095 - BIOREMEDIATION OF SOIL CONTAMINATED BY DIESEL B5

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In recent decades, environmental problems caused by accidents with fossil fuels become more frequent. Remediation of polluted sites by petroleum hydrocarbons with microbial preparations is of special importance because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminant. This study aimed to compare the biostimulation (BE) and bioaugmentation (BA) on the degradation of total petroleum hydrocarbons (TPH) in soils contaminated with 5% of biodiesel in diesel (B5). All experiments were conducted in duplicate with the following treatments: control (C) (contaminated soil); biostimulation (contaminated soil with nutrients $(NH_4)_2SO_4$ and K_2HPO_4 giving a final C: N: P of 100:10:1); bioaugmentation (contaminated soil with nutrients and inoculation of indigenous microorganisms cultivated in BH medium with 1% of B5). Contaminated soils (300 g samples) were placed in a set of polystyrene pan, and aerated by mixing. The microcosms were kept at room temperature (27°C), moistened every week and were sampled at 0, 34 and 62 days for chemical and microbiological analyses. Total petroleum hydrocarbon (TPH) was extracted from soil by Soxhlet extraction (USEPA 3540C) and analyzed by gas chromatography with mass selective detector (USEPA 8015). Dilutions of the soil suspension were spread-plated (three replicates/dilution) on the PCA media for total aerobic heterotrophs. In relation to soil microorganisms was found for all microcosms an increase in the heterotrophics population, reaching 2.6×10^7 UFC g^{-1} soil (BE) and 4.2×10^7 UFC g^{-1} soil (BA). In both experiments, a higher proportion of the hydrocarbon component of B5 was degraded compared to control. On average, 43.6% and 56.0% of TPH were degraded respectively for biostimulation and biaogmentation experiments. Indigenous microorganisms are well adjusted to their own environment. An immediate increase in the population density of these microbes could ensure rapid degradation of the pollutant. We conclude that the techniques employed (BE and BA) are feasible to be used in bioremediation of soil contaminated with B5, with superior results than natural attenuation (control).

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096 - *IN VITRO* CLONING AS CONTRIBUTION TO *ELAEIS* BREEDING PROGRAM

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Elaeis guineensis intraspecific hybrids tenera (dura x pisifera) are considered the best producers of oil that is used for food, biofuels and cosmetics. In Latin America, tenera plantations are harmed by an anomaly named lethal yellowing but F1 hybrids between *E. guineensis* and *E. oleifera*, the Amazon native species, survive in areas strongly affected. In addition, E. oleifera has a lower rate of annual shoot growth and a higher content of unsaturated fatty-acids. To maintain high yields *guineenis* x *oleifera* hybrids are retrocrossed with *E. guineensis*. Indeed progenies resulting from this complex breeding strategy present high variability. Cloning RC embryos can reduce "intraprogeny" variability and facilitate comparison among progenies allocated to different experimental areas. The objective of this work was to test different auxins and auxin concentrations to induce somatic embryogenesis in zygotic RC embryos. Fruits from three RC progenies (named 585, 586 and 321) and a F1 were collect 100+2.4 days after pollination, at Embrapa Western Amazon's Rio Urubu Experimental Station, Rio Preto da Eva, Amazonas, Brazil. Seeds were treated with 50% commercial bleach for 10 minutes and excised embryos with 5% bleach for 5 minutes.

Explants were cultivated in MS *medium* and vitamins supplemented with 110, 150 and 200 mg.L^{-1} 2,4-diclorophenoxiacetic acid (2,4-D) or 186 mg.L-1 naphtalenacetic acid (NAA). Frequencies of primary (PC) x embryogenic *calli* (EC) observed in six months were compared by chi-squared tests. In the sixtieth month 29% (108/375) explants were alive. The rest was discarded by oxidation, hardening and stop reacting, rooting and contamination. Regardless the auxin used, the frequency of PC (18%) was higher than that of EC (11%) what differed among progenies (P=0.005). 150 mg.L^{-1} 2,4-D influenced the frequency of EC in different progenies (P=0.005) and the induction of 24% EC in progeny 585 (P<0.001). For progenies F1 and 586, different concentrations of 2,4-D were not related to *callus* types. For progeny 321, 2,4-D and NAA were compared and none of them could be linked to different *callus* types. *Calli* induction with 2,4-D was simultaneous to development of shoots. Granular yellowish *calli* were more frequent. NAA induced compact, white, opaque nodular calli more frequently, which are apparently polyembryogenic complexes resulting from direct embryogenesis. In conclusion we suggest that differences among progenies are more pronounced than the effects of different auxins. Concerning is the low frequency of embryogenic *calli* obtained because it can turn difficult to represent satisfactorily the genotypes within progenies.

Funding Source: FINEP.

097 - PRODUCTION OF AN ANTIAPOPTOTIC RECOMBINANT PROTEIN BY AN BACULOVIRUS/ INSECT CELLS SYSTEM

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Apoptosis plays a central role in a variety of biological processes such as differentiation and embryonic development of diseases like cancer and Alzheimer's. In biotechnological processes, apoptosis is a limiting factor to be considered especially when cell cultures is used. Already is demostrated the presence of active principles in the hemolymph of the *Lonomia obliqua* caterpillar, including a potent antiapoptotic protein. These active ingredients were biologically characterized and have potential as candidates for development of pharmacologically active drug and innovative biotech products. The occurrence of apoptotic cell death in cultured mammalian cells and insect depleted of nutrients has been studied In these culture the supplementation of cultured insect cells and mammalian with *Lonomia obliqua* hemolymph extended culture viability avoiding death by apoptosis. The baculovirus / insect cell expression system described by Smith et al. in 1983 is widely used as a tool for producing complex recombinant proteins that require post-translational modifications for its correct use therapy, besides allowing an excellent cell growth in bioreactors, aimed at increasing scale. Thus, the aims of this study is to produce na antiapoptotic protein in a recombinant baculovirus system / insect cells (Invitrogen™). In this system, the recombinant virus produced was used to infect Sf9 and UFLAG cells. Three passages of recombinant viruses in cells grown in spinner were performed. These cultures were maintained in agitation at 100 rpm with a working volume of 15 ml. We observed an intense granulation in the infected cells after 48 hours in Sf9 cells. Little grain was observed in infected UFLAG cells. After 7 days, all infected cells died, while in control cell viability was over 80% in the same time. Samples of the supernatant of infected cultures were collected daily, concentrated and subjected to SDS-PAGE chromatography. A protein band around 20 kDa was observed. A sample of the infected culture was subjected to an analysis in electron microscopy for visualization of viral particles. We also observed an increase in volume of the cell nucleus in cells infected. Currently, the recombinant proteins obtained in the supernatant is being purified by affinity chromatography and its activity is being tested in cell cultures where apoptosis was induced by chemical agents.

Financial Support: FAPESP (08/57263-5), CAPES.