

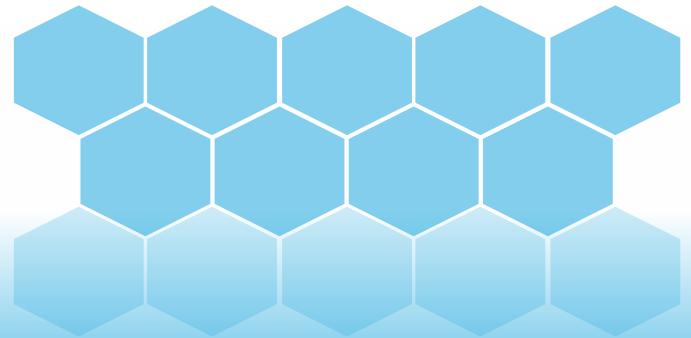


19th World Congress on

Biotechnology

November 13-14, 2017 Osaka, Japan

Scientific Tracks & Abstracts (Day 1)



Mehmet Cengiz Baloglu et al., J Biotechnol Biomater 2017, 7:5 (Suppl)
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miRNome analysis of Einkorn Turkish Wheat Cultivar (Siyez) under drought stress

Mehmet Cengiz Baloglu, Necdet Mehmet Unel and Ferhat Ulu Kastamonu University, Turkey

Viticum monococcum is the domesticated form of Einkorn wheat which is a staple food of early farmers for many thousand 🗘 years. Siyez is one of the most famous einkorn wheat cultivar and domesticated in Kastamonu, Turkey. MicroRNAs (miRNAs) are known as short non-coding RNAs that regulate gene expression at post-transcriptional level. In this study, miRNAs and their target genes were determined after drought stress in leaf and root samples of Siyez. Four small RNA libraries were constructed. Sequencing was performed on the Illumina HiSeq 2500. All bioinformatics analysis was carried out using CLC Genomics Workbench v.10. Although there are many studies related with transcriptome analyses in wheat, microRNA deep sequencing under drought stress in einkorn wheat has been firstly performed. After drought treatment, expression level of 30 and 69 miRNAs were increased and decreased, respectively. Expression level of tae-miR9672a-3p, tae-miR9666a-3p, tae-miR9773, tae-miR5048-5p and tae-miR167c-5p were significantly raised, whereas tae-miR395a/b, tae-miR9674a-5p, taemiR9662b-3p, tae-miR159a/b and tae-miR5200 expressions were remarkably reduced after drought stress application in Siyez. To identify potential regulatory effects of miRNAs on mRNAs, target genes were also predicted for all libraries. A detailed bioinformatics analysis showed that miRNAs up-regulated in response to drought stress, such as tae-miR9661-5p, tae-miR156 primarily targeted transcripts associated with activation of immune regulator SRFR1 and SBP-box genes. In the current study, a temporal miRNA-guided post-transcriptional regulation responded to drought was observed in leaf and root tissues of einkorn wheat. This kind of stress induced miRNA identification studies open new perspectives for designing novel wheat breeding strategies. This work was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK) with Grant Number 215Z354.

Biography

Mehmet Cengiz Baloglu is an Associate Professor of Department of Genetic and Bioengineering at Kastamonu University. He has received his PhD from Middle East Technical University and has established molecular biology, transcriptomics and bioinformatics research laboratories at the Kastamonu University. He has published over 50 peer-reviewed manuscripts on topics concerning with gene identification in plants, transcriptome and miRNA analysis using NGS and abiotic stress in plants. He is serving as an Associate editor and Editorial Board member for International journals with high impact factors.

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Cell function-based high-throughput screening of natural and synthetic compounds

Amandio Vieira

Simon Fraser University, Canada

Modulators of cell functions such as transport pathways, signal transduction and redox balance may have biomedical applications as pharmaco-therapeutics. We have developed biochemical and cellular high-throughput screening assays to assess activities of natural products, standardized plant extracts, as well as synthetic compounds and biosimilars. Several of these assays will be discussed in the context of (1) pro-oxidative and other pathological effects of misfolded and aggregated polypeptides in amyloidogenic diseases, (2) infectious diseases involving endocytosis of the microbe and (3) epigenetic regulation in metabolic disorders. Medicinal and dietary plant extracts, purified phytochemicals including flavonoids and nutrients, as well as combinations of purified compounds, are currently undergoing screening in our laboratory and the latest results will be presented.

Biography

Amandio Vieira has completed his BSc and PhD studies in Alberta, Canada and Postdoctoral studies in California, USA. He is currently Associate Professor and the Director of the Nutrition and Metabolism Research Laboratory, Biomedical Physiology department, Simon Fraser University, Burnaby, Canada. He has over 90 publications, including research papers in major International journals, with over 1500 citations. He has served as Reviewer and Editorial Board member for journals related to biomedical research, molecular and cellular biology, as well as educational and scientific books.

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Gladys Hayashida Soiza, J Biotechnol Biomater 2017, 7:5 (Suppl)

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Microalga isolated from a microbial mat in Salar de Atacama (northern Chile) as a potential source of compounds for biotechnological applications

Gladys Hayashida Soiza University of Antofagasta, Chile

Microalgae are an important source of unsaturated fatty acids, phospholipids, glycolipids, and carotenes, which are useful compounds for the food and pharmaceutical industries. The Atacama Desert of northern Chile is one of the driest deserts on Earth and as such, it is a great natural laboratory in which to study new microorganisms adapted to extreme environments. A microalgal strain, referred to here as CH03, was isolated from a microbial mat in salt flat water in Salar de Atacama. Genetic analysis of the 18S ribosomal RNA gene showed that the strain had homology with other known sequences of the species *Chlorella sorokiniana*. Results revealed the adaptability of this microalga to freshwater medium under laboratory conditions, despite coming from an extremely high-salinity environment. The fatty acid profile of CH03(A) newly isolated in Bold's basal medium differed from that of CH03(B) cultured *in vitro* in modified F/2 medium and from another five strains of *C. sorokiniana* and three strains of *Chlorella vulgaris* in that it had a high stearic acid content and had no polyunsaturated fatty acids. The major biochemical components observed in this strain were proteins (64.3-73.6%) and lipids (26.6-32.6%). This study suggests that the strain CH03 could be a protein source and that this oleaginous microalga is easy to grow *in vitro* as a biological model for future studies.

Biography

Gladys Hayashida Soiza has completed her PhD from Antofagasta University and her Masters studies from Kyoto University. She is the Director of the Associative Regional Project Explora of CONICYT, a Science Promotion Grant supported by the Government of Chile and implemented by the Antofagasta University. She has published scientific articles related to bioactive substances from marine bacteria and microalgal biotechnological applications, in reputed journals and has been her participating as researcher in several scientific studies.

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Enhancing animal welfare for good science through guidelines and regulations in Singapore

Leslie Retnam

Biological Resource Centre, Singapore

The use of animals in any field-be it food, work, entertainment or scientific purposes-continues to attract the attention of individuals with interest in their welfare. Of relevance to the biotechnology industry is the use of live animals for testing, research and training because of the potential harm and distress which may compromise their wellbeing. In Singapore, national guidelines regulating the use of animals for scientific purposes are currently being revised to further enhance animal welfare and promote good science. The National Advisory Committee on Laboratory Animal Research (NACLAR) was established in 2003 to formulate a set of national policies and standards regulating the acquisition, housing and utilization of laboratory animals in biomedical research. It also sought to address related scientific, ethical and legal issues pertaining to the use of animals within the biotechnology industry. Shortly after the publication of NACLAR's guidelines, the Agri-Food and Veterinary Authority announced-The Animals and Birds (Care for the use of animals for Scientific Purposes) Rules which legislated compulsory licensing of animal research facilities. To this day, these measures maintain a high standard of animal welfare within Singapore's biotechnology industry. This poster depicts a historical perspective on the development of guidelines and regulations in Singapore relating to the use of animals for scientific purposes and illustrates enhancements to the first edition of the NACLAR Guidelines currently being deliberated to improve animal welfare.

Biography

Leslie Retnam has completed his Bachelor of Veterinary Science from the University of Queensland, Australia and Masters in Laboratory Animal Science from Hahnemann University, Philadelphia, USA. He is Director of Veterinary Services at Biological Resource Centre, Agency for Science, Research and Technology, Singapore. He is also Council Member with the AAALAC International and serves on a number of animal and veterinary related committees in Singapore.

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CRISPR-PCS: An efficient and versatile chromosome splitting technology in Saccharomyces cerevisiae

Saeed Kaboli

Shahid Beheshti University, Iran

PCR-mediated chromosome splitting (PCS) was developed in the yeast *Saccharomyces cerevisiae*. It is based on homologous recombination and enables division of a chromosome at any point to form two derived and functional chromosomes. However, because of low homologous recombination activity, PCS is limited to a single site at a time, which makes the splitting of multiple loci laborious and time-consuming. Here we have developed a highly efficient and versatile chromosome engineering technology named CRISPR-PCS that integrates PCS with the novel genome editing CRISPR/Cas9 system. This integration allows PCS to utilize induced double strand breaks to activate homologous recombination. CRISPR-PCS enhances the efficiency of chromosome splitting approximately 200-fold and enables generation of simultaneous multiple chromosome splits. We propose that CRISPR-PCS will be a powerful tool for breeding novel yeast strains with desirable traits for specific industrial applications and for investigating genome function.

Biography

Saeed Kaboli has completed his PhD and Postdoctoral degrees in Department of Biotechnology, Osaka University, Japan. Presently, he is a Postdoctoral Researcher in Sciences and Biological Technologies, Shahid Beheshti University, Iran. Also, he is engaged in a project entitled "Development of novel genome engineering technology and its application in bioscience and biotechnology".

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Cloning and sequence analysis of bZIP and WRKY transcription factor genes in Einkorn Wheat (Siyez)

Pinar Baloglu and Mehmet Cengiz Baloglu Kastamonu University, Turkey

Wheat is an annual herbaceous plant whose breeding is done all over the world. Siyez wheat (*Tiriticum monococcum*) with 2n chromosomes is the first wheat variety which is known the oldest and taken culture. bZIP transcription factors have DNA binding motifs and form the leucine zipper dimerization. Most of WRKY type transcription factors control regulation of important functions for the development of plants. In this study, transcription factor genes were firstly cloned form Einkorn wheat. For this, genes were amplified in PCR using gene specific primers with Pfu Taq Polymerase enzyme. PCR products were transferred to pENTR*/D-TOPO* input vector. Plasmids containing genes were sent to sequencing. Finally, the sequences of the genes were aligned using CLC Genomics Workbench bioinformatics program. bZIP gene with 450 bp in length and WRKY gene with 672 bp in length were successfully cloned in a Gateway-compatible input vector. DNA sequence of genes was translated to protein sequence. A total of 150 amino acids in length for bZIP proteins and a total of 224 amino acids in length for WRKY proteins were determined. 3-D structure of proteins was modeled using Phyre2 program. The structural differences of these proteins in Siyez were identified. With this study, the sequence of bZIP and WRKY type transcription factors genes were firstly determined in ancestral wheat cultivar, Siyez and tried to obtain information about the function of these genes. Obtained results from this study may be used for development of abiotic stress resistance plants.

Biography

Pinar Baloglu is a Biologist at Kastamonu University in Research and Application Center. She has received her MSc at Department of Genetic and Bioengineering from Kastamonu University. In her thesis, she has identified 79 *LEA* (Late Embryogenesis Abundant) genes in cucumber genome. She has published 2 manuscripts on topics concerning with gene families identification in different plants. She has also attended many congress related with Molecular Biology and Bioinformatics. Presently, she is a PhD candidate in same department.

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Genome wide identification of important DNA sequences in plant genomes and its application in plant biotechnology

Mehmet Cengiz Baloglu Kastamonu University, Turkey

dvent of next generation sequencing (NGS) has been dramatically altered the fields of omics technologies including Agenomics, transcriptomics, proteomics and metabolomics. NGS provides mass analysis for genome and transcriptome of organisms including plants. This advanced technique accelerates identification of genes, regulatory sequences and biomarkers found in plant genomes. Although many draft plant genome sequences have been published over the past decade, all genes in genomes have not been completely detected. Therefore, gene family identification studies have been still continued. Gene families are often spread in the genome through tandem and segmental arrangements. Orthologous-paralogous genes, transcription factor family genes, some simple sequence repeats, microsatellites, microRNAs and long intergenic noncoding RNAs are in great demand for genome survey researchers. Development of new bioinformatics tools has caused detection of these important sequences in plant genomes. Transcriptome, molecular breeding, genetic transformation and genome editing studies can be considered as samples for this application in plant biotechnology, RNA-sequencing technology has been widely used for both measurement of gene expression levels and discovery of new genes and new alternative splicing isoforms. Aim of modern plant breeding programs is to improve and increase some agronomically important traits. The most significant contribution of genome wide investigation studies has provided precious source for functional genomics and plant breeding programs. Genome editing strategies including genetic transformation, RNA interference and CRISPR/Cas9 have a great potential to obtain new plant phenotypes with desirable characteristics. In the future, new omics analysis tools will be emerged and will provide deep investigation for plant genomes.

Biography

Mehmet Cengiz Baloglu is an Associate Professor of Department of Genetic and Bioengineering at Kastamonu University. He has received his PhD from Middle East Technical University (METU). He has established molecular biology, transcriptomics and bioinformatics research laboratories at the Kastamonu University. He has also published over 50 peer-reviewed manuscripts on topics concerning with gene identification in plants, transcriptome and miRNA analysis using NGS and abiotic stress in plants. He is serving as an Associate editor and Editorial Board member for International journals with high impact factors.

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Young Research Fourm (Day 2)



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Effect of water and super absorbent polymer (SAP) content on the efficiency of production biodiesel by liquid enzyme catalyst

Dinh Thi My Huong and Chia-Hung Su Ming Chi University of Technology, Taiwan

Water is the factor need to attend on the esterification to produce biodiesel by enzyme catalysts because of its complicated influences on the catalyst activity and stability of enzyme as well as the productivity of reaction. Some studies conclude that some lipases need to be maintained for its proper structure by the certain amount of water. On the other hand, water is the by-product of the ester reaction, hence the high-water content exists in the reaction media causes the back-forward reaction, consequences to reduce the yield. Therefore, this study drives to identify the suitable amount of water for assisting activation of liquid enzyme catalyst and investigate the using SAP to absorb the produced water. The effect of water content on the enzyme activity was assigned through discussed the efficiency of the esterification with molar ratio of oleic acid to methanol 1:3 at the temperature of 25 °C, catalyst amount of 10 wt.% and varied water amount of 0 to 30 wt.% (base on the fatty acid mass) for 1 hour and 3 hours. Another reaction was carried out with the condition similar to above reactions and added 5% (w/w oleic acid) of SAP amount to study the ability of maintaining the esterification. Results illustrated that the conversion was increased obviously from 0% to 95 % with added water content increased from 0 wt.% to 30 wt.%. Meanwhile the conversion rate was improved significant with using SAP, the yield reached to 95% with added water amount of 10 wt.% for only 1 hour.

Biography

Dinh Thi My Huong has completed her Bachelor's degree from Da Nang University of Technology, recently pursued Master's program of Biochemistry at from Ming Chi University of Taiwan. She is currently the Lecturer of Da Nang College of Technology.

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Characterization of microbial diversity influenced by natural radon source in soil environment

Kyu Yeon Lee and Chang Gyun Kim Inha University, South Korea

Radon is an inert gas with no color and odor having a half-life of 4 days, which is a radioactive element produced by the decay of Uranium. Recently, public interest about indoor radon presence has been increased over decade. In general, high concentrations radon is generally known to be making deleterious effects on plants, animals and humans, which can cause cell viability disruption, cell morphological changes or hormonal disorders. On the contrary, lower concentration of radon may nevertheless improve crop growth while disabling pest activity. This study shows how much of lower level concentrations of radon in natural soils affect microbial community and their diversity with regard to basal soil physicochemical characteristics. Microorganisms exposed to low radioactivity, such as low-level radon, can have strong viability and high biodiversity. Soil physicochemical parameters such as pH, electrical conductivity, moisture content and soil particle size were measured according to Korean Standard Analytical Methods for Soils. Gas phase of radon concentration was measured for 1 hour (FRD-400, FT-Radon Lab., Korea) while the concentration of it has been varied in lower, equal and greater level compared to the natural source of radon origin in the field. In the meantime, colony enumeration, dehydrogenase activity and identification of species were performed. In the long run, there were relatively greater extent of diversity and population density being observed when microbes were exposed to relatively lower or equal level compared to the natural origin. In response, they revealed higher enzymatic activity under the given lower level radon exposure.

Biography

Kyu Yeon Lee has been studying on the neutralization ability of acid soil and the environmental impacts and decomposition mechanisms of micro pollutants such as medicines and micro-plastics in Soil Groundwater Laboratory of Inha University. Also, investigating on the characteristics of microbial diversity in natural radon soil environments verifying microbial differences at various radon concentration conditions.

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Combination of bacterial cellulose wound dressing, silk sericin and polyhexamethylene biguanide for wound healing

Supamas Napavichayanun and Pornanong Aramwit Chulalongkorn University, Thailand

Wound dressing is an important factor for wound treatment. The good properties of wound dressing will lead to accelerate wound healing. Therefore, the objective of this study was to develop the bacterial cellulose wound dressing containing silk sericin and polyhexamethylene biguanide (PHMB) for wound treatment. Coconut is one of the value products of Thailand's industrial harvests. Unfortunately, ripe coconut water is usually a waste product from coconut milk production that is discarded into the environment and results in pollution. Bacterial cellulose is produced by *Acetobacter xylinum* which is fermented in ripe coconut water. It has many advantages for wound healing: transparency, autolytic debridement, acceleration of re-epithelialization and fewer daily wound dressing changes. Because of the many benefits of bacterial cellulose, it is used in many applications including cosmetics and medical devices such as wound treatment dressing material. Silk sericin is a protein from silk cocoons which can accelerate the proliferation of fibroblast cells and activate collagen synthesis for wound healing. PHMB is a broad-spectrum antimicrobial agent with high efficacy and low toxicity. Accordingly, the combination of bacterial cellulose wound dressing containing silk sericin and PHMB will have many benefits to the wound. The safety and efficacy of the dressings, *in vitro* and *in vivo* was investigated. The results showed the dressing has good physical, mechanical and biological properties. The wound size of wounds treated with the dressing showed a significantly less than control. No inflammation or irritation was shown in rats. Moreover, in the clinical study, the dressing also showed many benefits for split-thickness skin graft wound treatment without any toxicity.

Biography

Supamas Napavichayanun is a PhD student, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. She has obtained BSc from Faculty of Phamaceutical sciences, Chulalongkorn University. Her research experience ranged from protein including silk proteins and biomaterials. She also did clinical researches in the area of dermatology especially materials for wound healing application.

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A novel marvelous evolutionary detection: Lack of a large 2888 bp intron region within *HA03* gene from *Hyalomma anatolicum anatolicum*, unlike its commercial recombinant anti-tick orthologue, *Bm86*, from *Boophilus microplus*

Khosrow Aghaiypour Kolyani and Mohsen Aali Razi Vaccine and Serum Research Institute, Iran

Hyalomma anatolicum (H. a. anatolicum) as the most widespread tick species in Iran and other parts of the middle east is responsible for the hugely serious economic losses in livestock industry. This study was conducted to investigate genetic variability of the Bm86 orthologous gene, HA03, in five different Iranian H. a. anatolicum isolates including Kordan, Qom, Boinzahra, Lorestan and Bushehr. Likewise, a number of in silico analyses were performed in order to predict the possible impact of the amino acid substitutions on antigenicity of the protein. Comparative sequence analysis of the Bm86 orthologous gene sequence among five tick isolates allowed for identification of four non-synonymous single nucleotide polymorphisms (SNPs) including c.995A>C, c.1150G>C, c.1151A>C/T and c.1152G>T which would result in p.Asn 332 Thr, p.Glu 384 Leu and p.Glu 384 Ala substitutions. As much as antigenicity is concerned, based on our in silico studies, the amino acid position 384 was located in a putative antigenic peptide of the protein. Our subsequent physicochemical and structural analyses illustrated that two out of three amino acid substitutions including p.Glu 384 Leu and p.Glu 384 Ala considerably influenced the 3-dimensional structure and physicochemical properties of HA03 protein including hydrophobicity, amphiphilicity and net charge; thus, they might affect the antigen-antibody reaction and consequently immunogenicity of the antigen. In conclusion, it is a rational measure not only to replace Bm86 with HA03 in formulation of the recombinant anti-tick vaccine, but also to combine various antigens extracted from different isolates of the tick species.

Biography

Khosrow Aghaiypour Kolyani has completed his PhD from Tehran University of Medical Sciences and Postdoctoral studies from National Institute of Health, National Cancer Institute at frederick, USA. He is the Head of Genomics and Genetic Engineering, Department of Razi Vaccine and Serum Research Institute, which organizes the main human and animal vaccine research in Iran. He has published more than 30 papers in reputed journals and has been serving as an Editorial-Board member of repute.

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Bioactivity of different bee honey samples

Mahasin Ahmed Wadi

Princess Nourah Bint Abdulrahaman University, KSA

Background: The therapeutic value of honey was underlined in various literatures. Honey was widely used in folk medicine throughout the world. Honey has been used to treat a number of clinical conditions: Treatment of burns, wounds, peptic ulcers, gastritis, eye infection and sore throat. Antibacterial activity is attributed partially to the high osmolality of the sugar content of honey. Additional antibacterial activity of honey was investigated by extraction and fractionation of honey by organic solvents. Honey ethyl acetate extract revealed potent antibacterial activity.

Objectives: The objective of the current prospective study is to determine the antimicrobial activity of fifteen (15) different honey samples collected from different floral origin and to verify the nature of active fraction of bee honey.

Methods: Fifteen different raw bee honey samples were obtained from different countries, as well as commercially sold honey samples from the local market different brands, of different floral origin. *In vitro* antibacterial activity of bee honey, petroleum ether, diethyl ether and ethyl acetate extracts of honey samples were tested against five standard organisms; *Staphylococcus aureus*: ATCC 29213, *Staphylococcus* Methicillin Resistant (MRSA), ATCC: 23591 *Escherichia coli*: ATCC 25922 *Klebsiella pneumoniae*: ATCC 700603 and *Pseudomonas aeruginosa*: ATCC 27853.

Results: All honey samples exerted inhibitory effects on both Gram-positive and Gram-negative organisms. The petroleum ether and diethyl ether fraction exerted no activity, while the aqueous residue exerted strong antibacterial activity. Ethyl acetate fraction showed strong antibacterial activity.

Conclusion: The findings of the current study confirmed that all honey samples exerted strong antibacterial activity. The chemical findings of this study indicate the presence of polar antibacterial agent(s) which is characterized by its extractability by ethyl acetate to the organic phase.

Biography

Mahasin Ahmed Wadi has completed her PhD in Medical Microbiology and is currently working at College of Nursing, Princess Nourah Bint Abdulrahaman University, Riyadh, Saudi Arabia. She has published a numbers of papers in reputed journals and participated in many internationals and national conferences. She issued a patent research about the antimicrobial activity of Sudanese bee honey. She is a Member of many international associations: German Apitherapy Society, American Apitherapy Society, International Bee Research Association, European Society of Clinical Microbiology and Infectious Disease ESCMID. She has served as a Reviewer of various journals and has her research interest in the antibacterial and haling effects of Bee honey as natural product.

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Effect of surfactants on the production and structure of curdlan from Agrobacterium sp. ATCC 31749

Xiaobei Zhan, Ying Liang and Li Zhu Jiangnan University, China

The serious envelopment of curdlan on *Agrobacterium* sp. ATCC 31749 during fermentation is a major obstacle to increase curdlan production and the compact macrostructure of curdlan produced from *Agrobacterium* sp. ATCC 31749 limits its application in preparing curdlan β -glucooligosaccharides. In this study, the effects of different surfactants on curdlan production by *Agrobacterium* sp. ATCC 31749 was investigated and several new perspectives concerned with the effect of Tween 80 promoting curdlan production and altering curdlan structure were presented. Maximum curdlan production (51.94 g/L) was achieved when 16 g/L Tween-80 was added at the beginning of the cell growth stage. The addition of Tween-80 at higher concentration inhibited cell growth. However, the addition of 16 g/L Tween-80 enhanced the production of curdlan with a looser ultrastructure, significantly weakened the envelopment of curdlan on *Agrobacterium* sp. ATCC 31749, altered the fine structure of cell membrane and increased the cell membrane permeability. Compared with commercial curdlan, the curdlan with a looser ultrastructure exhibited higher substrate-binding affinity and maximum reaction rate when it was used as the substrate for preparing curdlan β -glucooligosaccharides. These findings demonstrate the mechanisms by which Tween-80 enhances curdlan production and provide a cheap and feasible approach to weaken the envelopment of water-insoluble polysaccharides on bacteria. Meanwhile, this paper provides an effective method to produce an ideal substrate so as to prepare oligosaccharides using enzymic degradation.

Biography

Xiaobei Zhan has completed his PhD from Kansans State University in 2004 and is a Professor of Fermentation Engineering from Jiangnan University. He is the Director of Industrial Microbiology and Biological Reaction Engineering Research Center, School of Biotechnology, Jiangnan University and the deputy Director of Key Laboratory of Carbohydrate Chemistry and Biotechnology of Ministry of Education. He is also the ASAE and IFT Institute of United States. His research focus is Technology of high-viscosity fermentation, biosynthesis of microbial polysaccharide, preparation of functional oligosaccharide and design of bioreactor. He has published more than 30 papers in reputed journals.

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