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Isolation, Identification, Enzymatic and Molecular Characterization of Intestinal Bacteria of Goldfish *(Carassius Auratus)* and its Role on Growth

KEYWORDS: Isolation, enzymatic, molecular characterization, intestinal bacteria, gold fish, growth.

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ABSTRACT *The present study deals with the isolation, identification, enzymatic and molecular characterization of intestinal bacteria of goldfish and its role on growth for a period of 45 days. Five distinct colonies such as GB1 (Pseudomonas sp.,), GB2 (Streptococcus sp.,), GB3 (Bacillus sp.,), GB4 (Proteus sp.,) and GB5 (Vibrio sp.,). were isolated from the intestine of gold fish and based on biochemical tests, enzymatic productivity and molecular characterization new bacterium was identified as Pseudoxanthomonas kalamensis and mass multiplied. Six different feeds having different concentration of bacterial sp. such as feed I (Control), Feed II (1ml), Feed III (2ml), Feed IV (3ml), Feed V (4ml), and Feed VI (1ml yeast) were prepared and given to gold fish for a period of 45 days. The feed consumption, feed conversion ratio, feed conversion efficiency, growth, percentage growth, relative growth rate, assimilation, metabolism, gross growth efficiency and net growth efficiency was higher in feed V.*

INTRODUCTION

Ornamental fish culture in India has shown a rapid progress during past few years but some major problems are hindering the progress path and disease being one of them. Disease outbreak is being increasingly recognized as one the major constrains in ornamental fish production which affects the trade and economic development. Virus, bacteria, and parasitic diseases cause immense damage in host metabolic process by producing toxic substances. So for conventional approaches similar to disinfection, vaccines, antibiotics and chemotherapeutics are continued to be an important disease measures in ornamental fishes. However conventional approaches have limited success in preventing or cure of aquatic disease. The use of beneficial bacteria to display pathogens by competitive process is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture (Prabu Narayanan Marimuthu et.al., 2012). Probiotics as a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community by ensuring improved use of the feed or enhancing its nutritional value by enhancing the host response towards disease or by improving the quality of its ambient environment (Fuller,1987). Beneficial microorganisms or their products in aquaculture control disease, improve growth and in some cases as a mean of replacing antimicrobial compounds (Tahere Bagheri et.al., 2008). The probiotic bacteria enhance digestion, high feed conversion, high specific growth rate and survival. The study related to the isolation, identification conversion, enzymatic and , molecular characterization of intestinal bacteria of goldfish (Carassius auratus) and its role on growth is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

For the present study Goldfish Carassius auratus were collected from Angel Aquarium, Dindigul, Tamil Nadu and transported to the laboratory in Polythene bags filled with aerated water. Intestinal contents of the goldfish were serially diluted with sterile water and plated on nutrient agar, plates were incubated at 37°C for 24 hrs (Pour plate, Spread plate and Streak plate method). After incubation, bacterial colonies were invalid at random from each plate, and examined for gram reaction, spore formation, cellular morphology, motility and identified at the genus and species level. (John G. Holt et al.,1994). The isolated Pseudomonas sp., (10^5 Cells) was mass multiplied by inoculating them into the nutrient broth. For growth studies, goldfish fingerlings (5 ± 0.30 g) were collected from Angel Aquarium, Dindigul, Tamil Nadu and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in glass aquaria (60 × 45 × 45 cm) for a period of 10 days at 28 ± 2° C. During acclimation, fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour and rice bran in the form of dry Pellets. One control (without bacteria), four experimental feeds by using different quantity (1,2,3,& 4 ml) of isolated bacteria and one feed by using commercially available probiont (yeast) was prepared according to square method (Ali.1980). Composition of different ingredients in experimental feeds given in Table 1.

TABLE 1: COMPOSITION OF DIFFERENT INGREADIENTS IN EXPERIMENTAL FEEDS (g/100gm)

EXPERIMENTAL FEEDS							
S. No	INGREDIENTS	Feed I control	Feed II	Feed III	Feed IV	Feed V	Feed VI 33.75
1	Fishmeal	33.75	33.75	33.75	33.75	33.75	33.75
2	GNOC	33.75	33.75	33.75	33.75	33.75	11.25
3	Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
4	Topioca	11.25	11.25	11.25	11.25	11.25	2
5	Fish oil	2	2	2	2	2	2
6	Sunflower oil	2	2	2	2	2	4
7	Suppelvite mix	4	4	4	4	4	1
8	Sodium chloride	1	1	1	1	1	1
9	Sodium benzoate	1	1	1	1	1	1ml Yeast
10	Microbes (10^5 Cells)	-	1ml	2ml	3ml	4ml	

GNOC – Groundnut Oil Cake.

Experimental design for growth studies:

For the present study uniform size of goldfish (Carassius au-

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ratus) (5 ± 0.30 g) were selected and then the fishes were introduced in the rectangular glass tanks (45 cm L × 22 cm B × 22cm H) having a capacity of 18 liters. Five fishes were introduced in each tank. For each treatment triplicates were maintained. During rearing, the fishes were fed on ad – labitum diet of the prepared feed twice a day for 1 hour each from 9 – 10 am and 4 – 5 pm . The unfed were collected after one hour of feeding without disturbing the fishes. The unfed was dried to constant weight. The faecal matter was collected daily before changing the water with least disturbance to the fishes and dried at 95^{0}C. Approximately 70 % of water in the tank was replaced with tap water. The experiment was continued for 45 days. On the 45th day length and weight of the fishes were measured in live condition for calculating condition factor (K)(Weatherley and Gill 1987) for individual fish before and after the experiment and other feed utilization parameters.

The experimental results are presented in the form of tables and graphs using Microsoft Excel (Version 2007). Mean, Standard deviation and T- test were also calculated with the help of the same tool, One-way ANOVA method was used for the analysis using DMRT (Version 2005) according to Sendecor & Cochran (1961). The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. Sum of square variations (SS), Degree of freedom (DF), Variability of sample means (MS), Critical probability value (F) and Probability (Prob.) were also obtained.

RESULTS AND DISCUSSION

The organism isolated from the intestinal content was identified using enzymatic productivity and biochemical tests (Table 2 & 3). Genetic code (sequance) of isolated Pseudoxanthomonas kalamensis sp. from intestinal content of gold fish is presented in Fig.1.

TABLE 2: Enzymatic productivity of intestinal bacteria of gold fish

S. No	Intestinal Bacteria	Amylase	Cellulase	Lipase	Protease
1	GB1(Pseudomonas sp.,)	+++	+++	+++	++
2	GB2 (Streptococcus sp.,)	++	+++	++	+
3	GB3 (Bacillus sp.,)	++	+++	++	+
4	GB4 (Proteus sp.,)	+	+++	++	++
5	GB5 (Vibrio sp.,)	++	+	++	++

+ - Nil (Absent) or (Negative) ++- Low productivity (Positive) +++ - Higher productivity (Positive)

Table 3 : Biochemical Characterization of Intestinal Bacteria of Gold Fish

Test	GB1	GB2	GB3	GB4	GB5
Simple staining	Rods	Cocci	Rods	Rods	Rods
Gram's staining	Negative	Positive	Positive	Negative	Negative
Motility Test	Motile	Non- motile	Motile	Non- motile	Motile
Indole test	Negative	Negative	Negative	Positive	Positive
Methyl Red	Negative	Negative	Negative	Negative	Positive
Voges Prokauser	Negative	Positive	Negative	Negative	Positive
Citrate test	Positive	Positive	Negative	Negative	Positive
Catalase test	Positive	Negative	Positive	Positive	Positive
Starch test	Positive	Not Performed	Positive	Positive	Positive
Gelatin hydrolysis	Positive	Not Performed	Positive	Negative	Positive
Oxidase Test	Positive	Not Performed	Negative	Negative	Positive
Lactose Test	Negative	Not Performed	Positive	Negative	Positive
Sucrose Test	Negative	Not Performed	Positive	Negative	Positive
Identification result	Pseudomonas sp.,	Streptococcus sp.,	Bacillus sp.,	Proteus sp.,	Vibrio sp.,,

Fig. 1: Genetic Code (Sequance) Of Isolated Pseudoxanthomonas Kalamensis Sp. From Intesti Nal Content of Gold Fish.

ORIGIN

1 tacccatgca agtcgaacgg cagcacagga gagcttgctc tctgggtggc gagtggcgga
61 cgggtgagga atacatcgga atctactctg tcgtggggga taacg taggg aaacttacgc
121 taataccgca tacgacctac gggtgaaagc aggggacctt cgggc cttgc gcgattgaat
181 gagccgatgt cggattagct agttggcggg gtaaaggccc accaa ggcga cgatccgtag
241 ctggtctgag aggatgatca gccacactgg aactgagaca cggtcca-gac tcctacggga
301 ggcagcagtg gggaatattg gacaatgggc gcaagcctga tccagc cata ccgcgtgggt
361 gaagaaggcc ttcgggttgt aaagcccttt tgttgggaaa gaaatc cagc cggctaatac
421 ccggttggga tgacggtacc caaagaataa gcaccggcta acttcgt gcc agcagccgcg
481 gtaatacgaa gggtgcaagc gttactcgga attactgggc gtaaa gcgtg cgtaggtggt
541 cgtttaagtc cgttgtgaaa gccctgggct caacctggga act gcagtgg atactgggcg
601 actagaatgt ggtagagggt agcggaattc ctggtgtagc agt gaaatgc gtagagatca
661 ggaggaacat ccatggcgaa ggcagctacc tggaccaaca ttt gacactg agggcacgaa
721 agcgtgggga gcaaacagga

Escherichia coli is very commonly present in aquatic systems. Usually these organisms are not pathogens. The other organisms isolated such as Aeromonas, Pseudomonas, and Enterococcus act as causative agents of bacterial disease. According to Lewbert (1998) Aeromonas, Pseudomonas, and Enterococcus cause bacterial disease in ornamental fishes where there is stress. The presence of these bacteria in the present study confirms that there was no stress on animals due to use of probiotics. The enzyme producing intestinal bacteria and their significance in fish is rare. The intestinal microbiota in fish has been classified as indigenous when it is able to colonize the gut ecosystem (Ringo et.al., 2003) . The condition factor of gold fish Carassius auratus reared in different feeds were indicated in Table 4. Condition factor (K) of Gold fish Carassius auratus was estimated for comparative purposes to assess the feed. The average initial condition factor is 2.96 and the final condition factor increased in feed V (3.38) and in all others the final condition factor was decreased. Josephin Suganthi (2009) reported that the final condition factor was increased in feed III containing 2 ml of bacteria in the feed.

TABLE 4: CONDITION FACTOR (K) OF GOLD FISH

FEEDS	INTIAL	FINAL
EX. Feed I (Control)	2.61 ± 0.26	2.82 ± 0.17
EX. Feed II (1 ml)	2.81 ± 0.19	3.11 ± 0.21
Ex. Feed III (2 ml)	2.88 ± 0.63	3.21 ± 0.21
Ex. Feed IV (3ml)	2.62 ± 0.23	3.12 ± 0.13
Ex. Feed V (4ml)	2.96 ± 0.22	3.38 ± 0.29
Ex. Feed VI (1 ml Yeast)	2.78 ± 0.11	3.03 ± 0.06

The different feed utilization and growth parameters are indicated in Table 5. The ANOVA (Analysis of variance) of Growth parameters (Feed Consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) given table 6.

Table 5 Feed Utilization And Growth Parameters Of Goldfish Carassius Auratus In Relation To Different Concentration Of Pseudomonas. Spp.(Cells) Each Value Is The Average (± Sd) Performance Of 5 Individuals In Tripilicates Reared For 45 Days

S.NO PARAMETERS	EXPERIMENTAL FEEDS					
	FEED I	FEED II	FEED III	FEED IV	FEED V	FEED VI
1. Feed Consumption(FC) (g/g live wt/45days)	12.26 ± 0.80^a	13.11 ± 1.05^b	14.13 ±0.10^c	14.45 ± 1.41^d	15.31 ± 0.57^e	13.26 ±1.10^f
2. Feed Conversion Efficiency (FCE)	0.13 ± 0.02	0.18 ± 0.02	0.19 ± 0.07	0.21 ± 0.09	0.23 ± 0.02	0.19 ± 0.04
3. Feed Conversion Ratio (FCR)	9.54 ±0.89	11.49 ± 1.05	12.83 ± 0.43	13.30 ± 0.82	14.34 ±1.08	11.23 ± 0.32
4. Growth (G) (g/g live wt/ 45 days)	0.60 ± 0.03^a	0.69 ± 0.60^b	0.72 ± 0.20^c	0.78 ± 0.80^d	0.92 ± 0.12^a	0.82 ± 0.19^f
5. Percentage Growth (PG) (%)	26.30 ± 6.22	28.39 ± 4.84	30.37 ± 5.40	32.85 ± 3.72	35.77 ±3.77	29.45 ± 1.94
6. Relative Growth Rate (RGR)	0.30 ± 0.01	0.34 ± 0.30	0.36 ± 0.10	0.39 ± 0.04	0.46 ± 0.06	0.41 ± 0.09
7. Assimilation (A)	7.43 ±0.97	8.10 ± 0.52	8.50 ± 2.93	9.73 ±1.42	9.96 ± 0.11	8.06 ±1.16
8. Metabolism (M)	6.84 ± 0.96	7.40 ± 0.47	8.77 ± 1.30	8.96 ±1.37	9.04 ±0.15	7.24 ±1.33

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9. Gross Growth Efficiency (GGE) (%)	4.92 ±0.34[a]	5.07 ± 0.68[b]	5.22 ± 1.74[e]	5.30 ± 0.22[d]	6.12 ± 1.05[a]	5.31 ± 0.52[f]
10. Net Growth Efficiency (NGE) (%)	8.20 ± 1.09[a]	8.55 ± 0.39[b]	8.84 ± 1.17[e]	9.35 ± 0.77[d]	9.61 ± 0.76[a]	8.83 ± 0.76[f]

Feed consumption	Growth	Gross growth efficiency	Net growth efficiency
a vs b (P>0.05) S	a vs b (P>0.05) NS	a vs b (P>0.05) NS	a vs b (P>0.05) NS
a vs c (P>0.05) S	a vs c (P>0.05) NS	a vs c (P>0.05) NS	a vs c (P>0.05) NS
a vs d (P>0.05) S	a vs d (P>0.05) NS	a vs d (P>0.05) NS	a vs d (P>0.05) NS
a vs e (P>0.05) S	a vs e (P>0.05) NS	a vs e (P>0.05) NS	a vs e (P>0.05) NS
a vs f (P>0.05) S	a vs f (P>0.05) NS	a vs f (P>0.05) NS	a vs f (P>0.05) NS

Table 6 Anova (Analysis Of Vari Ance) Of Growth Parameters (Feed Con Sump Ti0n, Growth, Gross Growth Efficiency, Net Growth Efficiency) Of Gold Fish Carasis Sus Auratus

S.No	Parameter	Source	df	SS	MS	F	PROB
1.	Feed consumption	Tot	17				
		Rep	2	29.793200	1.752541		
		Trt	5	2.352933	1.176467		
		Err	10	16.723733	3.344747	3.1211	0.059 NS
				10.716533	1.071653		
		SEd= 0.8452 CD (.05)= 1.8833 CD (.01)= 2.6789 CV% = 7.55					
2.	Growth	Tot	17				
		Rep	2	0.400694	0.161056		
		Trt	5	0.055144	0.027572		
		Err	10	0.184494	0.036899	2.2911	0.124 NS
				0.161056	0.016106		
		SEd= 0.1036 CD (.05)= 0.2309 CD (.01)= 0.3284 CV% = 16.71					
3.	Gross growth efficiency	Tot	17				
		Rep	2	12.795028	0.752649		
		Trt	5	1.982211	0.991106		
		Err	10	2.609428	0.521886	0.6362	0.678 NS
				8.203389	0.820339		
		SEd= 0.7395 CD (.05)= 1.6478 CD (.01)= 2.3439 CV% = 17.01					
4	Net growth efficiency	Tot	17				
		Rep	2	13.400000	0.788235		
		Trt	5	5.916900	2.958450		
		Err	10	4.392267	0.878453	2.8421	0.075 NS
				3.090833	0.309083		
		SEd= 0.4539 CD (.05)= 1.0114 CD (.01)= 1.4387 CV% = 6.27					

Feed consumption of gold fish was higher in feed V (15.31 ± 0.57) containing 4 ml of Pseudomonas sp. and lower in feed I (control) (12.26± 0.80). Rajan and Revathi (2011) reported that the feed consumption of Platy Xiphophorus maculatus was higher in Ex. Feed V containing 10^4 cells of Bacillus subtilis .Feed Conversion Efficiency of gold fish was higher in feed V (0.23 ± 0.02) containing 4 ml of Pseudomonas sp. In feed I, II, III, IV and feed VI the feed conversion efficiency were gradually decreased. Asma chaudhary and Javed Iqbal Qazi (2007) reported the feed conversion efficiency of Labeo rohita was higher in SSF2 (44.09 ± 4.25) lower in control (35.97 ± 4.06). Feed Conversion Ratio was best in feed V (14.34 ± 1.08) and lower in feed I (9.54 ± 0.89) . Himabindu K. Venket et.al., (2004) reported the same result in Macrobrachium rosenbergii. The feed conversion ratio was higher in T5 (2.75 ± 0.06) and lower in T1 control (2.26 ± 0.01).Growth of goldfish was higher in feed V (0.92 ± 0.12) containing 4 ml of Pseudomonas sp. and in feed I, II, III, IV and VI was decreased. Same result was reported in Gold fish (Jeyachiristina Arockia Selvi 2005) But in case of Black molly the growth was higher in control without bacteria (Josephin Suganthi 2009). Several authors reported such higher growth in different fishes such as catla, koi carp and rainbow trout. (Parthasarathy and Ravi 2011., Dhanaraj et al., 2010 and Bagheri et al., 2008). Like the growth, the percentage growth of gold fish was higher in feed V (35.77 ± 3.77) and feed I, II, III, IV and VI was decreased. The Percentage growth was higher in koi carp fed with 1ml of Lactobacillus sp. (Chandra and Rajan 2009).The relative growth rate of gold fish was higher in feed V containing 4ml Pseudomonas. sp. and lower in feed I (0.30 ± 0.01) . Seenivasan et.al., (2012) reported that the relative growth rate of fresh water prawn Macrobrachium rosenbergii was increased when fed with Bacilllus substilis. Assimilation of gold fish was higher in feed V (9.96 ± 0.11 lower in feed I (7.43 ±0.97 Rajan and Revathi (2011) reported similar results for assimilation and metabolism in Platy. Metabolism of gold fish was higher in feed V (9.04 ±0.15) lower in feed I(6.84 ± 0.96). Same result was also reported by Chandra and Rajan (2009) in koi carp. Gross growth efficiency of gold fish was higher in feed V (6.12 ± 1.05) and lower in feed I (4.92 ± 0.34). Rajan and Revathi (2011) also reported higher gross growth efficiency when Platy was fed with higher levels of Bacillus subtilis in the feed. Net growth efficiency of gold fish was higher in feed V (9.61 ± 0.76) and lower in feed I(8.20 ± 1.09). Same result was also reported by Jeyachristina Arockia Selvi (2005) were higher in feed V and lower in feed I. From the results, it is inferred that the some of the feed utilization parameters such as Feed Consumption, Feed Conversion Efficiency, Growth, Percentage Growth, Relative Growth Rate, Gross growth efficiency, and Net growth efficiency were higher in feed V containing 4ml of Pseudomonas sp.

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REFERENCE

Ali, S.A. 1980. Feed formulation method. Manual of research methods for fin fish and shell | fish nutrition . CMFRI special publication, No. 8: 98. | Asma Chaudhary and Javed Iqbal Qazi 2007. Influence of a probiotic Pseudomonas pseudoalcaligenes fermented feed on growth performance of rohu (Labeo rohita) fingerlings. Punjab Univ.J.Zool. 22 (1-2) : 41 - 56. | Bagheri, T., Hedayati, S., Yavari, V., Alizade in and Farzanfar, A. 2008. Growth, survival gut microbial load of rainbow trout fry given diet supplement with probiotics. J. Fish Aquat. Sci., 8 : 43 – 48. | Chandra, R. and Rajan, M.R. 2009. Proiotics effect of industrial bacteria of koi carp | Cyprinus carpio var. koi. J. of Pure and Applied Microbiology, 3 (1) : 363 – 366. | Dhanaraj, M., Haniffa, M.A., Arun Singh, S.V., Jesu Arockiaraj, A., Muthu Ramakrishnan, C., Seetharaman, S. and Arthimanju, R. 2010. Effect of probiotics on growth performance of Koi carp (Cyprinus carpio). Journal of Applied Aquaculture, 22 : 202 - 209. | Fuller , R. 1987. Probiotics in man and animals. J.Appl.Bacteriol. 66: 365 – 378. | Himabindu K Venkat., Narottam P. Sahu and Kamal K. Jain 2004. Effect of feeding Lactobacillus based probiotics on the gut micro flora growth and survival of post larvae of Macrobrachium rosenbergii (de Man). Aquaculture Research, 35 : 501 - 507. | Jeyachristina Arokia Selvi, J. 2005. Probiotic effect of intestinal micro flora of Gold fish Carassius auratus. Dissertation submitted to Gandhigram Rural Institute - Deemed University Gandhigram. | John G. Holt., Noel R. Kerieg., Peter H.A.Sneath., James T. Stanley and Stanley T. Williams 1994. Bergey's Manual of Determinative Bacteriology. Lippincott Williams & Wilkins, USA. | Josphin Suganthi, A. 2009. Probiotic effect of intestinal bacteria of Black molly Pocillia sphenops. Dissertation submitted to Gandhigram Rural Institute - Deemed university Gandhigram. | Lewbert, G.A. 1998. Self - Assessment colour review of ornamental fish. Lowa state university press, pp.192. | Parthasarathy, R. and Ravi, D. 2011. Probiotic bacteria as growth promoter and biocontrol agent against Aeromonas hydrophila in Catla catla (Hamilton,1822). Indian J.Fish. 58 (3) : 87 -93. | Prabu Narayanan Marimuthu., Rajasekar Periyannan., Nisha Rajagopalan Girijakumari and Manikandan Ramar 2012. Isolation, Characterization of Vibrio and Pseudomonas sp., from infected fresh water ornamental fishes and evaluation of potential agents for its control. Research in Biotechnology, 3 (6) : 14 - 23. | Rajan, M.R., and Revathi, U. 2011. Role of Probiotics in Ornamental fish Platy Xiphophorus maculates. Journal of Pure and Applied Microbiology, 5 (2) : 819 – 823. | Ringo, E., Olsen, R.E., Mayhew, T.M. and Myklebust, R. 2003. Electron microscopy of the intestinal micro flora of fish. Aquaculture, 227 : 395 - 415. | Seenivasan, C., Saravanan Bhavan, P., Radhakrishnan, S. and Shanthi, R. 2012. Enrichment of Artemia nauplii with Lactobacillus sporogenes for enhancing the survival , growth and levels of biochemical constituents in the post-Larvae of the freshwater prawn Macrobrachium rosenbergii. Turkish Journal of Fisheries and Aquaculture Sciences, 12 : 23 - 31. | Sendecor G.W. and Cochran, G. 1961. Statistical methods. Oxford and IBH Publising, New Delhi, India, pp.593. | Tahere Bagheri., Seyed Aliakbar Hedayati., Vahid Yavari., Morteza Alizade and Ali | Farzanfar 2008. Growth, Survival and Gut Microbial Load of Rainbow Trout | (Onchorhynchus mykiss) Fry Given Diet Supplemented with Probioticc during the | Two Months of First Feeding. Turkish Journal of Fisheries and Aquatic Sciences, 8 : | 43 - 48 . | Weatherley A.H and Gill, H.S. 1987. The biology of fish growth. Academic Press, London, | UK. |