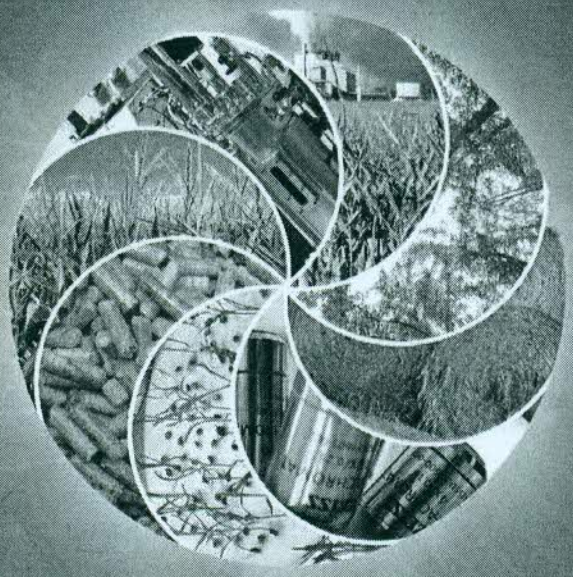
FerVAAP 2013

The 5th International Conference on Fermentation Technology for Value Added Agricultural Products

with Joint Session from
Research Group for Development of Microbial Hydrogen Production Process from Biomass - Khon Kaen University



August 21st - 23rd, 2013

Centara Hotel & Convention Centre, Khon Kaen, Thailand

BOOK OF ABSTRACTS

Dự Hội thảo quốc tế FerVAAP 2013

Đoàn Đại biểu của Viện NC & PT CNSH gồm 2 cán bộ, 1 nghiên cứu sinh và 6 sinh viên khóa 34-CTTT đi công tác tại Thái Lan từ ngày 20 đến 26/8/2013. Đoàn đã tham dự Hội thảo quốc tế FerVAAP 2013 "The 5th International Conference on Fermentation and Value Added Agricultural Products" và buổi trao đổi chuyên đề "The 5-Universities Joint Student Meeting" tại Khon Kaen. Trong hội thảo, đoàn tham gia 6 bài báo cáo oral và 5 poster trình bày về các đề tài nghiên cứu khoa học và trao đổi kiến thức chuyên môn với các nhà khoa học và sinh viên đến từ nhiều quốc gia khác. Đoàn cũng có đến thăm và làm việc với đội ngũ cán bộ và sinh viên của trường Đại học Khon Kaen và trường Đại học Kasetsart. Ngoài ra, đoàn còn có cơ hội được trao đổi, giao lưu văn hóa, thăm các di tích lịch sử và các địa điểm nổi tiếng ở Khon Kaen và Bangkok.

From August 20 – 26, 2013 a Delegation of BiRDI including 2 staff, 1 PhD candidate and 6 undergraduate students (class 34-Advanced training program) attended "The 5th International Conference on Fermentation and Value Added Agricultural Products" and "The 5-Universities Joint Student Meeting" held at Khon Kaen. In the conference and meeting, the BiRDI delegation contributed with 6 oral presentations and 5 posters reporting about the research results and exchanged the specialization knowledge with scientists and students coming from other countries. The delegation also visited and discussed with staff and students at Khon Kaen University and Kasetsart University. Besides, the delegation had an opportunity to visit some representative historical places, cultural and religious attractions in Khon Kaen and Bangkok.

Đoàn Viện NC & PT CNSH trong Hội thảo FerVAAP tại Khon Kaen -
The BiRDI delegation at the FerVAAP conference in Khon Kaen

Đoàn Viện NC & PT CNSH trong buổi họp mặt The 5-Universities Joint Student Meeting tại Khon Kaen

-

The BiRDI delegation at The 5-Universities Joint Student Meeting in Khon Kaen

Đoàn Viện NC & PT CNSH tại Đại học Kasetsart -
The BiRDI delegation at Kasetsart University

The 9 th Young Scientist Seminar

Establishment of International Network for Tropical Bioresources and Their Utilization

NOVEMBER 18 th-19 th, 2013
SEMINA[illegible]K, YAMAGUCHI, JAPAN

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The 9 th Young Scientist Seminar
Establishment of International Network for
Tropical Bioresources and Their Utilization

ISOLATION AND SELECTION OF LACTIC ACID BACTERIA HAVING ANTI-MOULD ABILITY FROM NEM CHUA

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Introduction

Lactic acid bacteria (LAB) play an important role in fermented food preservation thank to their anti-fungal ability and bacteriocin activity. They produce antifungal compounds like organic acids, hydrogen peroxide, cyclic dipeptide, hydroxyl fatty acid, etc. One of typical traditional fermented meat products in Vietnam, namely *nem chua* is usually placed under negative effects due to manual production process, uncontrolled ambient preservation condition, and consuming without cooking. In this study, the anti-mould ability of LAB isolated from *nem chua* was examined.

Objective: To screen LAB isolated from *nem chua* for their strong anti-mould ability.

Materials and methods

- Samples of *nem chua* were collected in Can Tho City, Vietnam. PCA used for total plate count; LAB counted and isolated on MRS agar; Moulds counted and isolated on Sabouraud Dextrose agar.
- All bacterial isolates were characterized by Gram staining, catalase and oxidase reaction, spore staining and disintegration of $CaCO_3$.
- Lactic acid content was analyzed by titrate method with NaOH 0.1N and also determined through Therner value.
- Dual culture overlay assay(*) applied to detect the inhibitory activity of LAB against mould. LAB inoculated in two 2 cm lines on MRS agar plates and allowed to grow at 30°C for 48 hrs. The plates were then overlaid with 10 mL of malt extract soft agar (0.05% malt extract, 1% agar) containing 10^6 spores/mL of mould. Measure the inhibition zone after incubation at 30°C for 48 hrs.

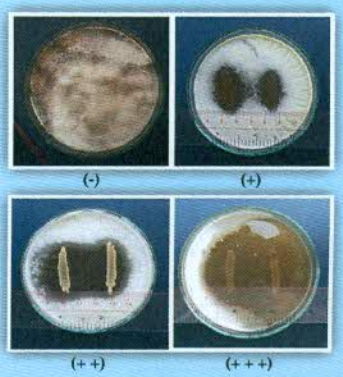

Fig. 1 Appearance of anti-mould ability by LAB
Levels of appearance of anti-mould ability ranging from - (nothing) to +++ (very much)

Table 1 Chemical and microbiological analyses of 5 *nem chua* samples

No	Names of *nem chua* samples	Chemical value		Microbiological Enumeration (CFU/g)		
		Lactic acid content (g) in 100 g sample	Therner value (°T)	Total plate count	Mould	LAB
1	Tư Kien	2.09 [b]	231.67 [b]	$2.8x10^7$	$1.9x10^3$	$1.7x10^7$
2	Thu Oanh	1.11 [d]	123.33 [d]	$2.2x10^7$	$2.0x10^3$	$1.4x10^7$
3	Co Phuc	1.35 [c]	150.00 [c]	$2.3x10^7$	$1.5x10^3$	$1.6x10^7$
4	Trang	1.15 [d]	127.50 [d]	$3.9x10^7$	$7.4x10^3$	$2.7x10^7$
5	Xuan Khanh	2.46 [a]	273.33 [a]	$3.3x10^7$	$3.8x10^3$	$3.5x10^7$

Note: Mean values of triplications with different subscripts within a column are statistically different at 95% confidence level.

- The target LAB isolates identified by molecular technique with the primers 1492R (5'-TACGGTTACCTTGTT ACGACT-3') and 27F (5'-AGAGTTTGAT CCTGGCTC-3').

Rtesults

- Results of chemical and microbiological analysis of 5 *nem chua* samples reported in Table 1.
- LAB isolates had round, smooth, white colonies, cocci or rods, producing typical smell of acid; Gram (+); catalase (-); oxidase (-); have ability to disintegrate $CaCO_3$. Mould isolates had spores, gray or black, branching mycelia, cell wall or no, sphere or pear versicle, single layer seriation.
- The anti-mould ability of LAB described in Table 2 and Figure 1.
- Isolates P32B and V13A characterized as *Lactobacillus plantarum*; and P41A as *Pediococcus pentosaceus*.

Table 2 Anti-mould ability of LAB isolates

LAB	MOULDS									Anti-mould ability [1]	Number of inhibited mould isolates
	L41	L42	A11	A24	C21	C32	T22	T71	X20		
V11B	- [2]	-	-	++	+	+	++	+++	-	9+	5/9
V13A	++	++	++	+	++	++	+++	+++	+	18+	9/9
V21B	-	+	-	+	-	-	-	+	-	3+	3/9
V31B	-	-	-	-	-	-	-	+++	-	3+	1/9
O22A	-	-	-	-	+	-	-	+++	-	4+	2/9
O32A	-	-	-	-	+	-	+	+++	-	5+	4/9
O33A	-	-	-	-	-	-	-	++	-	2+	1/9
P21B	++	-	+	+	+	+	++	+++	-	11+	7/9
P31B	++	-	++	++	++	++	++	+++	-	15+	7/9
P32B	++	+++	++	++	+++	++	+++	+++	++	22+	9/9
P41A	++	+++	+	++	-	++	+++	++	++	17+	8/9
R11B	++	-	-	++	++	++	+++	++	+	14+	7/9
R13A	+	-	+	-	++	+	++	-	-	7+	5/9
R14B	++	-	+	-	+	++	+	+++	+	11+	7/9
R22B	+	-	+	+++	+	+	++	+++	-	12+	7/9
R33B	-	-	-	++	+	-	-	+++	-	6+	3/9
K21A	+	-	+	-	-	+	+	+++	+	8+	5/9
K32A	-	-	-	+	-	+	+	++	-	5+	4/9
K34B	++	+	-	++	+	+	++	++	-	11+	7/9

Notes: [1] Total anti-mould ability of LAB isolate; [2] Ranging of anti-mould ability: (-) d = 0; (+) d ≤ 2 mm, (++) d ≤ 8 mm; (+++) d > 8 mm.

References: (*) Mayh-Harting A, Hedges AJ, Befikley F. Methods for Studying Bactoriocins. In Methods in Microbiology. Noris JB and Ribbons NW, Vol. 1972; 7A: 315-442.

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THE EFFECT OF pH, DARK-LIGHT CYCLE AND LIGHT COLOUR ON THE CHLOROPHYLL AND CAROTENOID PRODUCTION OF SPIRULINA SP.

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INTRODUCTION

Spirulina sp., multicellular filament algae, is helically coiled. This is a rich nutrition microalgae with protein, carbohydrate, vitamin, chlorophyll and carotenoid. Many researches and applications of *Spirulina* sp. have been studied by interested scientists, especially, pigment production. Application in human nutrition, medicine, cosmetic, waste water treatment.

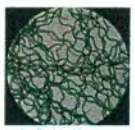

Figure 1. *Spirulina* sp. under microscope at magnification E40

OBJECTIVES

Determination of pH concentration, dark – light cycle and light colours appropriate for biomass growth rate, chlorophyll and carotenoid production in *Spirulina* sp.

MATERIALS AND METHODS:

Materials: *Spirulina* sp. was received from Microbiology Laboratory of Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam. Chemicals in Zarrouk media (1), acetone, alcohol 90 %, alcohol 70 %.

Methods

Spirulina sp.
↓ **Cell density**
Microalgae biomass increasing
↓ **Cell density**
Study the effect of pH
↓ **Clorophyll, Carotenoid, biomass, pH**
Study the effect of light colours
↓ **Clorophyll, Carotenoid, biomass, pH**
Study the effect of dark - light cycles
↓ **Clorophyll, Carotenoid, biomass, pH**
Study the growth recovery of *Spirulina* sp.

Figure 2. Microalgae increasing biomass

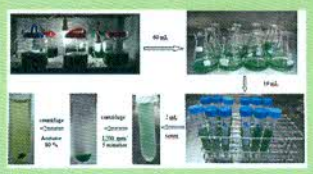

Figure 3. Pigment extraction method

RESULTS

The results showed that biomass, chlorophyll and carotenoid production of all treatments were highest in day 8 and pH 9 was the better condition for the growth of *Spirulina*.

Table 1. The effect of pH on *Spirulina* sp. biomass, chlorophyll and carotenoid production

	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Biomass (g/50mL)						
pH=8	0,08	0,12^{b}	0,13^{ab}	0,12^{b}	0,11^{ab}	0,09^{b}
pH=9	0,08	0,11^{bc}	0,16^{a}	0,14^{a}	0,13^{a}	0,12^{a}
pH=10	0,08	0,14^{a}	0,14^{ab}	0,10^{b}	0,07^{bc}	0,07^{c}
pH=11	0,08	0,10^{c}	0,11^{c}	0,08^{c}	0,06^{c}	0,05^{b}
	ns					
Chlorophyll a (µg/mL)						
pH=8	0,02	0,63^{a}	2,10^{a}	1,89^{ab}	1,90^{b}	0,93^{b}
pH=9	0,02	0,76^{a}	2,40^{a}	2,72^{a}	2,72^{a}	1,62^{a}
pH=10	0,02	1,10^{a}	0,94^{b}	0,78^{c}	0,65^{c}	0,54^{c}
pH=11	0,02	0,35^{c}	0,87^{b}	0,67^{c}	0,74^{c}	0,71b^{c}
	ns					
Chlorophyll b (µg/mL)						
pH=8	0,07	0,85^{c}	3,26	3,10^{a}	3,26^{a}	2,45^{a}
pH=9	0,05	2,60^{a}	3,35	3,35^{a}	3,35^{a}	2,71^{a}
pH=10	0,06	2,46^{ab}	2,35	0,93^{b}	0,63^{b}	0,51^{b}
pH=11	0,05	1,48^{bc}	2,38	0,39^{b}	0,49^{b}	0,32^{b}
	ns		ns			
Carotenoid (µg/mL)						
pH=8	0,02	0,45	1,13^{a}	0,77^{a}	0,90^{a}	0,58^{ab}
pH=9	0,03	0,51	1,43^{a}	1,32^{a}	1,08^{a}	0,79^{a}
pH=10	0,03	0,36	1,03^{a}	1,11^{a}	0,52^{b}	0,36^{bc}
pH=11	0,03	0,33	0,21^{b}	0,77^{a}	0,40^{b}	0,26^{c}
	ns	ns				

Note: mean values with different subscripts within a column are statistically different at the 95% confidence level, ns: no significantly different

In this experiment, three different colours (green, red and white) had significantly different effects in all treatments. In this case, *Spirulina* grew well at white light and slowly under green and red light.

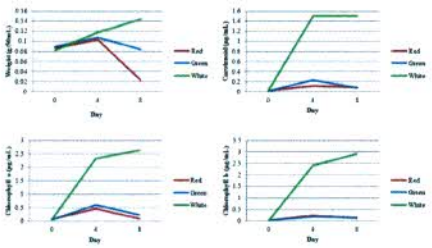

Figure 4. Biomass, chlorophyll and carotenoid in *Spirulina* under different light colours

In sufficient light regime (24/24 hour of illumination) *Spirulina* grew better than 12/24 hour of illumination. After 4 days, biomass was similar between two treatments but pigments of *Spirulina* were significant difference. In day 5, both biomass and pigments of *Spirulina* at 24/24 hour of illumination were higher than 12/24 hour of illumination (1.08 times in biomass, 2.36 times in chlorophyll a, 1.2 times in chlorophyll b and 1.7 times in carotenoid).

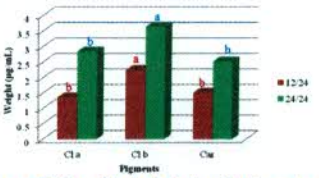

Figure 5. The effect of dark – light cycle on chlorophyll a, chlorophyll b and carotenoid in *spirulina* at day 5

After 5 days in Zarrouk media, pH 9, 24/24 hour illumination and continuous aeration, *Spirulina* grew and recovered very fast. The algae fiber was longer and biomass increased day by day.

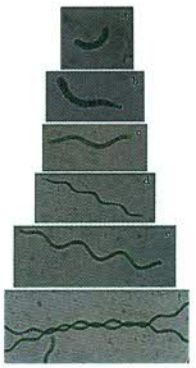

Figure 6. *Spirulina* growth during 5 days (magnification E40)
a: day 0; b: day 1; c: day 2; d: day 3; e: day 4; f: day 5

CONCLUSIONS

pH = 9, white light, 24/24 light illumination were appropriate conditions for biomass, chlorophyll and carotenoid production in *Spirulina*. During growth of *Spirulina*, pH remained stable from 10 to 10.18.

In inappropriate conditions, *Spirulina* was broken into small fragments, and quickly recovered when it was grown in appropriate conditions.

REFERENCE: (1) Zarrouk, C. Contribution à l'étude d'une cyanophycée. Influence de divers' facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. Ph.D. Thesis, Université de Paris, Paris; 1966.

EVALUATION OF EFFECTS OF LINGZHI MUSHROOM *(GANODERMA LUCIDUM)* ON NEURAL STEM CELLS ISOLATED FROM EMBRYONIC MOUSE BRAIN *(MUS MUSCULUS VAR. ALBINO)*

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INTRODUCTION

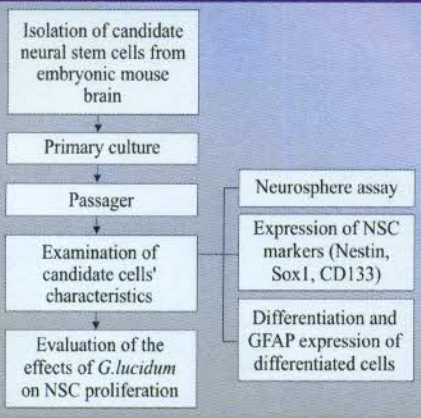
Neural stem cells possess the capacity to self renew and differentiate into astrocytes, neurons and oligodendrocytes. Moreover, neural stem cell proliferation and differentiation could be triggered by appropriate extrinsic factors in *in vitro* conditions (Reimers et al., 2008).

Ganoderma lucidum is a fungus widely used in Chinese, Japanese, Korean and Vietnamese medication for several years (Kleinwatcher et al., 2001). Triterpenes, polysaccharides and peptidoglycans are reported as the key components responsible for its important biological activities. Besides, Lingzhi-8 is documented as a mitogen-like protein that could promote cell division in *in vitro* conditions. Such mushroom extract was demonstrated to induce neuronal phenotype formation of pheochromocytoma cells and protect neurons from apoptosis due to NGF withdrawal (Cheung et al., 2000). According to Zhu et al. (2005), Lingzhi extract could prevent the loss of dopaminergic neurons in the striatum.

OBJECTIVES

- Evaluating the role of *Ganoderma lucidum* extract on neural stem cell proliferation.
- Determining the concentration of *Ganoderma lucidum* extract having the best growth stimulating effects.

METHODS



RESULTS

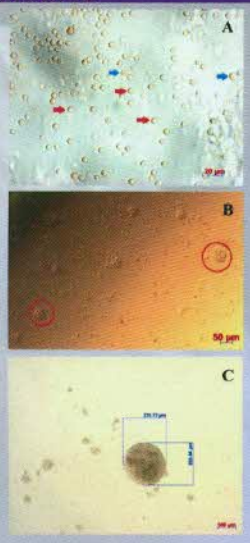


Figure 1. Sphere formation in primary culture. (A) Candidate cells with round shape (red arrows) and erythrocytes (blue arrows). (B) Small clusters of cells formed after 3 days of culture. (C) Spheres with compact structure formed after 11 days of culture.

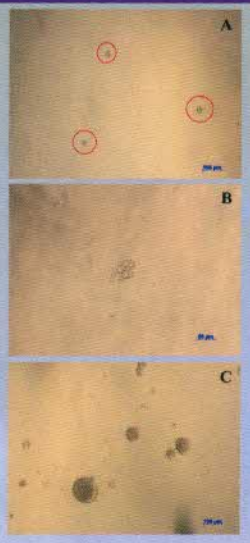


Figure 2. Sphere formation in neurosphere assay. (A) Clusters of 3-4 cells after dissociation. (B) A cluster of cells after 24 hours. (C) Spheres with distinct structure on day 7 of culture.

REFERENCES

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Reimers, D., R. Gonzalo-Gobernado, A.S. Herranz, C. Osuna, M.J. Asensio, S. Baena, M. Rodríguez and E. Bazán. 2008. Driving neural stem cells towards a desired phenotype. *Current Stem Cell Research & Therapy*, 3(4):247-253.

Zhu, W.W., Z.L. Liu, H.W. Xu, W.Z. Chu, Q.Y. Ye, A.M. Xie, L. Chen and J.R. Li. 2005. Effect of the oil from *Ganoderma lucidum* spores on pathological changes in the substantia nigra and behaviors of MPTP-treated mice. *Academic Journal of the First Medical College of PLA*, 25(6):667-671.

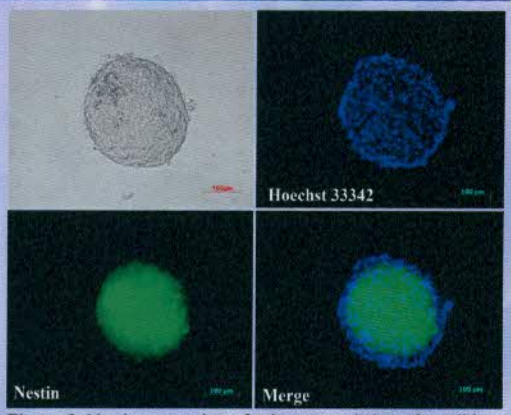

Figure 3. Nestin expression of spheres consisting of candidate neural stem cells cultured in DMEM/F-12 serum-free medium modified with B27, N2, heparin, EGF and FGF.

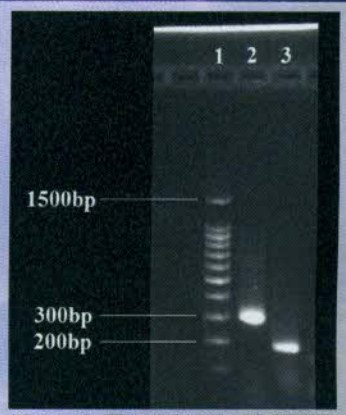

Figure 4. Electrophoresis of RT-PCR products. 1: Ladder, 2: *GAPDH*, 3: *Sox1*.

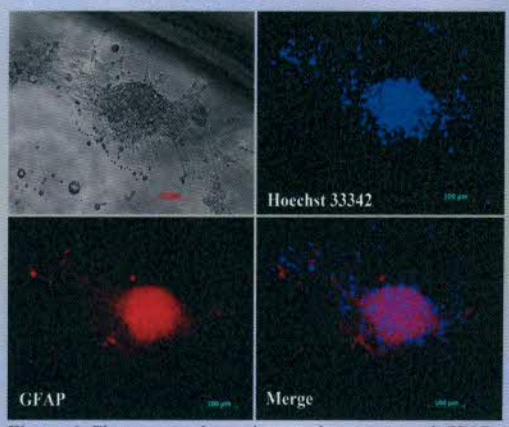

Figure 6. Fluorescent photomicrographs represented GFAP-positive cells after 10 days of non-orientable differentiation.

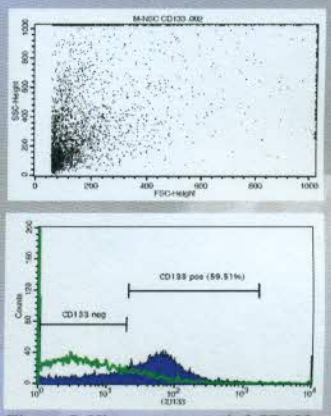

Figure 5. Histograms revealed CD133-positive cell population.

Table 1. The increase in neurosphere diameters after 72 hours of culture.

Treatments	Percentage of increase (%)
100 µg/ml	10.77 ± 0.61^{bc}
500 µg/ml	16.39 ± 0.78^{a}
1000 µg/ml	10.34 ± 1.18^{bc}
Control	10.33 ± 0.95^{c}

** Values were presented as Mean ± SD with 95% of confidence. Similar letters revealed that compared values were not significantly different ($p < 0.05$).*

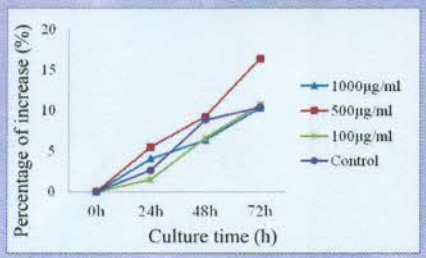

Figure 7. Changes in spheres diameter at different culture time.

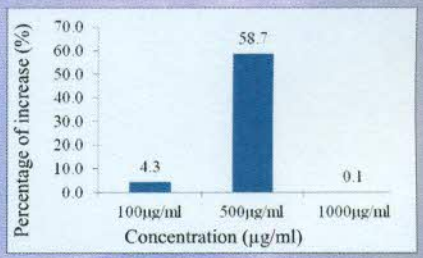

Figure 8. Size change of spheres in medium containing *G.lucidum* extract at different concentrations compared to the Control treatment after 72 hours of culture.

CONCLUSIONS

- 72% cell sample was successfully isolated and cultured in DMEM/F-12 serum-free medium containing B27, N2, heparin, EGF and FGF. Cells from 13.5 – 15.5 day embryos had a high growth rate.
- Candidate cells formed neurospheres, expressed Sox1 and were Nestin-positive, CD133-positive as well as differentiated into GFAP-positive cells.

Ganoderma lucidum extract at 500µg/ml showed the best effect on cell proliferation after 72 hours of culture.

INVESTIGATION OF EFFECTIVE MEDIA FOR IN VITRO PROPAGATION OF STEVIA

Nguyen Thanh Huy and Tran Thi Xuan Mai

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INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni), a natural sweetener, can be used to produce sugar replacement for patients suffering from diabetes, obesity, hypertension, or on-diet people. Stevioside compounds, found mostly in leaf content of Stevia, are 300 times sweeter than sugarcane, delicious, and non-caloric.

Large-scale production of this valuable herb is expanded in many provinces of Vietnam requiring much investment for not only growing techniques but also large number of homogeneous and disease-free plantlets. Propagation by seeds, however, is very poor and usually results in great variability in features like sweetening levels and composition. Moreover, vegetative propagation can be done from stem nodes but limitation in number is a facing problem (Guruchandran and Sasikumar, 2013). Plant tissue culture is thus an alternative way for rapid and mass production of Stevia.

MATERIAL AND METHODS

- *In vitro* grown Stevia .
- All media were prepared with 20g/l sucrose and solidified with 8g/l agar. The pH was adjusted to 5.8 before autoclaving at 121°C and 1atm pressure for 20 minutes. All the cultures were placed under stable conditions, at temperature 27±1°C, light intensity 1000lux, 16-hour illumination per day.
- **Callusing**: culturing 0.3-0.5cm^2 leaf explant on different media with the dorsal side being in contact with the medium surface.
- **Shoot multiplication from stem nodes**: 1-cm nodal segments were cultured on different media.
- **Root induction**: 4-cm-and-above shoots were subcultured in various media for rooting
- **Acclimatization**: 50-day-old rooted plants were taken out of the bottles and removed agar under tap water. The plantlets were then transplanted to plastic glasses containing mixture of soil, sand and decomposed rice straw (1:1:1 v/v/v). During the first week, plantlets were covered by plastic bags and kept under well-managed conditions (temperature 27±1°C, light intensity 1000lux, 16-hour illumination per day). In the second week, plastic bags were bored to allow air flow passing inside. From the third week, non-covered plantlets were transferred to the greenhouse.
- All data collected were assessed by analysis of variance for factorial complete randomized design (CRD) using computer software Statgraphics Centurion XV. Duncan's Multiple Range Test (DMRT) was applied for means separation.

RESULTS - SHOOT MULTIPLICATION

MS medium (including vitamins)+0.2mg/l NAA+0.15mg/l BAP resulted in 100% of nodal segment developed healthy shoots (1.15cm in average length) with hairy and obovate leaves after 21 days.

Table 2. The results of shoot multiplication after 21 days.

	C1	C2	C3
Shoots/explant	2.14^b	2.24^b	3.24^a
Shoot length (cm)	0.36^c	1.15^a	0.80^b
Note	Yellowish green and unhairy shoots, narrowed obovate leaves	Green and hairy shoots, obovate leaves	Bright green and unhairy shoots, oblanceolate leaves

a, b, c: means followed by the same letters in the same row were not significant difference ($p<0.05$).
C1: MS (Basal salt mixtures)+0.2mg/l NAA+0.15mg/l BAP
C2: MS (Including vitamins)+0.2mg/l NAA+0.15mg/l BAP
C3: MS (Basal salt mixtures)+3.5mg/l BAP

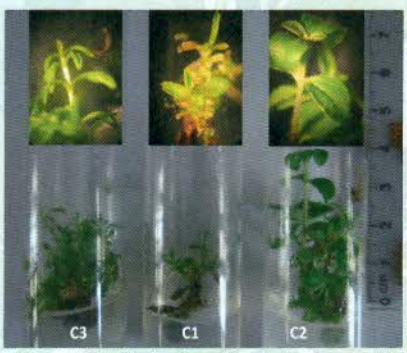

Figure 2. Shoots developed from nodal segments and observed under stereoscope at magnification 1,6x10x0,65.

RESULTS - ROOTING

MS medium (basal salt mixtures)+0.5mg/l IAA (R1) had resulted in 83.33% responses with fibrous roots (9.7 roots/explant and 2.2cm/root).

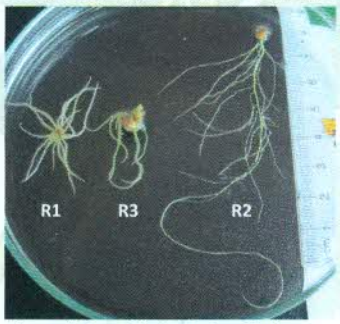

Figure 4. Roots formed after 25 days.

Table 3. The results of root induction after 25 days.

	R1	R2	R3
Rooting responded (%)	83.33^a	58.33^b	33.33^c
Number of roots/plant	9.57^a	1.67^c	3.50^b
Root length (cm)	2.20^b	7.02^a	1.39^c
Note	Hairy, thick fibrous roots	Less hairy, thin taproots	Hairy, thick fibrous roots

a, b, c: means followed by the same letters in the same row were not significant difference ($p<0.05$).
R1: MS (Basal salt mixtures)+ 0.5mg/l IAA.
R2: 1/2MS (Basal salt mixtures)+ 100mg/l activated charcoal.
R3: MS (including vitamins)+ 0.5mg/l IAA.

Figure 5. Roots observed under stereoscope at magnification 1.6x10x4.

OBJECTIVE

To find out a complete process for plant tissue culture of Stevia, starting from callusing, shoot multiplication to rooting and finally acclimatization.

RESULTS - CALLUSING

MS medium (including vitamins)+0.2mg/l NAA+0.15mg/l BAP was the best formula for callusing with 92.6% of explants forming profuse calli.

Figure 1. Callus formed in S5 medium

Table 1. The results of callusing experiment after 21 days.

Treatment	Callus responded (%)	Note
S1	33.33^b	Poor white callus
S2	0.00^d	Unswollen leaves
S3	0.00^d	Unswollen leaves
S4	3.70^c	Poor yellow callus
S5	92.60^a	Profuse yellow callus
S6	0.00^d	Swollen leaves

a, b, c, d: means followed by the same letters in the same column were not significant difference ($p<0.05$).
S1: MS (Basal salt mixtures)+1mg/l Kinetin+2mg/l 2,4-D
S2: MS (Basal salt mixtures)+0.75mg/l NAA+1mg/l 2,4-D
S3: MS (Basal salt mixtures)+3mg/l 2,4-D
S4: MS (Basal salt mixtures)+0.5mg/l BAP
S5: MS (Including vitamins)+0.15mg/l BAP+0.2mg/l NAA
S6: MS (Including vitamins)+0.2mg/l BAP+0.5mg/l IAA

RESULTS - ACCLIMATIZATION

Maximum percentage (100%) of plantlets was successfully acclimatized in the mixture of 1 soil: 1 sand: 1 decomposed rice straw (v/v) after 3 weeks.

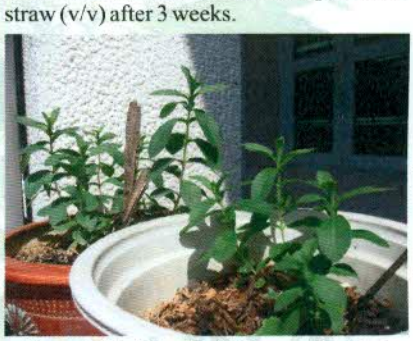

Figure 6. Plantlets after 80 days acclimatized.

REFERENCE:

Guruchandran V, Sasikumar C. Organogenic plant regeneration via callus induction in Stevia rebaudiana Bert. International Journal of Current Microbiology and Applied Sciences. 2013;2(2): 56-61.