Poster 3.14

DETECTION METHOD FOR AMINO ACID DECARBOXYLASE ACTIVITIES OF BACTERIA IMPLICATED IN FOOD POISONING USING MOELLER DECARBOXYLASE MEDIA IN MICROTITRE PLATE

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Test of amino acid decarboxylase activity has been done in tubed broth (2000 μl/test) or plated agar (30,000 μl/test). In the current study, the test was carried-out in wells (250 μl/test) of microtitre plate containing biogenic amines precursors. Wells of 96 well-microtitre plate containing Moeller decarboxylase base broth (MDB) with or without 1% amino acids were inoculated with E. coli, K. pneumoniae, A. anitratus and S. aureus. Uninoculated wells containing only MDB or MDB with amino acid served as controls. The absorbance of culture broth was read at 570 nm at 1-1.5 hour intervals for 7.5 hours. Comparison of means of %ΔA570 between 0 hour and the consecutive hours were determined statistically. Growth of E. coli and K. pneumoniae caused significant increase in absorbance starting from 2.5 h onwards in the medium containing histidine, lysine and ornithine, and 4 h onwards in the medium containing histidine and lysine, respectively (p<0.05). Positive decarboxylase activities were detected in E. coli and K. pneumoniae in shorter time (<6h) than the tube method (18-48 hours). The current method is suitable for detection of immediate producers of amino acid decarboxylase enzymes and has the potential to be used for screening bacteria in food materials. It is more cost-effective than tube or plate method for using less media and amino acids.

Poster 3.15

THE RESPONSE OF PHENOTYPICALLY SWITCHED CANDIDA KRUSEI TO THE EXTRACT OF PIPER BETLE

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Objective: To study the response of phenotypically switched Candida krusei to extract of Piper betle. Methods: Candida krusei (ATCC 14243) was revived from stock culture on YEPD agar containing 5 mg Phloxine B dye. Following a 5-days incubation period, colonies showing a different colony morphology were enumerated and considered as having a phenotypic switch from the original culture. These colonies were considered as the first generation of Candida krusei and subcultured on new media plates to
observe for subsequent switching. The phenotypic switch was repeated and observed for four generations. Each generation was tested for antifungal susceptibility towards Piper betle aqueous extract by disc diffusion test, minimum inhibitory concentration test (MIC), minimum fungicidal test (MFC) and growth profile. Results: Based on disc diffusion test, it was found that all switched generations were susceptible to Piper betle extract with an ascending sensitivity from the first to the fourth generation. The growth of each generation was influenced by Piper betle extract with decreasing in OD% at stationary phase from untreated Candida krusei with 16% (first generation), 20% (second generation), 18% (third generation) and 16% (fourth generation). Conclusion: The susceptibility and growth profile of each switched generations of Candida krusei was found to be greatly affected in the presence of Piper betle aqueous extract.

Poster 3.16

EVALUATION OF CHITINASE ACTIVITY AND RT-PCR OF ENDOCHITINASE ECH42 GENE FROM LOCAL TRICHODERMA ISOLATE

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Malaysian oil palm accounts for 51% of the world’s palm oil production. However, Ganoderma Basal Stem Rot (BSR) disease has reduced the yield from its full potential by 25-45%. Chitinases produced by Trichoderma spp. have been shown to have strong antifungal activity against wide range of pathogen. This study aimed to construct endochitinase ech42 cDNA by RT-PCR and evaluate the chitinase activity of local of Trichoderma spp. Cultures of Trichoderma spp. were isolated from soil at Balau Estate, Semenyih, Selangor, Malaysia. Mycelia were incubated in the presence and absence 0.75 % w/v of colloidal chitin prior to RNA extraction. Total RNA was extracted and PCR was conducted using primer sequence from Carollo et. al. (1994) on both genomic DNA and first strand cDNA. This produced a 900 bp band and BLAST results showed 98 % percentage of homology to 42 kDa endochitinase of Trichoderma spp. The effects of colloidal chitin on chitinase activity were investigated by chitinase assay using DNS reagent. Level of chitinase activity was significantly (P<0.05) higher in chitin induced culture. Induction of chitinase activity in local Trichoderma isolates indicate the potential antifungal activity of these chitinases. Further study will be conducted to determine the expression of chitinases induced by BSR pathogen, Ganoderma boninense.