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IN P09. Use of Azospirillum brasilense co-inoculated with rhizobia: alternative to enhance peanut yields

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Bacteria of the family Rhizobiaceae interact with leguminous plants in a host-specific manner and form N_2-fixing root nodules. On the other hand, bacteria of the genus Azospirillum are free-living, surface colonizing, sometimes endophytic diazotroph and plant growth promoting rhizobacteria (PGPR). They are capable of increasing the yield of important crops growing in various soils and climatic regions.

Arachis hypogaea L. (peanut) is one of the most important legume crops cultivated in the central area of Argentina. Inoculation of peanut is a controversial practice because nodulation by native bacteria is usually assumed to be sufficient. The experiments carried out in this work are in the context of a national project whose main goal is to optimize the symbiotic association between peanut/rhizobia using different co-inoculation methods. In this framework, it has to be provided that there are no problems of mutual exclusion, displacement, or competence between the inoculant strains. The aim of this research was to study the effect of co-inoculation of recommended and indigenous bradyrhizobia and a PGPR (Azospirillum brasilense Cd) on nodulation and symbiotic performance of peanut. There were inoculated at the time of transferring the pregerminated seeds with A. brasilense Cd at $1x10^6$ CFU ml^{-1} and rhizobia strains $1x10^5$ CFU ml^{-1}.

All values of root dry weight of inoculated peanut did not show significant changes with respect to the control conditions. A general positive effect on nodulation patterns number of nodules and growth was observed in the presence Azospirillum-PC34 strain co-inoculation. Co-inoculation with recommended bradyrhizobia (SEMIA6144 and TAL1000) and A. brasilense Cd caused a significant increase (80 % and 50 % respectively) in the total number of nodules per plant by comparison with the number for plants treated with SEMIA6144 or TAL1000 alone. Possible mechanisms involved in the influence of A. brasilense on this symbiotic system will be discussed.

Biotecnology and Fermentations

BF P01. SODIUM AZIDE BINDING TO GOAT PROBIOTIC

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In a previous paper the effects of probiotic supplementation on the diminution of mutagenic compounds in gut goat were determined in vivo (Apas et al 2010). In addition, the diminution of putrescine, a gut cancer marked was observed. In order to study if the antimutagenic effect could be due to the absorption of mutagen by probiotic strains, we study the probiotic binding.

The strains used in the probiotic mix, Enterococcus faecium DDE39, Lactobacillus alimentarius DDL48, Lactobacillus reuteri DDL 19, were challenged in the presence of sodium azide (15μg/mL) during two hours at 37°C. After this time, using the AMES test and the strains Salmonella typhimurium TA 100 and Salmonella typhimurium TA 98, as biological mutagen indicator, the binding was evaluated. The results indicated that the binding of Lactobacillus reuteri DDL 19, Enterococcus faecium DDE39 and Lactobacillus alimentarius DDL48 was of 74%, 69% and 59%, respectively using as bioindicator the strain Salmonella typhimurium TA 100 and 72, 72 and 44% using as indicator Salmonella typhimurium TA 98

The goat probiotic strains used in this study have shown high antimutagenicity and binding properties

BF P02. BIOSORPTION OF MOLASSES DYES BY VIABLE AND NON-VIABLE MICROBIAL BIOMASS

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Molasses, a by-product of sugar extraction process, is a common raw material used in several industrial fermentations. However, molasses contains colored substances, some of them with inhibitory activities. Thus, innovative technologies, such as biosorption, are needed as alternatives to conventional methods to find inexpensive ways of removing dyes. With respect to dye biosorption, microbial biomass (bacteria, fungi, microalgae, etc.) outperformed macroscopic materials (sea-weeds, crab shell, etc.). The reason for this discrepancy is due to the nature of the cell wall constituents and functional groups involved in dye binding. In this connection, the major objective of this study was to investigate the potential of viable and non-viable microbial biomasses as biosorbent for the removal of dyes from sugar cane molasses. Materials and methods: Both viable and inactivated by autoclaving biomasses of Aspergillus niger ATCC MYA 135, Saccharomyces cerevisiae and Brevibacillus agri MIR E12 were used. Microorganisms were grown on agarized medium as well as in liquid medium. Potato glucose, LB and YEPD were used for the cultivation of A. niger, B. agri and S. cereviciae, respectively. Decolorization experiments were conducted at 30 °C by shaking molasses solution at 300 rpm during 20 min in the presence of 1.5 or 3.0 % of microbial biomass (wet weight/v). The molasses pH was adjusted at 3, 7 or 9 being its initial reducing sugar concentration 10 g/l. Decolorization capacity

was determined by monitoring the absorbance at 475 nm. Results and conclusions: The initial molasses pH value was an important factor for the biosorption process. In the primary screening step, the inactivated biomass of B. agri grown in liquid medium showed the highest decolorization capacity (47 %) when a molasses at pH 3 was used. Interestingly, the total amount of reducing sugar did not significantly change. In addition, the production of an extracellular lipase from B. agri was significantly increased when this treated molasses was used as carbon source. On the other hand, the best decolorization capacity obtained with either A. niger or S. cerevisiae was detected with viable biomass growing on agarized medium and using molasses at initial pH 9. These results show the ability of microbial biomass to remove colored substances from sugar cane molasses. This work was supported by grands PIP-CONICET 297 and CIUNT 26/D409.

BF P03. A RAPID-BOD BIOASSAY BASED IN lyophilized klebsiella pneumoniae

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A microbial amperometric bioassay where Klebsiella pneumoniae strain employs ferricyanide as the last electron acceptor while degrading organic compounds has been developed in order to replace the established BOD_5 determination method when active interventions for environmental monitoring and control process are needed. The ferricyanide-mediated approach has been proposed years ago to overcome O_2 low solubility and long incubation times. Higher solubility of ferricyanide enables the increase of microbial load reducing determination times from days to hours.

The traditional BOD_5 test correlates biodegradable amounts of organic matter with dissolved oxygen consumed by microorganisms in samples after 5 days of incubation. Instead, our bioassay correlates easy biodegradable amounts organic matter with ferrocyanide concentrations, ferricyanide oxidized form, employing electrochemical techniques as amperometry. The used strain has been isolated from a commercial non-pathogenic consortium and identified by 16S rDNA sequencing process and BLAST sequence alignment tool as Klebsiella pneumoniae strain K30 or Klebsiella pneumoniae strain K8, both originally isolated from rhizosphere. Issues as replacement of centrifugation by the use of formaldehyde and avoidance of N_2 sparging of the samples, procedures usually made in ferricyanide-mediated approaches have already been successfully reached using freshly harvested cultures and a Pt electrode in order to develop a field dispositive to determine BOD values in-situ. This bioassay has given accurate $BOD_{K.\ pneumoniae}$ values of real municipal wastewater samples compared with the BOD_5 method. Now, the last improvements to the final dispositive is the employment of a disposable vibratory screen-printed Au electrode using freeze-dried bacteria suitable for a commercial bioassay mass production.

Several lyophilization methods have been assayed. Trehalose 100 mM used as lyoprotectant drove to a bioassay as sensitive as freshly harvested cultures (the slope of a GGA calibration curve was similar in both cases 10.4 and 9.6 -nA L mg^{-1} respectively) even when the growth rate decreased to 96%. These results enable the design of a disposable microbial bioassay for rapid BOD determination in wastewaters, treatment waters and water natural sources samples. Even when our Klebsiella pneumoniae is an environmental strain, these were the last assays done using it given the existence of pathogenic, antibiotic resistant strains of this species.

BF P04. DETERMINING BIOMASS IN SOLID STATE FERMENTATION CULTURES OF ASPERGILLUS TERREUS DURING LOVASTATIN PRODUCTION

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Lovastatin, a class of secondary metabolite typically produced by Aspergillus terreus strains, has become the focus of great attention due to its ability to block the de novo synthesis of endogenous cholesterol, which is nearly 2/3 of human total body cholesterol. Additionally, statins present potential applications for the treatment cancer, Parkinson and Alzheimer diseases, as well as viral and fungal infections, because of their capability to inhibit mevalonate derivatives biosynthesis. High lovastatin production yields may be microbiologically obtained by implementing culture systems in solid state. Since biomass levels are closely related to lovastatin productivity, appropriate methods for the determination of fungal growth are usually required. However, one of the main drawbacks is that the direct analysis of biomass by dry weight determination is not possible in solid state fermentation systems. Therefore, this work was aimed at comparing two indirect methods for biomass determination: one based on the N-acetylglucosamine content, a component of the fungal cell wall, and the other one, based on the ergosterol content, a fungal cell membrane steroid. N- acetylglucosamine was assessed by the colorimetric method with MBTH reagent, after sample acid hydrolysis. Extracted ergosterol, previous sample saponification, was analyzed by RP-HPLC with a photodiode array detection (PDA) system. A high correlation coefficient (r^2: 0.98) was found between fungal biomass dry weight and the ergosterol content as determined by HPLC, confirming the validity and reliability of this standardized technique. Additionally, the ergosterol method showed to be highly reproducible and time-efficient. On the other hand, the obtained results according to the N-acetylglucosamine method denoted that this technique would not be applicable to any solid culture system due to the false positive reactions (interference) observed for abiotic controls in complex systems with certain solid substrates.