23rd International Conference on

Pharmaceutical Biotechnology

December 10-11, 2018 | Rome, Italy

Scientific Tracks & Abstracts

Day 1

Pharma Biotech 2018

Day-1

SESSIONS

Formulation of Biotech Products | Biotechnology in Health Care | Industrial and Microbial Biotechnology Biotechnology and its Applications | Biopharmaceuticals | Biopharmaceutical Engineering | Nanoparticles in Biopharmaceuticals | Biotech Companies and Market Analysis

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Alejandro López Prieto et al., J Biotechnol Biomater 2018, Volume 8
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Effect of a biosurfactant extract obtained from corn on probiotic bacteria

Alejandro López Prieto, Rodríguez-López L, Rincón-Fontán M, Cruz J M and **Moldes A B**
University of Vigo, Spain

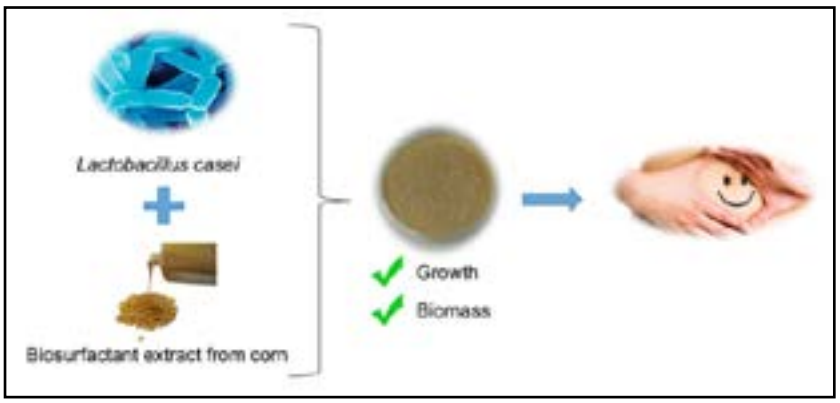
Statement of the Problem: Probiotics, prebiotics and nutraceuticals have been used over the years as supplements in several treatments and control of diseases providing important health benefits. Among the probiotic bacteria, *Lactobacillus casei* is one of the most important. On the other hand, there are some bioactive compounds produced by lactic acid bacteria or obtained from natural sources, with surfactant and antimicrobial pathogenic capacity, were named biosurfactants. These biosurfactants could increase the effect of probiotics when added to pharmaceutical formulations improving their adsorption and antimicrobial properties.

Aim: The aim of this study was to establish the effect of a biosurfactant extract obtained from corn, which is able to reduce the surface tension of water in more than 30 units, on the probiotic bacteria *Lactobacillus casei*.

Methodology & Theoretical Orientation: The biosurfactant extract was obtained from a corn milling industry stream by liquid-liquid extraction with ethyl acetate. After extraction, ethyl acetate was evaporated obtaining a concentrated biosurfactant extract that was added to an inoculum of *L. casei* at maximum concentrations of 0.5 g/L.

Findings: It was observed that the addition of the biosurfactant extract obtained from corn had a positive effect on the biomass of an inoculum of *L. casei*, maintaining and in some cases, favoring the growth of these probiotic bacteria, especially when this strain was under optimal temperature conditions.

Conclusion & Significance: This was the first study to assess the effect of a biosurfactant extract on probiotic bacteria. The positive effect of the evaluated biosurfactant extract on *L. casei* growth could open the door to the application of biosurfactants in pharmaceutical formulations although further studies will be necessary using other biosurfactants and probiotic bacteria.



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1. Banat I M, Makkar R S and Cameotra S S (2000) Potential commercial applications of microbial surfactants. App. Microb. Biotech. 53(5):495-508.
2. Kaur I P, Chopra K and Saini A (2002) Probiotics: potential pharmaceutical applications. Eur. J. Pharma. Sci. 15(1):1-9.
3. Rodrígez-López L, Vecino X, Barbosa-Pereira L, Moldes A B and Cruz J M (2016) A multifunctional extract from corn steep liquor: antioxidant and surfactant activities. Food Funct. 7(9):3724-3732.

4. Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz J M and Moldes A B (2014) Study of the surfactant properties of aqueous stream from the corn milling industry. J. Agric. Food Chem. 62(24):5451-5457.
5. Vecino X, Cruz J M, Moldes A B and Rodrigues L R (2017) Biosurfactants in cosmetic formulations: trends and challenges. Crit. Rev. Biotech. 37(7):911-923.

Biography

Alejandro López Prieto is a PhD student in the Department of Chemical Engineering at the University of Vigo, Spain. He has experience in food industry and in food and biotechnological research at Cranfield University, UK and the University of Vigo. He is currently focused on projects related with the application of biosurfactants extracted from agro-industrial residues in the food, environmental and pharmaceutical industries.

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Notes:

Rodríguez-López L et al., J Biotechnol Biomater 2018, Volume 8
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Effect of biosurfactants obtained from different sources on pathogenic microorganisms

Rodríguez-López L, Rincón-Fontán M, López-Prieto A, Vecino X, Cruz J M and **Moldes A B**
University of Vigo, Spain

Statement of the Problem: Biosurfactants are amphiphilic compounds with surface properties produced by microorganisms or obtained from biological cells. They have not only the same applications than their synthetic counterparts but also better characteristics in terms of biocompatibility and biodegradation. Although they have been proved in different areas such as bioremediation, their uses are increasing in food and cosmetic formulations. In these areas, microbiology properties are one of the most important parameter to control, so it is necessary to evaluate biosurfactants behavior in presence of microorganisms.

Methodology: Two biosurfactant extracts were produced following the methodologies established by Vecino et al. one obtained from corn steep liquor (CSL) and the other from *Lactobacillus pentosus*. Both biosurfactants were diluted up to 1 g/L of and put in contact with a known concentration of pathogenic microorganisms including *Candida albicans, Aspergillus brasiliensis* and *Pseudomonas aeruginosa*, at 22.5ºC. The effect of these biosurfactants on the microorganism growth was evaluated each 7 days during a month. The culture conditions for obtaining the inoculum of each microorganism were reflected.

Findings: The experiment carried out with biosurfactant from CSL, showed antimicrobial activity against *P. aeruginosa and A. brasiliensis*, thus the concentration of microorganisms was reduced from $2*10^6$ and $2*10^4$ UFC/mL, to 1 and $4*10^3$ UFC/mL, respectively. In the case of *C. albicans*, the amount of colonies slightly increased from $2*10^4$ to $8*10^4$ UFC/mL. For the biosurfactant from *L. pentosus*, the behavior observed was completely different, thus the number of colonies did not change significantly in any of the pathogens tested.

Conclusion & Significance: These results have demonstrated interesting effects of biosurfactant extract from CSL against pathogenic microorganisms, what is in concordance to its antioxidant properties. Furthermore, the biosurfactant from *L. pentosus* showed lower antimicrobial activity.

Microorganism	Culture Medium	Temperature (°C)	Time (Day)
Pseudomona aeruginosa	Tryptic Soy Agar	32.5	2
Candida albicans	Sabouraud Dextrose Agar	32.5	2
Aspergillus brasiliensis	Potato Dextrose Agar	22.5	5

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2. Santos D K F, Ruffino R D, Luna J M, Santos V A and Sarubbo L A (2016) Biosurfactants: Multifunctional biomolecules of the 21st century. International Journal of Molecular Sciences 17(3):401-432.
3. Luna J M, Filho A S S, Rufino R D and Sarubbo L A (2016) Production of biosurfactant from Candida bombicola URM 3718 for environmental applications. Chemical Engineering Transactions 49:583-588.
4. Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz J M and Moldes A B (2015) Optimization of extraction conditions and fatty acid characterization of *Lactobacillus pentosus* cell-bound biosurfactant/bioemulsifier. Journal of the Science of Food and Agriculture 95(2):313-320.
5. Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz J M and Moldes A B (2015) Optimization of liquid-liquid extraction of biosurfactants from corn steep liquor. Bioprocess and Biosystems Engineering 38(9):1629-1637.

Biography

Rodríguez-López L is a PhD student at the University of Vigo. She has completed her Master's degree in Advanced Biotechnology in 2016, at University of Vigo. She has co-authored six articles in JCR journals. Moreover, she has collaborated for three months in the Department of Pharmacy at University of Huddersfield, United Kingdom.

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Notes:

Myriam Rincón Fontán et al., J Biotechnol Biomater 2018, Volume 8
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Looking for more biocompatible sunscreen ingredients in pharmaceutical and cosmetic formulations

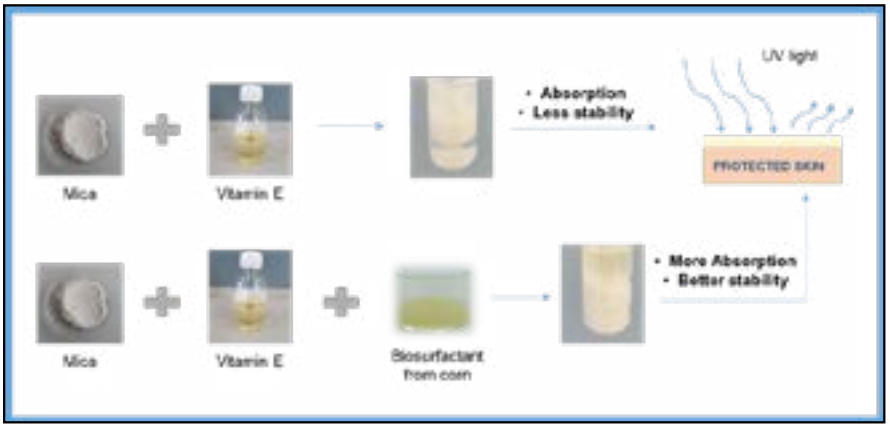
Myriam Rincón Fontán, L Rodriguez-Lopez, A Lopez-Prieto, X Vecino, J M Cruz and **A B Moldes**
University of Vigo, Spain

Statement of the Problem: Nowadays, cosmetic and pharmaceutical industries are looking for more natural ingredients to prevent the effects of sun damaging radiation (UV light) on skin. Usually, sunscreen formulations contain synthetic organic compounds, which can produce irritant effects on skin. Therefore, there is a need for the use of more natural and biocompatible ingredients. The utilization of a biosurfactant extract obtained from corn in combination with mica, could be an interesting alternative to replace chemical sunscreen ingredients. Additionally, vitamin E could be included as well in these formulations because of its antioxidant capacity. In this work, it is elucidated that the sun protection factor (SPF) and emulsion formation (EV) of different formulations were formed by a biosurfactant aqueous extract (obtained from corn), a mining silicate mineral (mica) and a non-aqueous soluble antioxidant (Vitamin E).

Methodology: Different formulations were prepared in presence and absence of biosurfactant, in order to see the effect of it as sunscreen and emulsifier. The SPF of each substance and of the different formulations was measured following the methodology of Mansur et al. using ethanol as solvent. On the other hand, the percentage of emulsion formation (EV) was measured as in previous studies, during one month.

Findings: It was shown that all substances tested exerted sun protection capacity. Particularly, the biosurfactant showed a better SPF than tocopherol, at the same concentration. As a consequence, the presence of the biosurfactant increased the SPF of the different formulations. Moreover, this biosurfactant was observed to exert emulsifier capacity during the experiment.

Conclusion & Significance: The preparation of formulations in presence of biosurfactant, mica and tocopherol can be a good alternative to obtain more biocompatible sunscreen formulations for the cosmetic and pharmaceutical industry, observing a synergistic effect of mica and biosurfactant on the stabilization of the emulsions.



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1. Rincón-Fontán M, Rodríguez-López L, Vecino X, Cruz J M and Moldes A B (2018) Design and characterization of greener sunscreen formulations based on mica powder and a biosurfactant extract. Powder Technology 327:442-228.
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3. Lin J Y, Selim M A, Shea C R, Grichnik J M, Omar M M, Monteiro-Riviere N A and Pinnell SR (2003) UV photoprotection by combination topical antioxidants vitamin C and vitamin E. Journal of American Academy of Dermatology 48(6):866-874.

4. Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz J M and Moldes A B (2015) Optimization of extraction conditions and fatty acid characterization of *Lactobacillus pentosus* cell-bound biosurfactant/bioemulsifier. Journal of Science of Food and Agriculture 95(2):313-320.

Biography

Myriam Rincón Fontán is a PhD student at the University of Vigo. She has completed her Chemical Engineering Degree at University of Santiago de Compostela in 2014. During the last year of the degree, she has carried out her Master Thesis at the Royal Institute of Technology (KTH) in Stockholm, Sweden in the Department of Biochemistry. In 2015, she has completed her Master degree in Chemical Industry and Research at the University of Vigo in the Physical Chemistry Department.

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Hanneke Later-Nijland, J Biotechnol Biomater 2018, Volume 8
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Expensive drug costs, compulsory patent licensing and the limits to compounding by pharmacists

Hanneke Later-Nijland
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Of late, the cost of medicines is a recurring subject of debate in Europe and it is anticipated that this topic will be discussed more intensely in the years to come. In this regard, the application of compulsory patent licensing and the (wider) application of the compounding exemption (formula magistralis) are seriously investigated by the Dutch Minister of Medical Care as an instrument to curb the cost of medicines. In respect to the latter, a legislative proposal is underway which would exonerate pharmacists from patent infringement when compounding medicinal products for direct use for individual cases on medical prescription in pharmacies. This presentation explores in view of the applicable legislation and case law whether these solutions have been correctly identified as the solution to the problem of expensive medicinal products. Pursuant to e.g. the TRIPS Agreement, compulsory patent licensing in view of the general interest is (at least) to be used in combination with an adequate remuneration. Nevertheless, it is worthwhile mentioning that a compulsory license in the public interest was recently granted (and upheld in appeal) in Germany for the HIV drug Isentress. The available case law with respect to compounding and the rationale thereof has demonstrated that it is solely to be utilized as an exception to the rule, which makes it unsuitable as a general solution. Patients are not guaranteed for the same quality control as authorized medicinal products and therefore a proper substantiated justification for this deviation is required. Such justification may when comparing European case law probably not be sought in financial gain leaving aside the fact that this affects the level playing field. This presentation is very relevant for parties manufacturing or marketing high-cost medicinal products.

Recent Publications

1. Later-Nijland (2018) Annotation to e.g. ECLI:NL:RBDHA:2017:12046 "The Alimta cases" (Patent infringement cases concerning Alimta) Jurisprudentie Geneesmiddelenrecht ('Case law Pharmaceutical law'), (Apr 6) 2018, Sdu [in Dutch]
2. Later-Nijland (2018) Annotation to ECLI:NL:RVS:2017:1175 "The Biodent case" (The Dutch Council of State rules that this caries protection product should be classified as both a medicinal product by presentation as well as a medicinal product by function) Jurisprudentie Geneesmiddelenrecht ('Case law Pharmaceutical law'), Apr, 2018, Sdu [in Dutch]
3. Later-Nijland H. (2016) Statutory prohibition inducements concerning medical devices upcoming, Life Sciences & recht, Jan 26, DeLex <https://www.lsenr.nl/artikelen/wettelijk-verbod-op- gunstbetoon-met-betrekking-tot-medische-hulpmiddelen-op- handen> [in Dutch]
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Biography

Hanneke Later-Nijland is an Attorney-at-law at Axon Lawyers, Amsterdam, Netherlands. Moreover, she has been trained as a Pharmacist. Furthermore, she has completed her PhD in Clinical Pharmacokinetics and is a former Inspector for Clinical Trials and Pharmacovigilance at the Netherlands Inspectorate for Healthcare, IGZ. She specializes in European and national legal and regulatory issues relating to medicinal products. In her practice, she advises life sciences and healthcare clients and litigates on a wide range of issues, often with a regulatory focus. Her areas of expertise in the medicinal products field covers marketing authorizations, reimbursement, compliance, pharmacovigilance and advertising issues. In addition, she also assists clients with product liability issues and IP and regulatory issues in transactions in the life sciences sector. Furthermore, she is a Lecturer at Leiden University Medical Centre. She regularly publishes on new European legislation and the impact of recent judgments in the sector.

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Notes:

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Epitranscriptomic blood biomarkers to manage psychiatric disorders

Dinah Weissmann
ALCEDIAG, France

Major depressive and bipolar disorders are leading causes of disability worldwide yet, many people remain undiagnosed or misdiagnosed or ineffectively treated. Diagnosis relies on the clinical assessment of symptoms and currently, there is no molecular diagnostic test available. Identifying and validating blood biomarkers could provide a more accurate and objective means of diagnosis. Genetic and epigenetic events are involved in psychiatric aetiology, among them RNA editing modifications have been associated with inflammation and neuropsychiatric disorders. Adenosine to inosine RNA editing constitutes a physiological cellular process that translates environmental cues by regulating protein function at the synaptic level in health and disease. RNA editing is post-transcriptional process that leads to functional diversity of proteins. These marks form the molecular interface between the genome and the environment. Of particular interest is the RNA editing modification that occurs on the *phosphodiesterase 8A* gene located on chromosome 15q25.3, a genomic region that has recurrently been associated with early onset of major depressive disorder. ALCEDIAG's test, EDITDIAG, identifies in blood specific signatures through the RNA editing modifications of patients in different pathological conditions such as a cohort of hepatitis C infected patients, treated with interferon alpha and ribavirin were followed during 16 weeks. This treatment is well known to trigger depression in 50% of patients. RNA editing modifications were measured each two weeks as well as clinical evaluations of depression (MINI). An algorithm was identified which allows to discriminate patients with depression from others with a high specificity and sensitivity; a cohort of depressed patients (n=163) was compared to controls (n=69). A specific RNA editing signature was identified in depressed patients. The test shows that RNA editing related blood biomarkers allow to stratify patients, characterizes psychiatric conditions and follows up the disease/treatment modifications along time. This test paves the way for a better management of psychiatric patients.

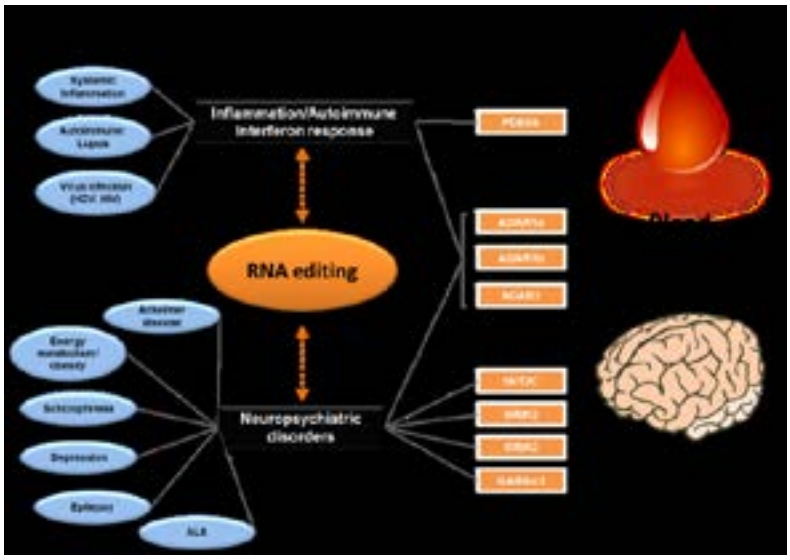

Figure: Schematic overview of the link between systemic inflammation and neuropsychiatric disorders. Blood and brain RNA editing biomarkers have been identified and characterised in various specimens and pathologies

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1. Weissmann D, Underwood M, Salvetat N, Cavarec L and Vincent L (2016) Region specific alterations of A-to-I RNA editin of serotonin 2c receptor in cortex of suicides with major depression. Translational Psychiatry 6(8):e878.
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3. Cavarec L, Vincent L, Le Borgne C, Plusquellec C and Ollivier N (2013) *In Vitro* screening for drug-induced depression and/or suicidal adverse effects: a new toxicogenomic assay based on CE-SSCP analysis of HTR2C mRNA editing in SH-SY5Y cells. Neurotox Res. 23(1):49-62.
4. Cambon K, Dos-Santos Coura R, Groc L, Carbon A and Weissmann D (2010) Aggressive behavior during social interaction in mice is controlled by the modulation of tyrosine hydroxylase expression in the prefrontal cortex. Neuroscience 171(3):840-51..

Biography

Dinah Weissmann is an Executive Vice President at ALCEDIAG's, biotech company dedicated to the development of innovative diagnostics based on epigenetic biomarkers mainly RNA editing. She is also Co-director of a public private laboratory, Sys2Diag that was created in 2015 and dedicated to the understanding of molecular basis of complex diseases. Her research fields are mainly in neuroscience which leads to the discovery of a specific plasticity in adult brain coined phenotype plasticity that gave rise to a novel class of antidepressant drugs. Later on, she focused on epigenetic mechanism understanding and developed new tests for diagnosis and management of psychiatric patients as well as prediction of psychiatric adverse effects. She has 15 years of research experience as Director of Research at the CNRS (French academic research institute) and has worked at various leader positions in pharma and biotech companies.

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Notes:

Kyo-Seon Kim et al., J Biotechnol Biomater 2018, Volume 8
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Development of monodisperse magnetic porous/hollow nanostructures for biomedical applications

Kyo-Seon Kim, Sang-Hyeok Yoon and **Nguyen D T**
Kangwon National University, South Korea

Recently, studies on therapeutic applications of the magnetic nanoparticles have gained more momentum. The porous/hollow structure also exhibited a great potential to encapsulate small drug molecules. Once inside the porous structures, small drug molecules would be shielded by the shell from fast reaction/deterioration in biological solutions. In our research, monodisperse magnetite Fe_3O_4 porous/hollow nanoparticles were successfully synthesized through one-pot solvothermal process without any surfactant and template as shown in Figure The Fe_3O_4 porous/hollow nanoparticles consisted of numerous tiny grains. Those particles were ferromagnetic with high saturation magnetization. The Fe_3O_4 porous/hollow nanoparticles were synthesized controllably with tunable particle size and porosity by adjusting the initial concentrations of Fe precursor and ammonium acetate. The formation mechanism of the magnetite hollow spheres comprised simultaneous chemical and physical processes including the formation of numerous tiny grains, the spherical assembly of those grains and the chemical conversion coupled with the relocation of the grains. The chemical conversion including a partially reductive reaction of the Fe (III) compounds and subsequent hydrolysis and dehydrolysis reactions of the Fe (III) and Fe (II) compounds to generate Fe_3O_4 caused the non-uniformities of tiny grains and the empty spaces within the spherical assemblies and thus enhanced the outward migration and relocation of the core grains toward the outer layer, resulting in the formation and expansion of the hollow core structure. The porous/hollow nanoparticles could be further coupled with a specific targeting agent and be concentrated around the area of interest, where drug molecules would be released either chemically via a pH control or physically through a magnetic stimulation and activation. Such a controlled drug release warranted the multifunctional porous/hollow nanoparticles a new class of carriers for simultaneous diagnostic and therapeutic applications.

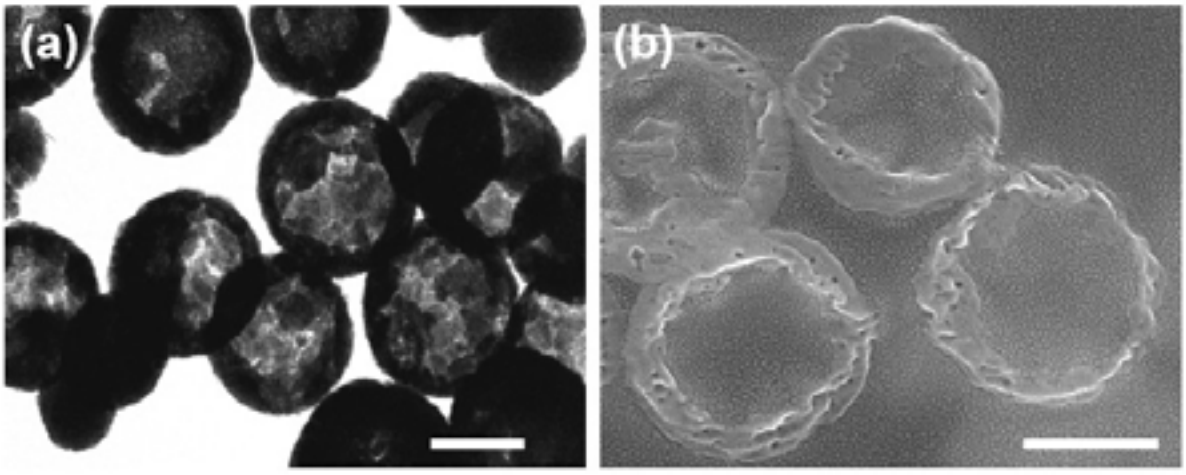

Figure: Representative (a) TEM image and (b) SEM image for the cross-section of the Fe_3O_4 porous/hollow nanospheres.

Recent Publications

1. Nguyen D T and Kim K S (2013) Template-free synthesis and characterization of monodisperse magnetite hollow nanoparticles through solvothermal process. J Nanosci Nanotechnol. 13(8):5773-5776.
2. Nguyen D T, Park D W, Kim T and Kim K S (2013) Controlled synthesis of magnetite porous/hollow nanoparticles through a template-free solvothermal process. J Nanosci Nanotechnol. 15(1):591-594.
3. Nguyen D T and Kim K S (2013) Analysis on development of magnetite hollow spheres through one-pot solvothermal process. AIChEJ. 59(10):3594-3600.

4. Nguyen D T, Charinpanitkul T, Park D W and Kim K S (2013) Preparation of magnetite hollow structure for drug delivery application. J Nanosci Nanotechnol. 14(10):7995-7999.
5. D T Nguyen and K S Kim K.-S (2015) Structural evolution of highly porous/hollow ZnO nanoparticles in sonochemical process. Chemical Engineering J. 276:11-19.

Biography

Kyo-Seon Kim is a Professor of Chemical Engineering at Kangwon National University, Chuncheon, South Korea, where he has been working since 1989. He has completed his BS, MS and PhD degrees in Chemical Engineering at Seoul National University, KAIST and University of Cincinnati, OH, USA in 1979, 1981 and 1989, respectively. His research interests are mainly focused on preparation and modification of nanoparticles for high-functional performances. The main applications of nanoparticles in his researches are in the fields of air pollution control, energy harvesting and development of medical devices.

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Notes:

Aliona Špakova et al., J Biotechnol Biomater 2018, Volume 8
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Synthesis and self-assembly of bacteriophage like particles in yeast: Novel molecular toolboxes

Aliona Špakova, Raminta Batiuškaite, Rasa Petraityte-Burneikiene, Eugenijus Šimoliunas, Lidija Truncaite, Vida Časaite and **Rolandas Meškys**
Vilnius University Life Sciences Center, Lithuania

Statement of the Problem: Current vaccines against infectious diseases have primarily relied on attenuated or inactivated pathogens. However, virus like particles (VLP) are used as vaccine platforms which are more favorable for their perfect defined structures; induction of strong immune response and also suitable for surface decoration by inserted foreign epitopes. While many icosahedral VLPs are synthesized in bacteria and the disadvantages such as lack of post-translational modifications are needed for eukaryotic proteins and contamination of purified VLPs with bacterial endotoxins are encountered. While icosahedral VLP platforms have been studied in detail but rod-shaped VLPs have been mostly forgotten. Until now, there is no information regarding the generation of tailed bacteriophage nanotubes in yeast.

Aim: The research aims to generate nanotubes using yeast expressed bacteriophage tail proteins and determine their tolerance for genetic introduction of foreign epitopes.

Methodology: DNA sequences coding tail proteins of bacteriophages NBD2, FV3 as well as RaK2 were cloned into yeast protein expression vectors. Synthesis of phage proteins was confirmed by protein electrophoresis and rod-shaped structures were analyzed by electron microscopy.

Findings: Our work has focused on developing an alternative epitope presenting rod-shaped platform which could be used for biomedical applications. To our knowledge, it is the first attempt to produce bacteriophage originated nanotubes in yeast cells which determines their tolerance for genetically incorporated foreign epitopes. Yeast protein synthesis system allowed efficient generation of long and flexible nanotubes originated from NBD2 tailed bacteriophage as well as tubes with different morphology from RaK2 and FV3 phages.

Conclusion & Significance: This work intends to show the suitability of yeast protein synthesis system to generate high yields of nanotubes that originate from tailed bacteriophages. The novel strategy presented here could provide safer vaccine candidates compared to the VLPs synthesized in bacteria.

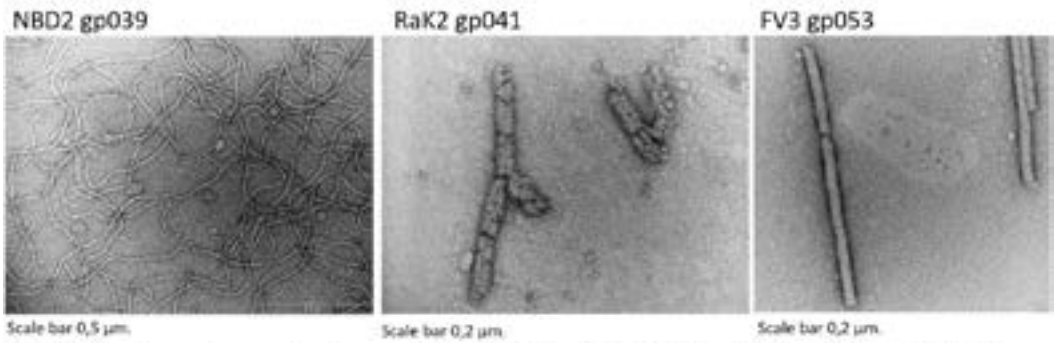

Fig. 1. Electron micrographs of recombinant NBD2 gp039, RaK2 gp041 and FV3 gp053 tail proteins generated in yeast.

Recent Publications

1. Kaliniene L, Truncaitė L, Šimoliūnas E, Zajančkauskaitė A, Vilkaitytė M, Kaupinis A, Skapas M and Meškys R (2018) Molecular analysis of the low-temperature Escherichia coli phage vB_EcoS_NBD2. Archives of Virology 163(1):105-114.
2. Schoonen L and van Hest J C (2014) Functionalization of protein-based nanocages for drug delivery applications. Nanoscale 6(13):7124-41.
3. Zeltins A (2013) Construction and characterization of virus-like particles: a review. Mol Biotechnol. 53(1):92-107.
4. Jennings G T and Bachmann M F (2008) The coming of age of virus-like particle vaccines. Biol Chem. 389(5):521-36.
5. Trivedi B, Valerio C and Slater J E (2003) Endotoxin content of standardized allergen vaccines. J Allergy Clin Immunol. 111(4):777-83.

Biography

Aliona Špakova is a PhD student at Life Sciences Center, Institute of Biotechnology-Vilnius University. Her research aims at generation of novel bacteriophage tail tube originated yeast expressed nanotubes as novel epitope presenting platforms.

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