生物技術在水產養殖上應用研討會論文集　56～67頁，1994年

基因重組生長激素促進魚類生長的研究

蔡懷楨

台大漁料所

一、前　言

生長激素(GH)是脊椎動物腦下垂體前葉細胞所分泌出來調節生長及代謝的一種蛋白質激素(Ganong 1983)。它會增強魚的食慾、飼料效率及生長速度(Donaldson et al. 1979)。所以Zohar(1989)認爲GH可能是水產養殖上用來促進生長的良劑。然而，若GH的獲得要是直接由腦下垂體粹取則相當地不經濟。

自從鮭魚的GH　cDNA基因被複殖、核苷酸被定序而且其基因重組GH(rGH)也證明有促進魚類生長的效力(Sekine et al. 1985)之後，rGH應用在水產養殖上的潛力大大的增加。因此，有價值的其他魚種的GH cDNA也陸續地被找出，且能在大腸菌中製造出來。但是，菌體表現外來基因的能力是個問題，例如，虹鱒GH在大腸菌所能表現的量卻低於總蛋白的1%(Agellon et al. 1988)，也一樣不經濟。所以，於79年向農委會申請研究採用不同的表現載體及菌株以提升虹鱒GH在大腸菌的表現能力（80農建－7.1－糧－121—73）。

大腸菌表現外來基因時，產物常以變性的狀態聚集在一起，稱爲內涵體(Petrov et al. 1987)，應用rGH時必需費時費力地去萃取、恢復活性後，再進行注射(Sekine et al. 1985, Agellon et al. 1988, Sato et al. 1988)，浸泡(Agellon et al. 1988)或包埋(Down et al. 1988)。但是這些方式在實際應用上稍感不便。若魚類GH能在酵母菌內表現出來，然後利用在飼料中添加這些基因重組酵母菌來達到促進，菌生長的效果，那麼就可省去萃取rGH的複雜工作，而變得相當方便。何況，酵母菌是眞核系統，所製造出來的外來基因產物沒有像大腸菌一樣有變性的缺點(Schaber et al. 1986)；同時，酵母菌又是食用微生物且常添加在飼料中，應算十分安全。然而，前人在這方面的研究相當有限。所以本人於80年開始連續兩年向農委會申請開發含魚類生長激素的酵母菌及其應用（81農建－12.1－糧－67－56及82－科技－1.1－糧－56）。

二、材料及方法

㈠、大腸菌表現虹鱒GH cDNA方面：

請參考Tsai and Tseng (1992).

㈡、酵母菌表現魚類GH cDNA方面：

請參考Kuo and Tsai (1993)及Tsai et al. (1993b)。

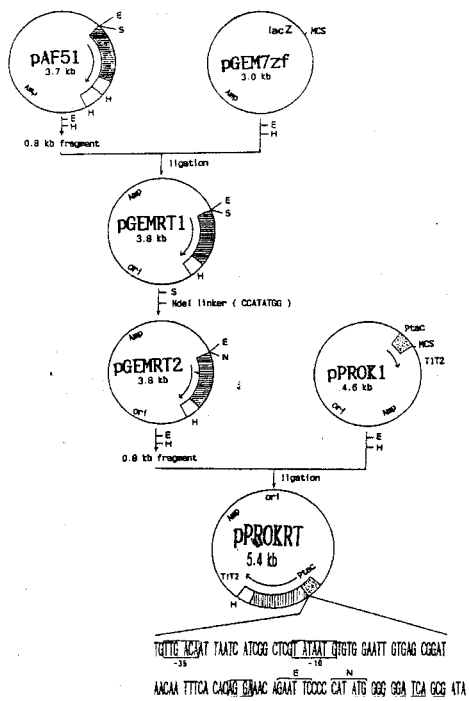
㈢、基因重組酵母菌促進魚類生長方面：

請參考Tsai et al. (1993a)及Tsai et al. (1994)。

三、結果及討論

㈠、大腸菌表現虹鱒GH cDNA方面：(Tsai and Tseng 1992)

用遺傳工程合成出來的生長激素已經證實可以促進魚貝類的生長。爲了要達到這種生長激素能普遍應用到養殖層面，我們構築一種在大腸菌能有較高表現虹鱒生長激素cDNA效率的表現質體，這種質體命名爲pPROKRT(圖一)，其表現效率要比原先報告的pAF51好。



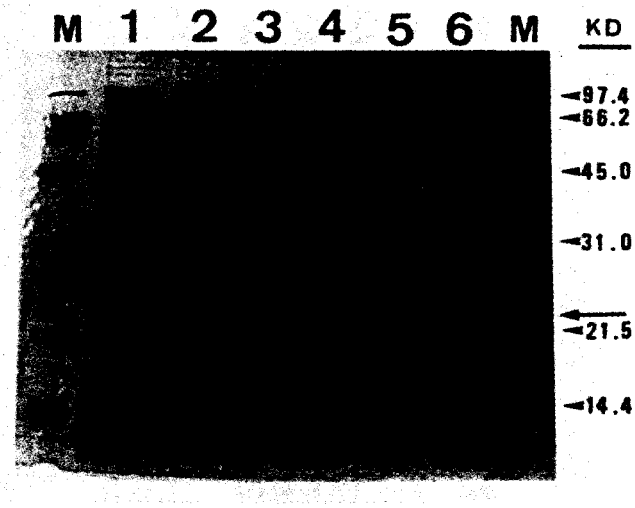
圖一 Scheme for construction of expression plasmid, pPROKRT. The stipple box, heavily hatched box and empty box represented the *tac* promoter, rtGH cDNA and 3′ untranslated region respectively. The arrow indicates the orientation of the *tac* promoter (Ptac). Amp, ampicillin resistant gene; ori, origin site of plasmid replication; T1 T2, T1 and T2 transcription terminator of *E. coli rrn*B gene. RBS, ribosomal binding site; E, *Eco*RI; H, *Hin*dIII; N, *Nde*I S, *Sma*I, MCS, multiple cloning site;

它是由*tac*起動子所引導，由*rrn*B終端子所結束。從核醣體接合處到第一個遺傳密碼的距離是16對鹽基。在生長激素分泌後第一個胺基酸－isoleucine－之前因遺傳工程所加入的胺基酸爲 Met－Gly－Gly－Ser－Ala。當 pPROKRT轉型到大腸菌JM109菌株時用SDS－多聚丙烯醯胺膠體電泳分析其蛋白質，發現比控制組的多一條相當明顯，其分子量約22000道爾頓的蛋白質出現，而且會隨induction的時間加長而增加(圖二)。再用Western blotting證實該蛋白質與抗chum salmon生長激素的抗體呈現正反應(圖三)。因此，這個表現質體證實可以在大腸菌內高效率製造虹鱒生產激素，其量約佔大腸菌總蛋白質的7%，這個幾乎是原先pAF51產量的7倍。

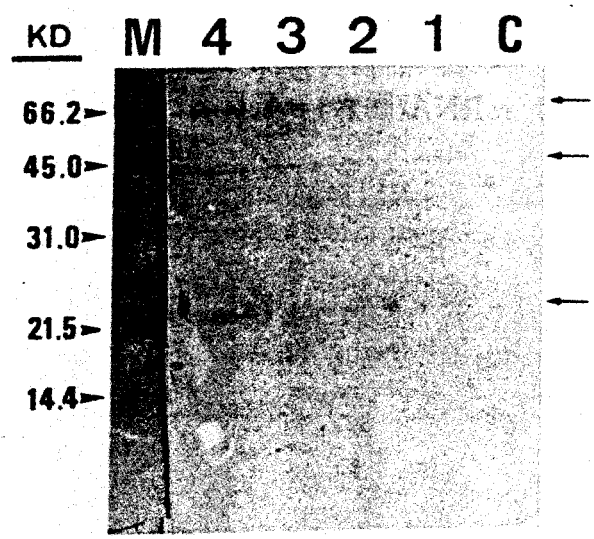
(二)、酵母菌表現魚類GH cDNA方面：

1. 虹鱒GH cDNA在酵母菌系統的表現(Tsai et al. 1993b)。

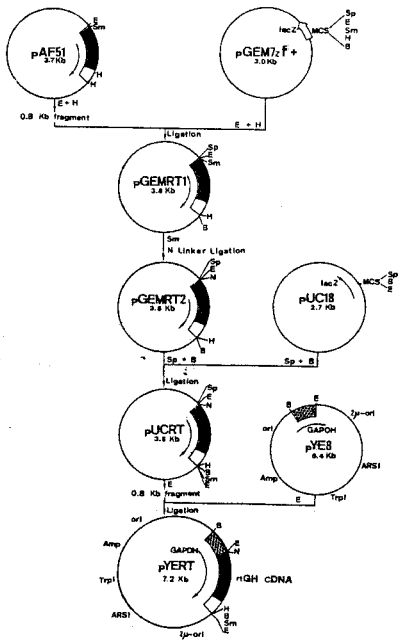
利用遺傳工程的技術將虹鱒生長激素cDNA剪接到酵母甘油醛磷酸去氫酶基因的起動子後，再接到含有2μm ori的episomal plasmid上。這個質體爲7.2kb的pYERT(圖四)。經核苷酸定序後推演這個基因組合生長激素爲Met－Gly－Gln－Gly－Ala－Ala再接上虹鱒生長激素(GH)不含分泌性訊號的188個胺基酸。當pYERT轉形到酵母菌(*Saccharomyces cerevisiae* 20B12)時，篩選到含虹鱒生長激素cDNA的酵母菌轉形株(strain Y－105)，並用南氏浸漬法，北方浸漬



圖二 Analysis of proteins by using SDS-PAGE and Coomassie blue staining. The gel was loaded with 30 to 40 μg total proteins extracted from *E. coli* JM109. Lane 1, carrying pPROK, vector with no insert, as control, induced by IPTG for 3h; lane 2, carrying pPROKRT, vector with rtGH cDNA, induced by IPTG for 1h; lane 3, carrying pPROKRT, 2h induction; lane 4, as lane 3, but two-fold amount of proteins; lane 5, carrying pPROKRT, 3h nduction; induction; lane 6, as lane 5, but two-fold amount of proteins; lane M, marker of molecular weights of proteins in kilodations (kD). The most obviously different band between the control sample and the experiment samples was indicated by arrow.

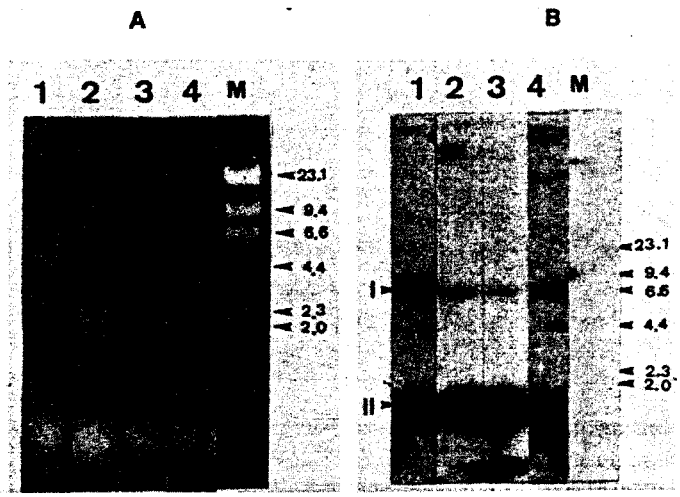


圖三 Western blotting analysis of proteins produced by *E. coli* JM109. Lane C, carrying pPROK, vector with no insert, as control, induced by IPTG for 3h; lane 1, carrying pPROKRT, vector with rtGH cDNA, induced by IPTG for 1h; lane 2 and 3, carrying pPROKRT, 2h induction; lane 4, carrying pPROKRT, 3h induction; lane M, marker of molecular weights of proteins kilodaltons (kD). The positive immunoreactive bands were indicated by arrows.

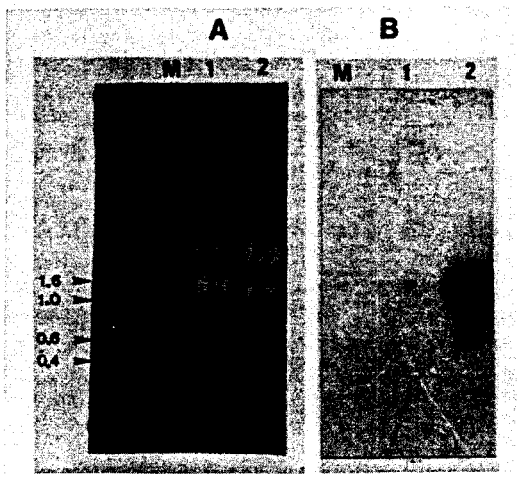


圖四 Scheme for pYERT construction. The GAPDH promoter (stipple box), rtGH cDNA (hatch box) and 3'-noncoding region of GH (empty box) are shown. ori,origin of replicon; Amp, ampicillin resisitance gene; Trp1, N-(5'-phosphoribosyl)-anthranilate isomerase; ARS, autonomous replicating sequences; 2μ-ori, *ori* site of 2μm-circle plasmid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; lacZ, encodes β-galactosidase; MCS, multiple cloning site. Arrow shows transcription direction. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nde*I; Sm, *Sma*I; Sp, *Sph*I.

法和免疫沉澱法，分別檢測strain Y－105的DNA, RNA及蛋白質。結果證實該轉形株每個細胞含有20－25個pYERT(圖五)；該段cDNA能被轉錄成GH mRNA(圖六)；並且在酵母菌萃取液中，有一條外來



圖五 Estimation of the copy number of pYERT harbored in *S. cerevisiae* Y-105. (A), the *Eco*RI-digestion pattern of total DNA extracted from Y-105 if cultured in YM medium at a density of: 3.2×10^6 cells/ml (middle-log, lane 1); 2×10^7 cells/ml (late-log, lane 2); 2×10^8 cells/ml (stationary, lane 3); and at 2×10^8 cells/ml after 10 generations (stationary, lane 4). (B), autoradiogram of Southern hybridization corresponding to (A). M, molecular marker of linear DNA fragments in kilo-base pairs. Positive band I and II represent the *leu2* gene and the GH cDNA, respectively. The copy number was determined by using a demsitormeter to compare the relative intensity of band I to and II.



圖六 Analysis of RNA obtained from the recombinant strains of *S. cerevisiae* 20B12 by hybridization with rtGH cDNA. (A): electrophoresis pattern shown on the 1.3% denaturing gel; 15 μg of RNA were loaded per lane. (B): autoradiogram of Northern blot hybridization corresponding to (A). Lane 1, strain C, harboring pYE8— a vector without insertion of foreign DNA; lane 2, strain Y-105, harboring pYERT— a vector with insertion of rtGH cDNA; lane M— molecular weight markers of RNA in kilo-nucleotides.

22kDa的蛋白質；此蛋白質與生長激素抗體有免疫反應(圖七)。

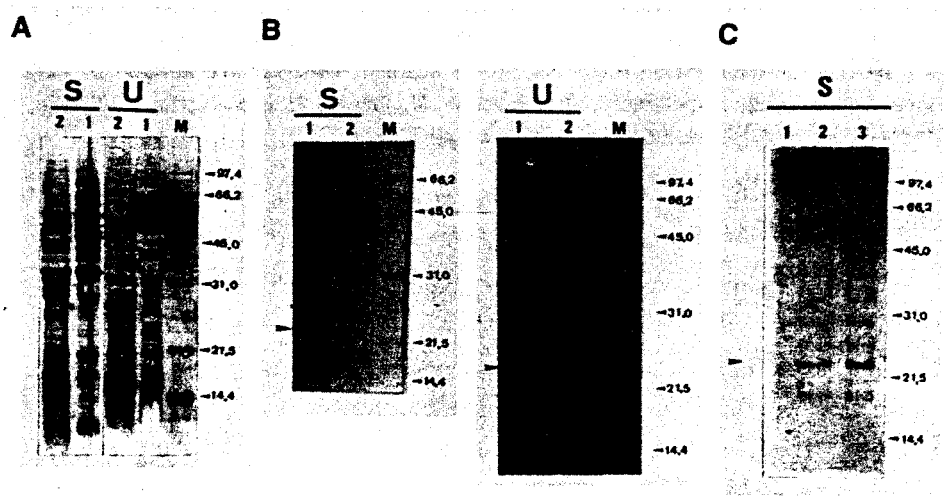
2. 黃鰭鯛GH cDNA在酵母菌分泌系統pre－α－factor的表現(Kuo and Tsai 1993)

利用聚合酶鏈反應，合成一段黃鰭鯛生長激素cDNA但不含分泌性訊號的片段，再連接到酵母菌pre－α－factor因子的前導序列後面，然後一起構築在2μm的表現載體(pMA56/α)上。這個新質體命名爲pMAyp(圖八和圖九)。含有pMAyp之轉形酵母菌(*S. cerevisiae* 20B12)的培養懸浮液在SDS－多聚丙烯醯胺膠體電泳圖上可以檢測到一條被分泌到細胞外的重組生長激素，其電泳移動位置與眞正生長激素相同--約22000道爾頓(圖十)。而該蛋白質條紋在只含pMA56/α但不含生長激素cDNA的轉形酵母菌懸浮液中並不出現。

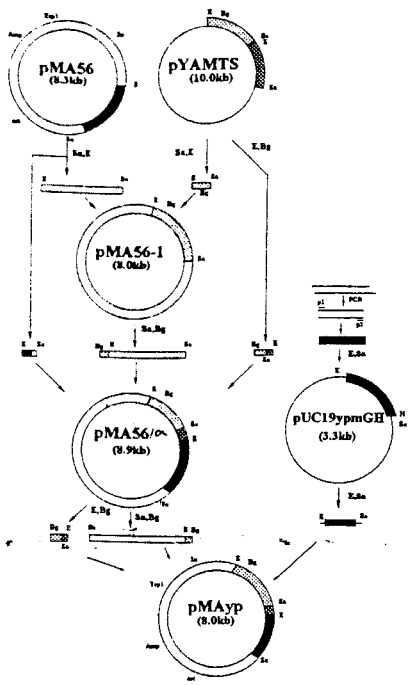
㈢、基因重組酵母菌促進魚類生長方面：

1. 基因重組酵母菌之菌體抽出液對吳郭魚魚苗生長之促進效力(Tsai et al. 1993a)

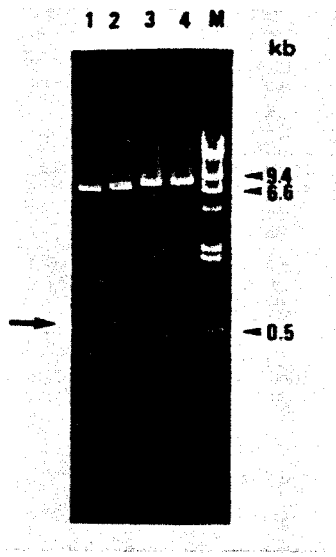
酵母菌(*S. cerevisiae* 20B12)帶有虹鱒生長激素(rGH)基因的轉形株（實驗組）和不含rGH基因的轉形株（控制組），分別經大量培養以後把酵母菌打破，其抽出液以各種比例(0.032, 0.16, 0.8及4%)與飼



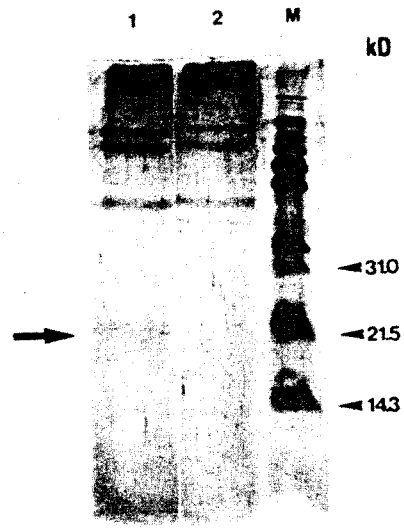
圖七 Protein analysis. (A), about 40 μg of proteins extracted from *S. cerevisiae* 20B12 transformants were analyzed by SDS-PAGE and Coomassie blue staining in a 13.5% acrylamide gel. (B), Western blotting analysis corresponding to (A). (C), Western blotting analysis for yeast cells treated with zymolase. Lane 1, strain C (harboring expression vector pYE8 without DNA insertion); lane 2, strain Y-105 (harboring pPYERT, a pYE8 with insertion of rtGH cDNA); lane 3, strain Y-122 (another transformant harboring pYERT); lane M, protein markers in kilo-daltons. The proteins in the supernatant fraction were obtained after yeast cells were lysed and centrifuged (S), or after the Triton-treated pellets were vortexed in the presence of 8 M urea, then centrifuged (U).



圖八 Plasmid construction. The prepro-α-factor leader sequence (hatched box), cDNA encoding for the mature GH (blacken box) and DNA fragment coding for the Col E1 plasmid replication and the selection marker (open box) are shown where 2μ, origin of yeast 2 μm circle plasmid repliciation; Trp1, N-(5′-phosphoribosyl)-anthranilate isomerase; Amp, ampicillin resistance gene; ori, origin of plasmid replication. The genes encoded in pMA56 and pYAMTS are surface antigen (stripe box) and pre-S2 segment with surface antigen of hepatitis B virus (cross box), respectively. Ba, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; Sa, *Sal*I; Sm, *Sma*I, Primers P1 and P2 were used for PCR amplification.



圖九 Restriction enzyzmes analysis of pMAyp. *Eco*RI (lane 1), *Sal*I (lane 2), *Cla*I (lane 3) and *Bgl*II (lane 4) were used to digest pMAyp. Lane M, molecular weights marker of linear DNA fragments in kilo-base pairs (kb). The arrow indicates the insertion of ypmGH cDNA.

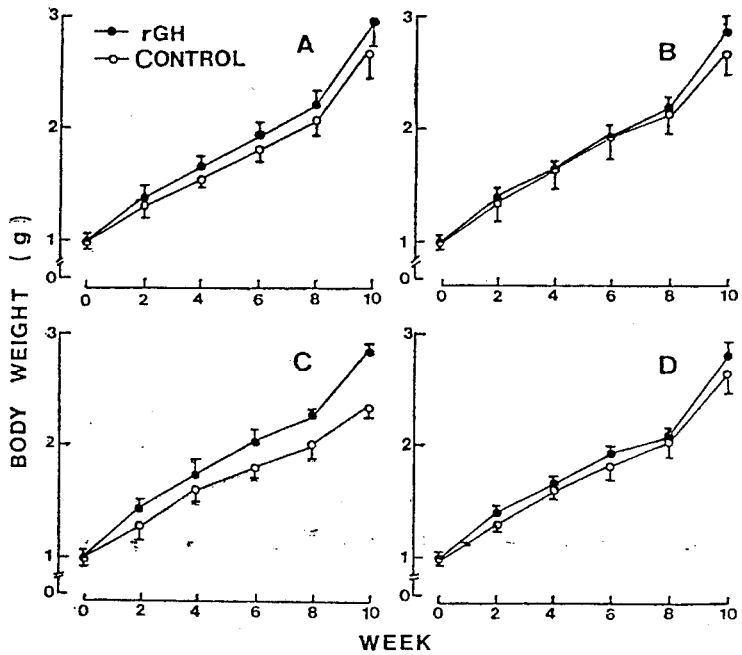


圖十 SDS-PAGE analysis of proteins obtained from the supernatant of yeast transformant cultures. The electrophorietic patterns of strain S-1 (lane 1; harboring expression vector with insertion of ypmGH cDNA) and strain SC (lane 2; harboring expressin vector without insertion of DNA) are shown, respectively. Lane M, protein makers in kilo-daltons (kD). The arrow indicates the rGH secreted by yeasts into the culture medium.

料混合製成顆粒狀，再餵食給1公克左右的吳郭魚幼魚。每組爲三重覆試驗，每天兩次餵以飽食，共十星期。結果顯示：平均體重在餵食含0.8% 含rGH酵母菌的抽出液與同劑量的控制組相比具有顯著性促進生長的效力；但在其他劑量的組別(0.032, 0.16及4%)則與同劑量的控制組相比差別不明顯(圖十一)。而餌料利用效率方面：四組添加rGH酵母抽出液(0.032, 0.16, 0.8及4%)與控制組相比較，則依次分別提高爲13.2, 17.5, 37.1及14%。顯示，0.8% rGH酵母抽出液的添加量能顯著性增加魚的成長速度，可能是由於適量的GH提高了餌料利用效率所致；而太多的rGH如（4%）則效果反而下降(表一)。

2. 基因重組酵母菌的直接添加於飼料中對烏魚魚苗成長的促進(Tsai et al. 1994)

含有7.2Kb質體（磷酸甘油醛去氫酶起動子，連接虹鱒生長激素cDNA）和含有6.4kb質體（只含磷酸甘油醛去氫酶起動子）的酵母菌(*S. cerevisiae* 20B12)轉形株，分別經大量培養後收集菌體，以0, 0.2, 2及8% 的量添加到一般飼料內，每日餵食一次。對480尾仔烏魚(*Mugil cephalus*)分24組（每組20尾）進行共四個星期的成長試驗。結果顯示(1)餵食含rGH的酵母菌比餵食不含rGH酵母的實驗組或未含酵母的控制組，成長顯著性的加速；(2)含rGH酵母菌實驗組中，又以添加2% 經sodium alginate及gelatin包埋處理的組別，其促進成長效力及飼料轉換率與未處理的控制組相比最爲顯著($P<0.01$)；(3)餵含酵母的組別比餵不含酵母的組別好(表二和三)。這個結果將提供



圖十一 Effects of rGH yeast lysate as a feeedstuff additive on the average body weights (mean ± SD; $n=3$) of 12 tilapia fignerlings for each treated dose. Open circle, fed with non-rGH yeast lysate (as the control groups); closed circles, fed with rGH yeast lysate (as the rGH groups). The weight percentage of yeast lysate added to the fish feed as a supplement was (A) 0.032% (B) 0.16% (C) 0.8% and (D) 4%.

表一 The feed efficiency (FE) of tilapia after 10 weeks treatment with yeast lysates of rGH and non-rGH as a supplement

Test groups		Amount of additive	Total food fed (g)	Weight gain (g)	FE	% of FE increase
1	A	0.032%	175.10	60.99	0.348	
	B	0.032%	181.42	71.42	0.394	13.2
2	A	0.16%	180.52	61.89	0.343	
	B	0.16%	170.29	68.62	0.403	17.5
3	A	0.8%	170.66	50.11	0.294	
	B	0.8%	168.66	67.90*	0.403*	37.1
4	A	4%	166.29	57.97	0.339	
	B	4%	165.46	65.81	0.398	14.0

A, the control groups which were fed the non-rGH yeast lysate (yeast transformant contained plasmid vector); B, the experimental groups which were fed the rGH yeast lysates (yeast transformant contained plasmid vector with rtGH cDNA insert). The survival rate of the cultured fingerlings examined through this study was 100%. The calculation of FE was described in text. Asterisk (*) indicates the significant difference from the control group, $p< 0.05$.

表二 Effects of recombinant growth hormone yeasts administered in the diet on the total wet weight of juvenile striped mullet in each group over a 4-week period[a].

Group	Weight (g) at week:		
	0	2	4
Control	42.7±0.3z	60.8±0.2w	75.5±0.1t
YGC-0.5	42.8±0.1z	61.6±0.1y	79.6±0.3x
YGC-2	43.0±0.2z	62.3±0.2z	85.1±0.2z
YGC-8	42.7±0.1z	61.4±0.1xy	78.3±0.3w
YOC-0.5	42.5±0.2z	61.1±0.1wxy	75.8±0.1t
YOC-2	42.5±0.3z	61.0±0.1wx	77.0±0.1uv
YOC-8	42.5±0.1z	60.9±0.1wx	76.9±0.1uv
YG-0.5	42.6±0.2z	61.3±0.1wxy	78.2±0.1w
YG-2	42.7±0.1z	61.4±0.1xy	80.2±0.1y
YG-8	42.6±0.2z	61.2±0.3wxy	78.4±0.0w
YO-2	42.5±0.2z	60.9±0.1wx	76.8±0.1u
YO-8	42.6±0.1z	60.8±0.1wx	77.4±0.1v

[a]Data are shown as average body weight (mean±SE) of duplicate groups of 20 fish in each treatment. Groups within the same column followed by different letters are significantly different ($P<0.01$). Fish were fed meal with coated recombinant growth hormone (rGH) yeasts (YGC), coated yeasts containing no rGH (YOC), uncoated rGH yeasts (YG), or uncoated yeasts containing no rGH (YO); doses were 0.5% (−0.5), 2% (−2), or 8% (−8). Control fish were fed the basal meal only.

表三 Effects of oral administration of recombinant growth hormone yeasts on specific growth rate and feed efficiency of juvenile striped mullet in each treatment over a 4-week period[a].

Group	Specific growth rate (%/d)	Feed efficiency (g gained/g fed)
Group	2.04±0.02s	0.38±0.004t
YGC-0.5	2.22±0.03xy	0.42±0.006xy
YGC-2	2.44±0.03z	0.47±0.005z
YGC-8	2.16±0.00vwx	0.41±0.002uvw
YOC-0.5	2.07±0.01st	0.38±0.002t
YOC-2	2.12±0.03tuv	0.40±0.005uv
YOC-8	2.12±0.02tuv	0.40±0.003uv
YG-0.5	2.17±0.01vwx	0.41±0.002vwx
YG-2	2.26±0.01y	0.43±0.003y
YG-8	2.19±0.02wx	0.41±0.005wx
YO-2	2.10±0.01stu	0.40±0.002u
YO-8	2.14±0.01uvw	0.40±0.001uvw

[a]Data are shown as the average (mean±SE) of duplicate groups in each treatment. Groups within in the same column followed by different letters are significantly different ($P<0.01$). Fish were fed meal with coated recombinant growth hormone (rGH) yeasts (YGC), coated yeasts containing no rGH (YOC), uncoated rGH yeasts (YG), or uncoated yeasts containing no rGH (YO); doses were 0.5% (−0.5), 2% (−2), or 8% (−8). Control fish were fed the basal meal only.

了以最經濟、方便的方法，把重組生長激素直接應用在水產養殖上一條新的途徑。

謝　辭

本人在此感謝美國馬利蘭大學陳鐵雄教授所提供的虹鱒GH cDNA；中研院分生所郭宗德教授所提供的酵母菌質體及變異株；海洋大學養殖系張清風教授、本校同仁羅秀婉教授、台西水試分所郭仁杰先生在成長實驗方面的協助；生物技術開發中心紀威光博士及張金全先生在酵母菌醱酵上的鼎力支持。同時，也謝謝兩位研究生—曾俊豐及郭榮烈的努力。最後更謝謝農委會給予本計劃的經費：80農建－7.1－糧－121－73（60萬），81農建－12.1－糧－67(56)（60萬）以及82－科技－1.1－糧－56（53萬）。

參考文獻

Agellon LB, CJ Emery, JM Jones, SL Davis, AD Dingle, TT Chen. 1988. Promotion of rapid growt of rainbow trout (*Salmo grirdneri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat, Sci., 45: 146－151.

Donaldson EM, UHM Fagerlund, DA Higgs, JR McBride. 1979. Hormonal enhancement of growth. In *Fish Physiology* (WS Hoar, DJ Randall, JR Brett, eds.). Academic Press, New York, 8: 455-597.

Down NE, EM Donaldson, HM Dye, K Langley, LM Souza. 1988. Recombinant bovine somatotropin more than doubles the growth rate of coho salmon (*Oncorhynchus kisutch*) acclimated to seawater and ambient winter conditions. Aquaculture, 68: 141-155.

Ganong WF. 1983. The pituitary gland. In *Review of Medical Physiology* (W.F. Ganong, 11st ed.) Lange Medical Publisher, Los Altos, 323-335.

Kuo JL, HJ Tsai. 1993. Electrophoretic analysis of recombinant fish growth hormone secreted from *Saccharomyces cerevisiae* by using α－factor－leader seguence. J. Fish. Soc. Taiwan 20:51-58.

Petrov P, E Jay, I Ivanov. 1987. Inclusion bodies in recombinant *E. coli* producing human calcitonin tetramer, as visualized by immunogold electron microscopy. Biol. Cells 61: 1－4.

Sato N, K Murata, M Nonaka, M Sakaguchi, Y Kariya, T Hayami, S Kimura, A Kimura, KWatanabe. 1988. Growth promoting activity of tuna growth hormone and expression of tuna growth hormone cDNA in *Escherichia coli*. Biotechnol. Appl. Biochem., 10: 385-393.

Sekine S, T Mizukami, A Saito, H Kawauchi. 1989. Isolation and characterization of a novel growth hormone cDNA from chum salmon (*Oncorhynchus keta*). Biochim. Biophys. Acta, 1009: 117-120.

Tsai HJ, CF Tseng. 1992. Expression of rainbow trout growth hormone cDNA in *E. coli* from vector utilizing *tac* promoter, J. Fish. Soc. Taiwan, 19: 45-53.

Tsai HJ, WK Chi, CC Chang, TT Kao, CF Chang. 1993a. Enhancement of tilapia growth by dietary administration of recombinant yeast lysates as a supplement. J. Fish. Soc. Taiwan, 20: 271-277.

Tsai HJ, JC Kuo, SW Lou, TT Kuo. 1994. Growth enhancement of juvenile mullet by feeding recombinant yeasts containing fish growth hormone. Prog. Fish Cult. 56: 7-12.

Tsai HJ, CF Tseng, TT Kuo. 1993b. Expression of rainbow trout growth hormone cDNA in yeast. Bull. Inst. Zool., Academia Sinica, 32: 162-170.

Zohar Y. 1989. Endocrinology and fish farming: aspects in reproduction, growth and smoltification. Fish Physiol. Biochem., 7: 395-405.