

5.0.14

Exploring signaling events surrounding extracellular amastigote invasion processes of *Trypanosoma cruzi***D. Bahia^{*}, E. Alves Da Silva, P. Oliveira, M. Cruz, E. Gaspar, S. Hernandez, R. Mortara**

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Trypanosoma cruzi is a protozoan pathogen that infects humans and other mammals, producing a pathology called Chagas disease. The disease is endemic in most of central and South America affecting ~18 million people, with an increasing number of cases in North America. The parasite's life cycle alternates between vertebrates and insects and comprises distinct developmental stages. Amastigotes which are generated by the extracellular differentiation of trypomastigotes are referred to as Extracellular Amastigotes (EA) and are able to invade cultured cells. EA of the G strain promptly aggregate actin filaments by attaching to dorsal microvilli of HeLa cells and, as a result, cup-like structures are formed underneath the parasite. EA is therefore dependent on host actin filaments polymerization to invade cells. EA invasion can be easily detected by several techniques, such as freeze-fracture replicas of recently infected HeLa cells. However, signaling events surrounding these processes are still obscure. In the present study, we aim to examine these events and EA invasion features by focusing on the following molecules: cortactin, ezrin, Protein Kinase D (PKD) and a set of kinase inhibitors. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion. The ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. Ezrin is characterized by an N-terminal FERM domain and a C-terminal actin-binding domain. Once activated, ezrin dissociates and acts as a plasma membrane-cytoskeletal linker and thereby affects a variety of cellular activities, such as actin cytoskeleton regulation, control of cell shape, cellular adherence and migration and the modulation of intracellular signaling pathways. It has been recently demonstrated that cortactin is a substrate of PKD phosphorylation *in vivo* and it also colocalizes with ezrin. HeLa, Vero and CHO cells were transfected with the following markers — cortactin, ezrin, RhoA and PKD GFP-vectors — infected with EA and examined for the acquisition of these markers. Cells were previously treated with kinase (PKC, MAP kinase, Src, PI3K and Rho) inhibitors and control cells were left untreated. Several initial assays have yielded encouraging results. For example, PKD is recruited to sites of actin remodeling at the leading edge of EA invasion, which also recruits cortactin. Colocalization of PKD and cortactin may be an indication that PKD plays a role in cytoskeletal reorganization. PKC—PKD signaling cascade is crucial to PKD function in cells. PI3K seems to interfere with neither ezrin nor cortactin recruitment. Here, we propose a signaling pathway model of EA entry. This pathway includes the above-mentioned kinases as upstream molecules.

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5.0.15

Investigation of differences in intestinal microbial composition between breast-fed and infant prebiotic formula-fed infants**O. Martinov^{1,*}, U.O.C. Lugonja², S. Snezana¹, G. Gojgic-Cvijovic¹, M. Vrvic²**¹ Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia² Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

Breast-feeding is the best way of feeding a newborn baby. The kind of delivery and feeding have important influence on the composition of the intestinal flora of newborns.

The aim of this study was investigation of possible differences between the composition of gut microbiota of breast-fed and formula (supplemented with prebiotic)-fed newborns (younger than six months).

Healthy, term born infants enrolled in a four-week study and were divided in two groups — prebiotic formula-fed group and breast-fed group.

Fecal samples were obtained before formula administration (0 day) and during formula administration (14 and 28 days). At study days 0, 14 and 28 total aerobic and anaerobic bacteria, fungi, *Lactobacillus* and *Bifidobacterium* counts were performed on the fecal samples of both groups.

Before (0 day) and after 14th day of feeding the median number of bifidobacteria did not differ among the group. At the end of the 28th day feeding period, the population of bifidobacteria was significantly higher in formula-fed infants versus breast-fed infants. There (during four-week feeding period) was no statistically significant difference in the number of lactobacillus between the babies and no statistically significant difference in the total number of aerobic and anaerobic bacteria and fungi between formula-fed and breast-fed babies.

Infant milk formula containing prebiotic is able to induce intestinal flora of newborns that closely resembles the microbiota of the breast-fed infants. According to obtained results we concluded that addition of prebiotic to infant formula have beneficial effect for the baby and thus minimizes differences in the composition of the intestinal flora between the breast-fed and formula-fed infants.

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Similar bifidogenic effects of the infant formulae with added inuline and breastfeeding on gut microflora**N. Lugonja^{1,*}, O. Martinov², S. Spasic², G. Gojgic², M. Vrvic¹**¹ Faculty of Chemistry, University of Belgrade, Serbia² Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia

At birth and in the next few days begins the microbial colonization of human intestine. The composition of the intestinal flora newborns is influenced by the kind of delivery and feeding. *Bifi-*

dobacteria, as probiotics, are dominant flora in gastrointestinal tract (GI) of newborn, and their development is stimulated by food — substrate, what is called bifidogenic effect. Infant formulae are 'substitutes' for breast milk.

The purpose of this study was to determine if there is and what are the differences in the bifidogenic effects and gut microflora of breast-fed and formula-fed newborns (younger than 6 months and children age from 6 to 12 months).

Healthy, term born infants were enrolled in a four-week study in four groups — formula-fed group (FF1 — infants younger than 6 months and FF2 — children 6—12 months old) and breast milk (control) group (BM1 — infants younger than 6 months and BM2 — children 6—12 months old). Fecal samples were obtained before formula administration (0 day) and during formula administration (14 and 28 day). At study day 0, 14 and 28 stool specimens were quantitatively cultured and evaluated count of *Bifidobacteria*.

Before (0 day) and after 14 days of formula administration, the median number of *Bifidobacteria* did not differ among the group infants younger than six months as in between the groups of older children. At the end of the 28-day feeding period, the number of *Bifidobacteria* significantly increased in both FF group versus BM group.

These data indicate that supplemented term infant's formula with inuline has a stimulating effect on the growth of *Bifidobacteria* in the intestine as on children younger than 6 months, that as on children 6—12 months old. We conclude that infant formula with inuline has similar effect on the intestine of new born as human breast milk.

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Treatment of oily sludges in soil — a field study

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Various activities of the oil industry such as, drilling, production, processing and distribution generate significant volumes of solid waste, containing various classes of hydrocarbons. Alternatives of treatment are varied processes including physical, chemical and biological agents to remove organic pollutants at concentrations that are undetectable or, if detectable, the concentrations below the limits established as well as acceptable by the laws. Among biotechnologies, process of *landfarming* presents a considerable emphasis on the basis of the low operational cost, and availability of treatment of large volumes of oily wastes. *Landfarming* is based on the potential of organizations and/or their products to assist in the removal of hydrocarbons, reactive layer in the prepared soil. This work aims the treatment of oily wastes at an area of 1000 m². Methodologies were used for this operational biostimulation through humidification and homogenization. Along side was an isolated area known as ground control. To evaluate the per-

formance of the bioprocess were monitored relevant parameters such as pH, humidity, levels of total organic carbon, phosphorus, nitrogen, metals, total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), total heterotrophic aerobic bacteria (THAB), filamentous fungi and total heterotrophic anaerobic bacteria (THANB) for seven months. Results of work were promising by considering the time and initial concentration of contaminants. Content of TPH decreased 89.6% in the treated soil, while the control soil was negligible degradation of the order of 22.4%. The concentration of PAH decreased 88.7% in the treated soil and control the degradation reached 25.1%. Thus, the techniques of biostimulation that is humidification and homogenization were essential for increasing biodegradation of hydrocarbons. Population of THAB, fungi and THANB showed the average value of 1.42×10^7 CFU g⁻¹, 2.67×10^5 CFU g⁻¹ and 2.24×10^6 cells g⁻¹, respectively. Test of ecotoxicity in treated soil was an indication of efficiency of the process.

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Iodide for the phytoextraction of mercury contaminated soil

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Mercury is a highly toxic element that is found both naturally and as an introduced contaminant in the environment. Although its potential for toxicity in highly contaminated areas is well documented, research has shown that mercury can be a threat to the health of people and wildlife in many environments that are not obviously polluted. Its exposure, its form and the geochemical and ecological factors that influence how mercury moves and changes form in the environment determine the risk.

Contamination of the soil with mercury has often resulted from human activities, especially those related to mining, industrial emission, disposal or leakage of industrial wastes and application of sewage sludge to agricultural soils. Many remediation techniques have been used for the removal of Hg from contaminated soil, like chemical extraction or electroremediation, but they are relatively expensive and they interfere with the natural environment, so their applications are limited. An alternative method, which is viewed as environmentally friendly, is phytoremediation.

Low bioavailability of Hg in soil is a restricting factor in phytoextraction of mercury contaminated soil. To enhance the phytoextraction potassium iodide (KI) has been used. The phytoextraction was carried out with the use of *Lepidium sativum* plant. The process was run under laboratory conditions, in a model soil. Inorganic forms of mercury (HgCl₂, HgSO₄ and Hg(NO₃)₂) were used to contaminate the soil. The phytoextraction for different mercury salt used for soil contamination was conducted before and after potassium iodide application to the soil. Iodide was applied in different amounts. In all variants of the process the total mercury concentrations in roots, stems and leaves were determined.

The result showed that *L. sativum* accumulated mercury from soil. The overall maximum concentration of mercury in its com-