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PRODUCTION BIOTECHNOLOGY WITH CONTROLLED ENVIRONMENTAL REGIMES, SUBSTRATES AND ENZYME MARKERS FOR THE SHIITAKE MUSHROOM, LENTINULA EDODES

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By

GANISAN KRISHNEN

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PRODUCTION BIOTECHNOLOGY WITH CONTROLLED ENVIRONMENTAL REGIMES, SUBSTRATES AND ENZYME MARKERS FOR THE SHIITAKE MUSHROOM, *LENTINULA EDODES*

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Faculty : Agriculture

Mycelial growth of *L. edodes* was optimum at 25°C in six of the seven strains of *L. edodes* tested. Mycelial biomass production was greater on sawdust than grain with 0.53 to 1.60 mg glucosamine per 200 g substrate derived from sawdust compared to 0.02 to 0.2 mg glucosamine per 200 g from grain. In fructification, a fluctuating temperature set of 15°C (8 h)/25°C (16 h), with continuous lighting at 25-35 lux, humidity of 80-95% and aeration of 1.3 - 2.5 m/s produced the highest yield or Biological Efficiency (B.E.) in 11 of the 13 strains tested at three temperature sets. Strains L38 (7.1-32.7% B.E.) and L300 (7.2-35.5% B.E.) were the best yield producers at all three temperature sets. Mycelial density during spawn-run was found to be uncorrelated with yield in the 13 strains tested.

In the present study, specific activities of three lignocellulolytic enzymes, laccase, carboxymethylcellulase (CMC'ase) and xylanase indicated that the second week and 17th week was the correct time to initiate fruiting on liquid and rubber sawdust medium respectively. In liquid medium, laccase specific activity reached its highest specific activity of 136.69 Umin^{-1} (mg protein)$^{-1}$ on the second week of

incubation before recording a sharp decline prior to primordia initiation on the third week and recorded its lowest activity of 2.88 $Umin^{-1}$ $(mg\ protein)^{-1}$ during the development of mature fruit body which occurred at the fourth week of incubation. In contrast, xylanase showed a decline in specific activity during the first two weeks of incubation but increased prior to primordia initiation on the third week and until the development of mature fruit body the fourth week where xylanase specific activity was at its highest at 39.6 $Umin^{-1}$ $(mg\ protein)^{-1}$. The drastic decline in laccase specific activity and increase in xylanase specific activity after the second week could be used as morphogenetic markers for primordia initiation. In solid medium the sudden increase in CMCase specific activity on the 17^{th} week from 0.11 $Umin^{-1}$ $(mg\ protein)^{-1}$ to 0.60 $Umin^{-1}$ $(mg\ protein)^{-1}$ on 18^{th} week corresponded to the onset and development of primordia.

C-serum supplementation in Leatham's medium and rubber sawdust had no promotional effect on fruiting. Supplementation of C-serum from *Hevea* latex on a Leatham's medium increased mycelial biomass production in the early stages of growth. Mycelial biomass production in supplemented medium ranged between 17.8 - 249.5 mg while in control, the mats weighed at 7.0 - 173.8 mg over the 13 weeks of incubation. Supplemented medium fruited at fourth week while the control only on the sixth week of incubation. Meanwhile, C-serum supplementation on *Hevea* wood sawdust significantly reduced yield from 24.4% B.E. to 8.3% B.E. even though there was a slight increase in biomass of mycelia. Supplementation of C-serum in PDB and *Acacia* sawdust medium stimulated both mycelial growth and fruiting. Supplemented PDB produced a yield of 2.8 - 49.8 mg while the control failed to produce any fruit bodies. To date, no fruiting of shiitake on PDB as a

medium has ever been reported. In *Acacia* sawdust, C-serum supplementation induced fruiting (18.1% B.E.) while the control failed to produce any fruit bodies.

Three strains of *L. edodes,* L38, L272, L2161 grown on rubber wood sawdust, palm pressed fibres (PPF) and five combinations of grass substrates showed that rubber wood sawdust was better for mycelial growth compared to PPF and grass substrates. Strain L38 required 36.0 days to colonize the rubber sawdust medium while both L272 and L2161 needed 37.0 days respectively. This growth was significantly faster compared than one on PPF substrates where strain L38, L272 and L2161 took 42.3, 41.0 and 38.5 days to colonize the substrate respectively. All three strains recorded significantly slower colonization in all the grass substrates compared to rubber sawdust and PPF. In fructification, only rubber wood sawdust and grass treatment C (*Imperata cylindrica*) produced fruiting in strains L38 and L2161 with no significant differences in fruit body yield between both strains on both substrates. Strain L38 recorded 21.8% B.E. and 23.3% B.E. on rubber sawdust and grass treatment C respectively while strain L 2161 recorded 11.9% and 12.8% B.E for the rubber sawdust and grass substrates respectively.

Knowledge of an optimal temperature - humidity - light - aeration regime for a particular strain, employing morphogenetic enzyme markers and using productive substrate and supplements all contribute to the development of a precision shiitake cultivation technology.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

BIOTEKNOLOGI PENGHASILAN CENDAWAN DENGAN PERSEKITARAN TERKAWAL, SUBSTRAT DAN PENANDA ENZIM UNTUK CENDAWAN SHIITAKE, *LENTINULA EDODES*

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Pertumbuhan miselium adalah optimum pada 25°C bagi enam dari tujuh strain *Lentinula edodes* (*L. edodes*) yang diuji. Habuk kayu menghasilkan lebih banyak miselia daripada bijirin di mana 0.53-1.60 mg dan 0.02-0.2 mg glukosamin dihasilkan masing-masing bagi setiap 200 g medium habuk kayu dan bijirin. Set suhu 15°C(8j)/25°C(16j), cahaya berterusan (25-35 lux), kelembapan sebanyak 80-95% dan kelajuan pengudaraan 1.3-2.5 m/s menghasilkan Keefisienan Biologi (K.B) yang tertinggi bagi 11 dari 13 strain pada tiga set suhu yang diuji. Strain L38 (7.1-32.7% K.B.) dan L300 (7.2-35.5% K.B.) merupakan penghasil K.B. yang terbaik bagi kesemua set suhu yang diuji. Kepadatan miselia yang dihasilkan semasa inkubasi adalah tidak berkolerasi dengan K.B. bagi kesemua strain yang diuji.

Aktiviti spesifik dari tiga enzim lignoselulolitik iaitu laccase, CMC'ase dan xilanase menunjukkan bahawa minggu kedua bagi medium cecair dan minggu ketujuhbelas bagi media habuk kayu adalah waktu yang sesuai untuk memulakan pembuahan cendawan. Aktiviti spesifik bagi laccase dan xilanase pada minggu kedua adalah 136.69 $Umin^{-1}$ $(mg\ protein)^{-1}$ dan 7.35 $Umin^{-1}$ $(mg\ protein)^{-1}$ masing- masing.

aktiviti spesifik laccase dan kenaikan aktiviti spesifik xilanase selepas minggu kedua sebelum penghasilan primordia pada minggu ketiga pengeraman, ini boleh digunakan sebagai penanda morfogenetik bagi penghasilan primordium. Bagi medium habuk kayu, hanya CMC'ase didapati sesuai digunakan sebagai penanda morfogenetik. Peningkatan mendadak aktiviti spesifik CMC'ase pada minggu ketujuhbelas (0.11 $Umin^{-1}$ $(mg\ protein)^{-1}$) sehingga minggu kelapanbelas (0.60 $Umin^{-1}$ $(mg\ protein)^{-1}$) adalah bersamaan penghasilan primordium.

Penambahan C-serum pada medium Leatham dan habuk kayu getah tidak menunjukkan kesan yang menggalakkan dari segi pembuahan cendawan. Penambahan serum dari lateks getah ini hanya menunjukkan peningkatan biomas miselia pada peringkat awal pertumbuhan. Miselia yang dihasilkan dengan penambahan serum adalah sebanyak 17.8-249.5 mg berbanding dengan kawalan sebanyak 7.0-173.8 mg sepanjang 13 minggu pengeraman. Media dengan penambahan serum menghasilkan cendawan pada minggu keempat manakala kawalan pula pada minggu keenam pengeraman. Manakala Habuk kayu getah dengan penambahan serum mengurangkan K.B. dari 24.4% (kawalan) kepada 8.3% (media ditambah serum). Bagi media Potato Dextrose Broth (PDB) dan habuk kayu *Acacia* dengan penambahan C-serum menggalakkan penghasilan miselium dan hasil cendawan. Media PDB dengan penambahahan serum menghasilkan cendawan sebanyak 2.8 - 49.8 mg manakala kawalan pula gagal menghasilkan cendawan. Sehingga kini tiada laporan tentang penghasilan cendawan pada PDB. Penambahan serum ini pada habuk kayu *Acacia* menggalakan penghasilan cendawan (18.1% K.B.) manakala kawalannya gagal sama sekali untuk berbuat demikian.

Tiga strain *L. edodes*, L38, L272, dan L2161 yang dikultur pada habuk kayu getah, serabut kelapa sawit (PPF) dan lima kombinasi rumput menunjukkan bahawa hanya habuk kayu getah sahaja menghasilkan pertumbuhan miselia terbaik dibandingkan dengan PPF dan substrat rumput. Strain L38 memerlukan 36.0 hari untuk mengkoloni habuk kayu getah manakala kedua-dua strain L272 dan L2161 memerlukan 37.0 hari untuk tujuan yang sama. Pertumbuhan ini adalah cepat jika dibandingkan dengan PPF, di mana strain L38, L272 dan L2161 masing-masing mengambil 42.3, 41.0 dan 38.5 untuk mengkoloni substrat. Kesemua strain menunjukkan pertumbuhan yang lambat pada kesemua kombinasi rumput yang diuji. Untuk penghasilan cendawan, hanya strain L38 dan L2161 berjaya menghasilkan cendawan pada media habuk kayu getah dan rumput kombinasi C (*Imperata cylindrica*) tanpa sebarang perbezaan signifikan. Strain L38 menghasilkan K.B. yang bernilai 21.8% dan 23.3% masing-masing bagi media habuk kayu getah dan rumput kombinasi C, manakala strain L2161 pula menghasilkan K.B. yang bernilai 11.9% dan 12.8% bagi habuk kayu getah dan rumput kombinasi C.

Pengetahuan tentang suhu-kelembapan-cahaya-pengudaraan yang optima terhadap strain yang tertentu, penggunaan penanda morfogenetik dan substrat yang produktif serta penambahan serum menyumbang ke arah penghasilan teknologi penanaman cendawan yang canggih.

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I certify that an Examination Committee met on 6th June 2001 to conduct the final examination of Ganisan Krishnen, on his Master of Science thesis entitled "Production Biotechnology with Controlled Environmental Regimes, Substrates and Enzyme Markers for the Shiitake Mushroom, *Lentinula edodes*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows :

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that this thesis has not been previously or concurrently submitted for any other degree at UPM or any other institutions.

Signed

GANISAN KRISHNEN

Date :

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