) is a valuable bulk-chemical with industrial applications as fuel additive and reagent in manufacturing of moistening and softening agents, perfumes, fumigants, insecticides, explosives, plasticizers and printing inks. The present work is dedicated to the development of bio-based process for its production by non-pathogenic strains from renewable, waste, abundant, and inexpensive substrate as the lignocellulosic biomass. Ten *Bacillus* sp. strains were isolated from different soil, rhizosphere, and yogurt samples and selected for their ability to produce 2,3-butanediol from glucose. Based on 16S rRNA gene sequences, seven of them (13, 14, 16, 24, 39, 49, and 55) were affiliated to *B. licheniformis*, two (1RA, 1RB) - to *B. cereus* group, and one strain (5RB) belonged to *B. amyloliquefaciens* group. Considering the strains potential to degrade lignocellulose, their hydrolytic enzyme activities were tested using AZCL (azurine cross-linked) substrates. Nine strains were able to degrade cellulose, since they liquefied HE-, DEAE-cellulose, and β-glucan. Several strains degraded the hemicellulose polysaccharides xyloglucan, xylan and arabinoxylan. Importantly, the strains fermented the main lignocellulose monosaccharide components D-xylose, L-arabinose, D-mannose, and D-galactose. Eight of the strains utilized branched arabinan, 7 of them - galactomannan, and 7 - inulin (all spread in the plant biomass). Disaccharides utilization profiles revealed that all novel strains were able to ferment sucrose, lactose, maltose, and cellobiose. In conclusion, promising non-pathogenic producers of 2,3-BD were isolated. Displaying wide spectrum of active hydrolytic enzymes, they could be successively used in the development of a new biotechnology for 2,3-BD production from lignocellulose, currently known as the world largest, but weakly explored biomass source.

Biography

Kaloyan K Petrov is Head of the Department of Chemical and Biochemical Reactors in the Institute of Chemical Engineering, Bulgarian Academy of Sciences. His work is devoted to optimization of downstream processes by the techniques of bioprocess and metabolic engineering. The team develops biotechnologies for microbial production of platform chemicals and fuels by conversion of waste or renewable energy resources, including the cutting-edge biotechnologies for 2,3-butanediol production from glycerol and starch by the use of natural and recombinant strains, and novel bio-processes for lactic acid synthesis by utilization of starch and inulin. Other topics of his work are the fermentative production of enzymes and valuable chemicals in respect of their industrial application, strain's improvement by gene engineering, microbiological and molecular biological tools, development and analysis of probiotics and prebiotics.

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Penka M Petrova et al., J Biotechnol Biomater 2018, Volume 8
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De novo whole genome sequencing of 2,3-butanediol producing *Bacillus* sp. strain 5RB

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Compared to the chemical synthesis, the biotechnological route for 2,3-BD production has numerous economical and ecological advantages. Microbial synthesis by non-pathogenic strains allows the use of renewable raw materials during cheaper and less complex industrial fermentation process. In the present work, the whole genome sequence (WGS) of a novel isolate *Bacillus* sp. strain 5RB, capable to produce 2,3-BD was completed. WGS was based on TruSeq DNA PCR-library, Illumina SBS technology and Prokka pipeline identifying the locations of protein-coding sequences, tRNA, and rRNA genes (Macrogen Inc.). The genome of *Bacillus* sp. strain 5RB is 3.91 Mbp in size, without plasmids, and contains 3839 genes. The largest part of the genome is connected with amino acid transport and metabolism (289 genes), carbohydrate transport and metabolism (225), inorganic ion transport (176), and energy production and conversion (170 genes). Cell wall, the membrane, and envelope were encoded by 176 genes; translation, ribosomal structure and biogenesis - by 161; post translational modifications and chaperones - by 96 genes. Interestingly, 871 genes are with unknown functions (23% of the genome). Considering the species affiliation of the novel strain, the sequences of 5S, 16S and 23S rRNAs showed that *Bacillus* sp. 5RB is a member of *B. amyloliquefaciens* group, closely related to *B. velezensis*, *B. siamensis* and *B. tequilensis*. Its carbohydrates utilization profile, however, is substantially different from these of the mentioned species, giving convincing evidence that the new isolate represents a new species, promising producer of 2,3-BD. The genes, involved in 2,3-BD synthesis by Bacullus sp. 5RB were identified in the chromosome. For the first step, the decarboxylation of pyruvate to α-acetolactate, is responsible α-acetolactate synthase (EC 2.2.1.6), encoded by alsS gene, and putatively - by ilvB, and ilvH genes. Acetolactate conversion to acetoin is performed by α-acetolactate decarboxylase (EC 4.1.1.5), encoded by alsD, adjacent to alsS. The last step, the reversible reduction of acetoin to 2,3-BD is putatively catalyzed by the enzyme 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), acting as acetoin reductase, and encoded by two remote genes - *bdhA*1 and *bdhA*2. Large spectrum of genes, encoding glycoside-hydrolases was presented: *amyE* and *malL* (amylases), *xynA* (xylanase), *xynB* (xylosidase), *xynD* (arabinoxylan arabinofuranohydrolase), *xynC* (glucuronoxylanase), *eglS* encoding endoglucanase (cellulase), *etc*.

Biography

Penka M Petrova is Head of Gene Expression Laboratory at the Institute of Microbiology, Bulgarian Academy of Sciences. Her main interests are in the area of microbiology and molecular biology of Gram-positive bacteria, including isolation and genetic characterization of LAB, searching for new enzymatic activities, prebiotics utilization and synthesis, genes cloning and expression. She is author of more than 60 scientific publications and book chapters, cited more than 530 times. She is a leader of a number of research projects, funded by the National Scientific Fund, Republic of Bulgaria, Chr. Hansen A/S, and State Key Laboratory of Dairy Biotechnology of Bright Dairy & Foods Co. Ltd.

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Monika Chovanova et al., J Biotechnol Biomater 2018, Volume 8
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Production strategies of thyroid peroxidase from *Branchiostoma belcheri* in *Escherichia coli*

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Peroxidases are catalytic enzymes that reduce hydrogen peroxide to oxygen and water and also oxidize a various substrates. They are widely used in various branches of biotechnological industry as they are also applicated in variety of high potential biotransformation reactions. Problem of using this enzyme is that it is usually difficult to obtain an enough yield of enzyme or for their high-cost production. The aim of our research is to produce thyroid peroxidase from *Branchiostoma belcheri*, for the structural studies and also because of its possible use in biotransformations. The BbePOX1 gene sequence was prepared synthetically, codon-optimized for expression in *Escherichia coli* and cloned into the pET-21a vector. The aims of the project are to select the optimal conditions for the production of thyroid peroxidase (e.g. expression vector selection, expression and purification conditions procedures, etc.). To optimize expression from vector and protein purification point of view we choose vectors for the high efficient expression of peroxidase (vector with T5 promoter and vector with fusion partner). To achieve the highest yield of protein with the highest degree of solubility and enzyme activity we have also focused on the optimal temperature of production, selection of suitable host strain, concentration of inductor, selection of cultivate media and fermentation conditions. After isolation of a sufficient amount of active recombinant thyroid peroxidase, we will be able to study its structure in the following steps, as well as potentially used it in oxidoreduction biotransformation reactions.

Biography

Monika Chovanova has completed her Master´s degree in Biotechnology at the Comenius University in Bratislava, Faculty of Natural Sciences, and Department of Molecular Biology. Theme of her diploma thesis was Heterologous expression and solubilization of synthetic peroxidase gene Mag2C8 from *Magnaporthe oryzae* in *Escherichia coli*. After graduation in 2017, she began the PhD study in the same department in the theme: Preparation of producers of biologically active substances.

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Notes:

Ekaterina S Shakhova et al., J Biotechnol Biomater 2018, Volume 8
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Luciferase of the syllid polychaete *Odontosyllis undecimdonta*

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Bioluminescence is one of the most beautiful and fascinating natural phenomena. Methods for biological and medical research and various techniques based on luciferin-luciferase reactions play an important role in modern science and are widely used from analytical methods *in vitro* and in vivo, including tests for various analytes, to real time bio-imaging of live systems. *Odontosyllis undecimdonta* is a marine syllid polychaete that produces bright internal and exuded bioluminescence. Despite over fifty years of biochemical investigation into *Odontosyllis* bioluminescence, the light-emitting small molecule substrate and catalyzing luciferase protein have remained a mystery. Here we describe the *O. undecimdonta* luciferase, its amino acid sequence and some biochemical properties. Moreover, no homologous proteins in publicly available datasets were identified. This suggests that the syllid polychaetes possess an evolutionarily unique luciferase among all characterized luminous taxa.

Biography

Ekaterina S Shakhova is student of biological faculty at Moscow State University

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Notes:

Eva Struharnanska et al., J Biotechnol Biomater 2018, Volume 8
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Thermostable catalase as an excellent supplement for growth media of industrially important lactic acid bacteria strains

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Lactic acid bcteria (LAB) are exceptionally important strains in food industry. It is a heterogeneous group of Gram-positive bacteria that are acid-resistant, generally non-sporing, microaerophilic bacilli or cocci which have the same metabolic and physiological properties including the ability to degrade carbohydrates and produce lactic acid. LAB include many genera, e. g. *Oenococcus, Sporolactobacillus, Teragenococcus, Vagococcus, Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Streptococcus, Aerococcus, Carnobacterium, Enterococcus* and *Weisella.* They are usually catalase-negative strains, which represents a big disadvantage in food production in comparison with pathogenic bacteria as staphylococci and listeria existing in the same environment, because one of the most used disinfection agent is hydrogen peroxide that is utilized by catalases. We focused on increase in LAB surviving through the disinfection using thermostable catalase (produced in our laboratory) in growth media. In our functional test 10 mM hydrogen peroxide was applied to bacteria and this suspension was cultivated for 60 minutes. 10 mM thermostable catalase AfKatG was added to solid media to cultivate bacteria afterwards. Our control samples were cultivated without catalase addition. As predicted there was no difference in the growth of pathogenic bacteria with or without catalase addition to media. However, we showed a huge positive effect on surviving LAB. With addition of catalase to solid media we gained 2-38 times higher number of surviving colonies than in control samples without catalase. We can assume catalase as an excellent supplement for growth media of food strains.

Biography

Eva Struharnanska has completed her Master degree in Biotechnology at the Comenius University in Bratislava, Faculty of Natural Sciences, Department of Molecular Biology in 2015. She has also completed her Master degree in Pharmacy at the Comenius University in Bratislava, Faculty of Pharmacy in 2017. She started her PhD studies in Biotechnology after graduation in 2015 at the same Department of Molecular Biology. The theme of her studies: New strategies in production of recombinant proteins; and she focuses on production and purification of a thermostable catalase.

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Notes:

Lydia Bondareva et al., J Biotechnol Biomater 2018, Volume 8
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Herbicide absorption and translocation in plants and soil using radioisotopes

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Understanding pesticide metabolism in plants and microorganisms is a key component for the development, the safe and efficient utilization of these compounds, and for bioremediation of these chemicals in contaminated soil and water. The use of isotopes as tracers may offer additional information, since they allow and may be differentiated with great accuracy from the ions of the compound, which are already in the environment, even when both show a similar chemical behavior. The liquid scintillation spectrometry technique (LSS) is knowingly the most adequate one in order to determine the radiation with low penetration power, such as β radiation, emitted by ^{14}C, since it shows adequate sensitivity and reproducibility. The use of a certified pattern for the studied herbicide is indicated for the adequate calibration of the liquid scintillation equipment (scintillation counter). In order to quantify the radiolabeled herbicide, the main purpose of preparing samples is obtaining a homogeneous and stable solution that is adequate for the LSS analysis. Thus, radioisotopes was used for environmental behavior and in plants studies, since they provide some advantages in comparison to chemical measures, including greater sensitivity, stepwise description of a particular element in a metabolic system, and pesticide position and detection through x-ray films and/or radio image (in plants) and liquid scintillation (in plants and soil), respectively.

Biography

Lydia Bondareva has completed her PhD from Lomonosov's State University, Moscow, Russia. She is Senior Researcher in F.F. Erisman Federal Scientific Centre of Hygiene, Department of the Analytical Methods of Control, Russia. She has more than 70 papers in reputed journals and has been serving as an Editorial Board Member of repute.

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Notes:

Djalila Boudemagh et al., J Biotechnol Biomater 2018, Volume 8
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Synthesis and physicochemical characterization of hydroxyapatite HAP nanoparticles

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Over the last ten years, significant research efforts are focused on the development of the nanoparticle hydroxyapatite (HAP). This biomaterial is used widely as dental filler material, bone graft substitutes in orthopedic applications, hard tissue paste and tissue engineering. The research works has been directed toward the control of the composition, the morphology, and the physico-chemical properties in order to obtain biocompatible biomaterials. However, the difficulty is limited by the synthesis of hydroxyapatite nanoparaticle as a pure compound; the reduction of particle size from micro to nano level also significantly improves their biological activities. In this study, we were interested in preparing a pure phase, crystalline HAP with required morphologies and particle sizes under our experimental conditions. The effects of the mode of preparation, duration of aging, calcinations temperature, pH and concentration of precursors are investigated. The prepared samples are characterizated by Fourier transform infrared (FTIR), X-ray diffract ration (XRD), Transmission electron microscope (TEM), IR and TEM. Nanohydroxyapatite was successfully synthesized by sol gel method and result conforms functional groups like (-OH) and presence of a phosphate group. The XRD patterns reveal that hydroxyapatite is a major phase presented in samples. The crystallite size of the HAP nanoparticles increased with the temperature.

Biography

Djalila Boudemagh is an Associate Professor at the University Ferhat Abbas of Setif. In 2010, she completed her PhD in Material Physics at University of Grenoble, France. Her research activities are focused on the conception, characterization, and evaluation of the behavior of pharmaceutical compositions able to improve the efficiency and/or decrease the side effects of pharmaceutical drugs.

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Notes:

Mihai Gidea et al., J Biotechnol Biomater 2018, Volume 8
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Research on the use of amino acid products in the treatment of sunflower crops

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Due to the high oil content and ecological plasticity, sunflower is grown on large surfaces, reaching 20 million to 26 million ha worldwide (FAOSTAT). Due to the biological peculiarities, sunflower is a culture that requires special attention from the farmers because it has sensitivity to imbuking, to drought, to the preparation of germinative bed, and some hybrids are susceptible to diseases and Orobanche sp. Lately, specialists in agriculture have proposed using amino acid products as a solution to reduce the stress caused by external factors on sunflower crops. The researches were carried out in the Romanian Field at Experimental Farm M Domneasca, under the conditions of the process, in a monofactorial experience with the following variants: 1. Witness; 2. Raiza mixed seed treatment 4l / t (RM); RM + Naturamin WSP (NW) x 2 x 0.5 l / ha; 4. RM + NW 2x0.5 + Retenol 1% o (R); 5. Rm + NW 2x0.5 + R 1% o + Pleniflor 0.8 l / ha (P); 5. RM + NW 2x0.5 + R 1% o + P + Terrenova 1l / ha. During the research, the uniformity of growth, biomass accumulation, chlorophyllity and yields were monitored. Research has shown that all treatments with amino acid products have had a stimulating effect on sunflower culture. The production growes obtained were statistically assured.

Biography

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Accepted Abstracts

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Genetics of seed phenolic content and antioxidant activity in diallel crosses of sesame, *Sesamum indicum* L.

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Proper understanding of genetic mechanisms involving in the expression of total phenolic content and antioxidant activity would help in planning effective breeding programs in sesame. The objective of this study was to evaluate genetic variability for total phenolic content and antioxidant capacity in twelve sesame lines and investigate gene action in 6x6 diallel analysis. The field experiment was conducted at Mora during cropping season from 2011 to 2013 in randomized complete block design (RCBD) with three replicates. Data analysis showed significant ($p<0.05$) variability in the sesame cultivars for these traits. Broad sense heritability was high for total phenolic content ($h2=0.96$) and for antioxidant activity ($h2=0.99$), indicating preponderance of genetic factors controlling these traits. The value of 2GCA/2SCA ratio was less than one for total phenolic content (0.38), suggesting the prevalence of non-additive gene effects in the genetic control for this trait, while for antioxidant activity, its value was greater than one (3.73) showing the preponderance of additive genes effects. The parents differed for their general combining ability (GCA) and the crosses showed specific combining ability (SCA). Dominant genes have positive effects for these traits. Genotype variation for phenolic content and antioxidant activity indicates that, it would be possible to select for these quantitative traits in a breeding program.

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Application of the standardized form magnetite nanoparticles (ICNB) in creature simple and practical method of additive modernization of preservation solutions for red blood cells

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This study was devoted to the learning of the use of nanotechnology to correct the functional activity of red blood cells (RBCs) at the storage stages at a positive temperature. It was established that saline NaCl which had previously been processed by magnetite nanoparticles (ICNB) had a marked membrane-stabilizing effect, inhibits hemolysis and increasing the sedimentation stability of preserved RBCs. The complex analysis of the obtained data allowed to determine the primary mechanisms effect of the saline NaCl which had previously been processed by ICNB on the preserved RBCs. The proposed method of additive modernization of preserved RBCs was adapted to the production process. The optimisation results were obtained in creating a simple and practical method of additive modernization of preservation solution that does not violate the compliance requirements, improves the quality, efficiency and safety transfusion of RBCs.

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In vitro studies in *Celastrus paniculatus* Willd., and green synthesis of silver nanoparticles

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Celastrus paniculatus Willd., belongs to the family Celastraceae is one of the important medicinal plants. Various parts of the plant are used in indigenous systems of medicine for their immense health benefits. In Ayurveda, the seeds are recognized as an effective nervine tonic. Apart from seeds, other parts like those that bark, leaf and root are used to overcome pain and local inflammations. Because of its extensive medicinal uses, overexploitation has led to the threatened status of the taxon. *In vitro* culture technique is one of the promising methods for conservation and propagation of threatened taxa. Nodes, internodes and leaf were cultured on Murashige and Skoog's medium supplemented with various concentrations. Both direct and indirect organogenesis was obtained from the cultures. The effective combinations of hormones for direct and indirect organogenesis were found to be MS+NAA+BA/BAP and MS+NAA+BAP+TDZ+ - glutamine respectively. Thus, obtained shoots were rooted on MS+IBA/IAA medium and further successfully acclimatized on soilrite. About 50% of survival rate was recorded. In view of its threatened status and medicinal properties, synthesis of nanoparticles has a significant and promising role to play in pharmaceutics. Hence, silver nanoparticles from seed extracts were synthesized and standard tools such as UV-Vis spectrophotometer, SEM and XRD, characterize them. Efficacy of these nanoparticles against known pathogens is under study.

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Creating a sustainable green planet (SGP) by generation of solar bio-energy using wastewater

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Author has invented a new technology to create a sustainable green planet by generation of solar bio-energy in the form of healthy vegetation, soil, water and air from wastewater and waste (SGP). Our current sanitation methods decompose valuable organics in waste and wastewater to foul gases and acids that degrade air, land and water. They are unsafe for workers; require skilled staff and immense electricity. They lead to major problems such as water pollution, water scarcity, loss of soil fertility, global warming, climate change, poor economy, poor health and loss of life. SGP solves these problems. In SGP, wastewater is not decomposed but, recomposed efficiently to said bio-energy products in aerobic conditions using photosynthesis, gravity and biofiltration through building debris. The products are excellent for afforestation, farming, animal husbandry, biodiversity and aquaculture amongst other uses. Thus, atmospheric carbon dioxide can be lowered and stored in the soil carbon pool by growing vegetation. This is encouraged by the Kyoto protocol. This stabilizes the planet and averts natural hazards. SGP has been operated successfully at a large lab and 200-people field scale. SGP also treats raw water. SGP units can be made on any scale: a single family to a large municipal size plant. SGP is sustainable because it is safe, ecological, economical, efficient and being simple, it has the potential to create human equity. SGP and other such technologies can foster a sustainable green planet if we all synergies our interests and strengths to adopt and promote them.

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Multiple inhibitory activities of *Urgenia* species

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The genus *Urginea* belongs to family Asparagaceae is found growing in India, Africa, and Mediterranean regions of the world. It is one of the medicinally important genera showing multiple activities to cure various deadly diseases. In the present study anti-inflammatory, anti-diabetic, anti-Alzheimer's, anti-cancer, anti-oxidant and anti-osteoporosis activity of *Urginea* species have been discussed. Methanol extract of *U. wightii* has shown inhibition against raw 264.7 cell lines in lipoxygenase, inhibition assay and exhibited the IC value of 200.7 µg/ml with quercetin as standard. Anti-oxidant activity was determined by nitric oxide free radical scavenging assay where curcuminoids was used as standard. *U. indica* and *U. wightii* showed significant anti-oxidant activity with IC value of 174.1 µg/ml and 371.9 µg/ml respectively. Anti-diabetic activity was detected using alpha amylase inhibition assay with acarbose as standard. Both *U. indica* and *U. wightii* have shown significant results with IC value of 253.7 µg/ml and 202.2 µg/ml respectively. Anti-Alzheimer activity was analysed using acetylcholinesterase assay with neostigmine as standard. *U. indica* showed IC value 121 µg/ml against standard 0.04 µg/ml. Cell proliferative activity of the extraction on UMR 106 lines against osteoporosis, has revealed. IC_{50} value of 185.9µg/ml in *U. indica* and 69.75µg/ml in *U. wightii*. Anti-cancer activity was determined against cell line MCF-7 using MTT assay. Both the species have showed significant results inhibiting cell growth in MCF-7 cell line and exhibited IC_{50} value of 325.9µg/ml in *U. indica* and 424.3µg/ml in *U. wightii*. These results show *Urginea* species have a magical potential to heal deadly diseases. Further work is in progress.

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Molecular analysis of human parainfluenza viruses (HPIV) associated with acute respiratory infections (ARI) among children in AL-Muthanna/ Iraq

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Human parainfluenza viruses are important cause of respiratory tract diseases including lower respiratory infections which is a leading cause of deaths in infants and young children worldwide. This study was conducted among children in Iraq to evaluate the unclear epidemiological features of human parainfluenza viruses (HPIVs) and their role in acute respiratory infections (ARIs). Three hundred nasopharyngeal samples were collected from hospitalized pediatric patients in Al-Muthanna/Iraq at the period from January to March/2015 and screened for HPIVs by reverse transcription real time-polymerase chain reaction (RT-PCR), specific forward, reverse primer F- ACTGGAAGCACGGAAAGAAG, R-TTGTTGGTGAGCTTGTTGCC and TaqMan prob 5-FAM-TGAGCTGGAGACATCCACAGCCA-BHQ1-3 were used for detection of HPIV nucleoprotein (NP) gene. The total percentage of positive results was (45.38%). While the HPIV-1 virus was the predominate (32.17%) as compare with (13.21%) of HPIV-3 virus. Conventional end point PCR by using specific forward primer F-GCCCGAGTGTGACAGATGAT and R- GTGTCTCCCGTGAAGACCAG was applied. Ten randomly selected PCR products were purified, sequenced for GenBank submission, these our clones were recorded in GenBank with accession numbers (KT763053, KT763054, KT763055, KT763056, KT763057, KT763058, KT763060, KT763052, KT763059 and KT763061). The result of sequence alignment of our HPIV clones by using ClustalW2 with global reference strains showed high homology phylogenetic analysis with MEGA V6.0 showed that the clones of HPIV-3 (KT763052, KT763059, KT763061) were located in the same branch with (EU346887.1, M14552.1, X04612.1) isolated in Lithuania, Chile, India respectively, and has identity with other global strains isolated in USA, China and While our HPIV-1 (KT763053, KT763054, KT763055, KT763056, KT763057, KT763058, KT763060) located in the same branch with (JQ901971.1, EU346886.1, M62850.1, M62850.1, M62850.1, M62850.1, M62850.1) isolated in USA, Lithuania. And related to other strains isolated in USA, Thailand, and Japan real time RT-PCR is beneficial for epidemiologic studies as well as genotyping of the virus, the results indicate that HPIV is one of the important causative agents of ARI in infants and young children in Al-Muthanna. This is the first study in Iraq to detect HPIV clones and confirm homology and to generate sequence data that may help in understanding virus diversity and evolution.

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Active metabolites of the marine strain *Streptomyces sundarbansensis* with highest and selective antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA)

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Four polyketides were isolated from the algal-derived endophytic actinomycete *Streptomyces sundarbansensis,* which represents the lacking member in the recently reported series of phaeochromycins A–E. We also proposed a method based on the comparison of experimental IR spectra with the DFT ones calculated in order to establish the tautomeric forms for these metabolites. The results indicated a γ-pyrone structure for these compounds, in analogy to the related polyketides mutactin and SEK34. Due to this study, it is possible to suggest that also the known phaeochromycins were isolated mainly in this tautomeric form, differently by the structures reported until now. Evaluation of IC50 values on the pure and structurally defined metabolites as inhibitors of Gram positive and Gram negative bacteria, from where the new compound showed the highest and selective antibacterial activity against MRSA.

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Biodegradation of plastic waste by using microalgae and their toxins

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Today the use of plastic wastes (high density and low density polyethylene) have become an unavoidable entity of human life, these wastes continuously accumulating in the environment and becoming worldwide ecological threat. This accumulation can be hazardous and may cause some environmental disturbances. The conventional methods used for polymer degradation including landfill, incineration and chemical treatment are causing harmful effect to the surrounding environment and living things due to their non-degrading nature. A better idea for the complete degradation of plastic has not yet been identified; so their complete disposal is still a major problem. Although to solve this tremendously growing issue, biological mode of polythene degradation protocol may be discovered and expanded in the future. Biodegradation is an effective option for eco-friendly degradation of plastic waste because biodegradable plastics are environment friendly; they have an expanding range of potential application and are driven by the growing use of plastics in packaging. In recent years considerable attention has been focused on biodegradability of polymeric materials mainly due to the pollution in the environment created by plastic waste and no protocol has yet been developed to feasibly degrade polyethylene by biodegradation on commercial scale. Polyethylenes are carbon and hydrogen polymers, exceptional resistant to biological decay. It is estimated that polythene would degrade less than 0.5% over 100 years, degradation mainly depends on temperature, light exposure, oxygen and moisture availability. The two possible approaches to reduce the plastic waste are: to develop biodegradable commodity plastics from fossil fuel and /or renewable resources building blocks (hydro-biodegradable) or reengineering of full carbon backbone commodity polymers (Oxo-biodegradable) and to identify potential micro-algae and its toxins to develop protocol to effectively biodegrade the polymeric materials. The present study is an attempt to assess algal diversity in plastic contaminated area using molecular approach and to isolate potential indigenous microalgae and its toxins for the efficient degradation of plastics. Biodegradation is promoted by various microalgae and simple or multiple toxin systems, with the enzymes being synthesized by microalgae involving reduction in the energy of activation and weaken the chemical bonds in the polymer, thereby decreasing the energy required for degradation. Our main goal is to conversion of these plastics by microalgae into metabolites such as CO_2, H_2O and new cell biomass (i.e. mineralization).

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Enhancement of morphogenetic potential to obtain elite varieties of *Sauropus androgynus* (L.) Merr. through somatic embryogenesis

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S. androgynus is a member of Euphorbiaceae, popularized as multivitamin plant and consumed as green leafy vegetable due to its rich nutritional profile including proteins, vitamins, minerals, essential amino acids, etc. The plant is cautioned for excessive consumption due to the presence of papaverine alkaloid which, at higher concentration leads to bronchiolitis obliterans. In the present study morphogenetic potential of shoot tip, leaf and nodal explants of *Sauropus androgynus* was investigated to develop and enhance the reliable plant regeneration protocol via somatic embryogenesis. Somatic embryos were derived directly and from the embryogenic callus derived from shoot tip, node and leaf cultures on Phillips and Collins (L_2) medium supplemented with NAA at various concentrations ranging from 5.3 µM/l-26.85 µM/l within two months of inoculation. Thus obtained embryos were sub cultured to modified L_2 media supplemented with increased vitamin level for further growth. Somatic embryos with well-developed cotyledons were transferred to normal and modified L2 basal medium for conversion. The plantlets thus obtained were subjected to brief acclimatization before transferring them to land. About 95% of survival was recorded. Optimized techniques of various explant cultures on Phillips and Collins media with various growth regulators has supplemented the conventional propagation methods in commercial production resulting in availability of improved *Sauropus* through somatic embryogenesis. The development of regeneration systems for *S. androgynous* has opened possibilities for developing genotypes with novel characters including low quantity papaverine content which has facilitated conventional improvement programs there by providing a valuable resource to the food and pharmaceutical industry. Based on this research, plant tissue culture techniques show promise for economical and convenient application in *Sauropus androgynus* breeding.

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Formulation and anticancer evaluation of beta-sitosterol in henna methanolic extract embedded in controlled release nanocomposite

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In the present study, beta-sitosterol in Lawsonia methanolic leaf extract embedded in controlled release nanocomposite was prepared and evaluated for in vivo anticancer efficacy in dimethyl hydrazine (DMH) induced colon cancer. In the present study, colon cancer was induced by subcutaneous injection of DMH (20 mg/kg b.wt) for 15 weeks. The animals were divided into five groups as follows control, DMH alone, DMH and beta sitosterol nanocomposite (50 mg/kg), DMH and Beta Sitosterol nanocomposite (100 mg/kg) and DMH and standard silymarin (100 mg/kg) and the treatment was carried out for 15 weeks . At the end of the study period the blood was withdrawn and serum was separated for haematological, biochemical analysis and tumor markers. Further, the colonic tissue was removed for the estimation of antioxidants and histopathological analysis. The results of the study displays that DMH intoxication elicits altered haematological parameters (RBC,WBC and Hb), elevated lipid peroxidation and decreased antioxidants level (SOD, CAT, GPX, GST and GSH), elevated lipid profiles (cholesterol and triglycerides), tumor markers (CEA and AFP) and altered colonic tissue histology. Meanwhile, treatment with beta sitosterol nanocomposites significantly restored the altered biochemicals parameters in DMH induced colon cancer mediated by its anticancer efficacy. Further, beta sitosterol nanocomposite (100 mg/kg) showed marked efficacy.

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Evaluation of diagnostic potential of recombinant D-erythrulose 1-phosphate dehydrogenase using indirect enzyme-linked immunosorbent assay for diagnosis of bovine brucellosis

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Serological tests used for diagnosis of bovine brucellosis are usually depending on smooth lipopolysaccharides (S-LPS) as a diagnostic antigen which usually gives false positive reactions. So, our study aims to produce and evaluate a diagnostic kit for accurate diagnosis of bovine brucellosis differentiating between vaccinated and infected cattle and exclusion of false positive cases. Idea of this kit depends upon the fact that The *EryC* gene is absent in *Brucella abortus* S19 only but it is present and functional in all other *Brucella* strains and isolates so according to these facts, the use of ELISA kit coated with single subunit (recombinant) *EryC* protein may be useful, rather than S-LPS, as an alternative diagnostic antigen in diagnosis of bovine brucellosis and differentiation between S19 vaccinated and *Brucella* infected cattle. The present study evaluated antibody responses of brucellosis infected and S19 vaccinated cattle to purified recombinant *EryC* protein in an indirect enzyme-linked immunosorbent assay (I-ELISA). Cattle sera were screened using Rose Bengal Plate test (RBPT). 114 samples of naturally infected cattle (Rose Bengal test positive), 78 sera from S19 vaccinated cattle and 25 sera samples from *Brucella* free cattle were used in this study. I-ELISA using S-LPS and periplasmic proteins as a coating antigen were used as a gold standard test. The results revealed that in case of sera of naturally infected cattle, sero-positivity was 94.7%, 100%, 100% and 100% with *EryC*-ELISA, LPS-ELISA, periplasmic-ELISA and Rose Bengal test respectively. Where in case of sera of S19 vaccinated cattle, all samples were negative when tested with *EryC*-ELISA while in case of LPS-ELISA, periplasmic-ELISA and rose Bengal test, sero-positivity was 92.3%, 84.6% and 100% respectively. It could concluded that the *EryC* protein could be used in serological tests for diagnosis of bovine brucellosis and differentiation between infected and *Brucella abortus* S19-vaccinated cattle but more studies are needed to be done on large cattle populations accompanied with bacteriological isolation to detect the sensitivity and specificity of this protein as a diagnostic antigen and also for validate this test.

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