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NATURAL PRODUCTS

WITH PHARMACEUTICAL, NUTRACEUTICAL,
COSMETIC AND AGROCHEMICAL INTEREST



PK24

Automated Cherry Picking Process of natural products extracts by using remote data on a robotic platform

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The Natural Products effort at CIBE covers the initial steps of the current research program on new Antiinfectives at Merck. Since the introduction in October 2005 of a new High Throughput platform for the preparation and evaluation of extracts from fungi or actinomycetes, the number of extracts processed has increased considerably. Current numbers reach the 20,000 complex extracts evaluated per month.

After primary screening assays some of the extracts are selected for being confirmed. The unexpected growth in the number of extracts made necessary an automated way of managing these extracts whose activities needed to be confirmed and tested in our secondary screening platforms.

In response to this challenge, a new system was proposed for performing a full automated Cherry Picking at CIBE. The initial objectives were to avoid manual procedures and reduce the risks of mistakes by using Barcodes. To decrease the manpower needed per extract for primary screening was a secondary benefit of the system where the active extracts are transferred between positions of 96 Deep Well plates with a robot.

For this new process the robot selected for the development of the application and Workflow was determined to be a BiomekFX + Cytomat platform due to its availability in the lab and the capability of this equipment to manage high numbers of plates without human interaction.

A key obstacle in the implementation of the new automated Cherry Picking resulted to be the impossibility of finding a commercial solution. Requirements of connecting our Oracle databases to determine which where the source and destination positions, and the idea of tracking any plate by barcodes forced us to design a proprietary VB application as a way to create working lists and send 'ready to use data' to the robot.

PK25

In vitro propagation of *Piper nigrum* L. (Black pepper)

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Piper nigrum (black pepper) is one of the most important spices medicinal plants used by man since historic times. It is given in dyspepsia and flatulence, as an anti-periodic in malarial fever, alternative in paraplegia and arthritic diseases. The present system available for multiplication is vegetative propagation viz. rooting of cuttings is inadequate and diseases caused by virus could be spreaded, *In vitro* propagation of (*Piper nigrum* L.) was carried out using nodal explants. The main problems of culture establishment of *in vitro* propagation were browning due to phenolic compounds, internal bacterial and fungus contamination in the initial stage. Browning of explants was overcome by incorporation of PVP (Polyvinyl Pyrrolidone) at the rate of 200 mg/L initial culture incubation in low light and frequent transferring of explants to the fresh medium and fungal contamination was overcome by proper maintaining of mother plants in the green house. Nodal explants were successfully established in MS (Murasige and Skoog, 1962) medium supplemented with 2.5 mg/LBA after sterilization with 0.1% HgCl₂. Shoot proliferation was compared using MS medium supplemented with different BA, Kinetin and 2ip levels. BA was the superior for shoot multiplication and the suitable BA concentration was 3.5 mg/l. A higher shoot multiplication observed in liquid medium (6.57) over solid medium (4.0). Shoots were rooted MS medium with 1 mg/LIBA and charcoal. The rooted plantlets were transferred to soil more than 80% success. Thus a protocol for *in vitro* propagation of *Piper nigrum* was developed.

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2. Bat et al; (1995) Plant cell reports 14: 398-402

PK26

Evaluation effect of media formulation, pH and temperature on "shiitake" mycelium growth on solid and liquid culture conditions

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Shiitake mushroom [*Lentinula edodes*(Berk) Singer/Pegler] has the third production class among the most important other edible mushrooms. For a long time this mushroom has drawn attention due to its unique flavor and taste and also therapeutic properties. The presented research aimed at the optimization of the environmental conditions for shiitake production. In the first experiment the effect of four medium types in solid form, three media pH (4.5, 5.5 and 6.5) and two temperatures regimes (25 and 27 °C) were evaluated on mycelium radial growth of the mushroom. In the second experiment the effect of medium types and pH was evaluated on mycelium yield in liquid form at 25 °C. The obtained results of the first experiment showed that medium type, pH and temperature significantly affect mycelium growth rate at $p \leq 0.01$. Interaction between medium \times pH, temperature \times medium and pH \times temperature also was significant at $p \leq 0.01$. The highest growth rate (8.548 mm/day) was detected on 1 and 2 "hand making" media and the lowest one (6.201 mm/day) was observed on MEA alone. The higher the temperature (27 °C) the lower the mycelium growth rate. Increasing the medium pH from 4.5 to 6.5 also decreased mycelium growth rate and the highest growth rate was observed at pH=4.5. In the second experiment (liquid medium), medium type and pH also affected mycelium yield significantly ($p \leq 0.05$). The highest mycelium yield was observed in hand making media and on the contrary to the first experiment, the best mycelium yield was obtained at pH=5.5

PK27

Green synthesis of gold nanoparticles using *Camellia sinensis* ethanol extract

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Today, nano metal particles, especially gold, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of medicine, chemistry and biotechnology at the nanoscale [1]. Also development of non-toxic, clean techniques for the synthesis of metal nanoparticles such as gold has attracted increasing attention in recent years. Although many reports have been published about the biogenesis of gold nanoparticles using several plant extracts such as Neem leaf broth (*Azadirachta indica*), the capacity of a large number of such extracts to form gold nanoparticles has yet to be elucidated. In this research an extract provided from *Camellia sinensis* is employed for green synthesis of gold nanoparticles. This extract was used for the synthesis of gold nanoparticles through the reduction of aqueous AuCl₄⁻. Transmission electron microscopy, energy-dispersive spectroscopy and visible absorption spectroscopy confirmed the reduction of gold ions to gold nanoparticles. This extract produced gold nanoparticles in the size ranges of 2-16 nm with an average size of 3 nm. To the best of our knowledge, this is the first report on the synthesis of gold nanoparticles using a total extract of *Camellia sinensis*.

References: 1. Magudapathy, P. et al. (2001) Physics B. 299: 142-146.

PK28

Induction of nutritive corms through in vitro culture of *Gastrodia elata* immature tubers

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Gastrodia elata Blume, a parasitic herbaceous plant, belongs to Orchidaceae, and its dried roots (tubers) have been used as a traditional Chinese medicine for various human diseases such as vertigo, blackout, headache, hemiplegia and convulsions epilepsy. *G. elata* is devoid of chlorophyll and exhibits symbiosis with the fungus *Armillaria mellea* (myco-heterotrophy). However the yields of *G. elata* have been decreased, mainly due to the degeneration of spawn tubers caused by repetitive asexual reproduction. This study was conducted to know the possibility of asexual reproduction through *in vitro* culture of *G. elata* immature tubers.