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Isozyme Patterns at Five Loci in Salmonids and Their Hybrid(I)

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연어류에서의 종간교배 및 Isozyme Pattern에 관한 연구

홍경표 · 명정구 · 김병기 · 손진기

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초 록

연어류의 종간교배 실험과 LDH, MDH, IDH, αGPDH, ME 등 다섯가지 isozyme에 대한 genetic marker로서의 활용 가능성을 알아보고자 1차적으로 연어, 산천어 및 무지개송어를 이용하여 종간교배를 실시하였고 이들 세 종의 isozyme pattern을 비교하였다.

교배실험은 종간교배, 정역교배 및 allotriploid 구간 등 12 구간으로 나누어 실시하였으며 연어 암컷과 산천어 수컷의 교배결과가 초기성장 단계적에서 가장 우수하게 나타났고 이들의 allotriploid도 부화율이 28.1%로 가장 우수하였다.

Genetic identification을 위한 isozyme loci 분석결과 연어와 산천어는 대부분의 loci에서 거의 찾아볼 수 없었고 무지개송어는 MDH-B와 IDH에서 다형현상을 확인할 수 있었다. 특히 MDH-B loci는 b 유전자의 출현 빈도에서 세 종간의 식별이 기능하였으며, IDH pattern을 산천어와 무지개송어의 비교에 유효한 것으로 나타났다. 이들 두 loci는 hybrid의 genotype 분석시 유용한 marker로 활용할 수 있는 가능성을 보였고, 앞으로의 어류 육종에 좋은 기초 자료로 이용될 것으로 사료된다.

INTRODUCTION

Interspecific hybrids between different species play an informative role in the study of the onset of the genomic function in early development, because they can be determined by detecting the appearance of paternal characters. On the other hand, extreme mortalities and abnormalities are frequently found in hybrids between phylogenetically distant species. When such phenomena are specific to a certain hybrid combination, an examination of the hybrids will provide another useful tool for gaining insight into the mechanisms responsible for abnormal development in fishes(Arai, 1984).

The use of isozymes in hybrid experiments provided a effective methodology for the study of gene activation during early developmental stages including embryogenesis. Such experiments on hybrids using isozymic gene markers may be used in determining hybrid incompatibilities between the two parental genomes as well as between exotic genome and maternal cytoplasm. According to the gene duplication at several loci, LDH, MDH, etc., salmonid fishes were suggested tetraploid (Okazaki, 1982). But Allendorf(1975) insisted that there was no evidence of a tetrasomocally

inherited locus in salmonids. May *et al.*(1979) insisted that there was no evidence of a tetrasomocally inherited locus in salmonids. May *et al.*(1979) reported an extensive survey of joint segregation involving 37 pairwise comparisons of 12 loci in 11 single pair matings of brook trout(*Salvelinus fontinalis*) and 38 pairwise comparisons of 9 loci in an F_1 splake (lake trout, *Salvelinus namaycus* × brook trout) backcross to brook trout.

Walter *et al.*(1989) proposed the potential applications of electrophoretically detectable genetic marker in triploid studies of brown trout. In recent years, many applications of genetic engineering techniques to fish breeding have been reported. Among them are mitochondrial DNA analysis(Hynes *et al.*, 1989; Palva *et al.*, 1989) and genomic DNA analysis of rainbow trout(LLoyd and Fields, 1989; Ferreiro *et al.*, 1989).

METERIALS AND METHODS

1. Hybridization

For the artificial hybridization in salmonids, mature parental fishes of the following three species were selected: chum salmon, *Oncorhynchus keta*, masu salmon, *O. masou* and rainbow trout, O. mykiss. Specimens of chum salmon were captured during their spawning season(Nov. 1988) from rivers in the east coast of Korea, and the other specimens were provided from the rearing stocks of the fish ponds of Yang-yang Inland Fisheries Institute.

Artificial hybridizations and their reciprocal crosses were carried out as Figure 1. Hybrids and control groups were prepared by fertilizing eggs using each parents(Table 1). Each group was reared in seperated incubation tanks at the research station of KORDI in Yang-yang, Korea. For the induction of allotriploid(the triploid of hybrid) fertilized eggs were treated

♀ \ ♂	*Oncorhynchus keta*	*O. masou*	*O. mykiss*
Oncorhynchus keta	Control	MC MC-t*	RC RC-t
O.masou	CM CM-t	Control	RM RM-t
O. mykiss	CR CR-t	MR MR-t	Control

* -t : Triploidization of hybrid

Figure 1. Schematic diagram for crossing experiment.

at 30℃ for 10 minutes on and after 15 minutes from fertilization. In all matings, eggs from females were stocked together because of insufficient numbers of ova were obtained from a single female. The combined eggs were divided into several groups for mating as shown in Figure 1. The sperm from males of each species were inseminated to eggs in the same.

Rearing water used was underground water, and the temperature during hatching were ranged 9.6 ~ 13.8℃.

2. Collection and preparation of samples

Tissues of skeletal muscle, liver and heart were cut from the parental fishes in order to examine the tissue specific and interspecific distribution of several isozymes. The fingerlings of hybrid and control groups were taken as whole bodies. The part or whole bodies used were kept frozen below −30℃ until they were analyzed electrophoretically. The extracts were prepared by placing equal amounts of tissue and distilled water into glass tubes and squashing these mixtures with a glass rod. They were centrifuged for 10 minutes at 3,000 rpm and the supernatants were analyzed.

Table 1. Summary of specimens used as parental fishes for hybridization

Species	Sex	Fish No.	Total length (cm)	Fork length (cm)	Body length (cm)	Body weight (g)	Total wt. of egg (g)	Egg size (cm)	Individual egg wt (g)*	Year of sampling	n**
Chum	F	1	57.5	53.5		1,430	290	0.63	0.15	1987	
salmon	F	2	51.5	48.5		1,300	250	0.62	0.15		
(Oncohy-	F	3	60.0	56.6		1,360	250	0.62	0.15		
nchus	M	4	46.7	42.8		730					
keta)	M	5	64.8	61.8		2,370					
	M	6	55.0	50.6		1,200					
	M	7	62.0	56.5		1,560					
	F	8			68.0	3,200		0.69	0.19	1988	10
	F	9			65.0	3,450		0.69	0.19		13
	F	10			76.0	4,000		0.70	0.21		13
	M	11			68.5	3,100					
	M	12			63.0	3,400					
	M	13			60.0	2,200					
Masu	M	1	44.2	42.8		765				1987	
salmon	F	2			27.0	210		0.518	0.096	1988	
(O.	F	3			25.0	200		0.524	0.100		
masou)	F	4			27.0	240		0.466	0.081		
	F	5			30.0	330		0.486	0.085		
	F	6			27.5	240		0.492	0.088		
	F	7			23.5	160		0.506	0.091		
	F	8			27.0	240		0.511	0.100		
	F	9			29.0	300		0.526	0.096		
	F	10			26.8	230		0.533	0.111		
	F	11			30.0	310		0.552	0.119		
	F	12			23.5	160		0.500	0.095		
	F	13			23.5	150		0.486	0.078		
	M	14			23.5	195					
	M	15			24.5	215					
	M	16			24.3	205					
	M	17			25.0	220					
	M	18			22.7	190					
Rainbow	F	1			50.0	1,400		0.425	0.052		
trout	F	2			48.0	1,450		0.431	0.055		
(O.	F	3			44.0	1,550		0.375	0.039		
mykiss)	M	4			49.5	1,250					
	M	5			45.0	1,100					
	M	6			51.0	1,500					

* Values are means

** Number of eggs sample

3. Electrophoresis and staining procedure

The supernatants of prepared samples were subjected to starch gel electrophoresis. The gels were made using hydrolyzed starch(Sigma, S-4501) at a concentration of 13% with appropreate buffer systems(Shaw & Prasad, 1970; Ridgway *et al.*, 1970; Clayton & Tretiak, 1972).

RESULTS

1. Hybridization

Table 2 shows the rates of eyed eggs and hatchings in each crossing. The hybrid between chum salmon(♂) and masu salmon(♀) had the highest survival rate of eyed stage(83. 5%), and its triploid(allotriploid) showed the best result in hatching rate of 28. 1%. The triploid of hybrid groups were better than untreated groups in hatching rate. The survial rate of crosses, between chum salmon(♀) and rainbow trout(♂), chum salmon(♀) and masu salmon(♂) and between rainbow trout(♀) and masu salmon(♂) were significantly higher than other crosses.

Table 2. The rate of eyed egg and hatching in each corssing

	Crossing (♀×♂)	No. of egg	Rate of eyed stage(%)	Hatching rate (%)
Cont.	M×M	796	91.8	86.1
	C×C	4026	67.5	61.5
	R×R	1117	71.6	45.8
Exp.	M×C	1023	–	–
	M×C-t	1145	–	–
	M×R	989	–	–
	M×R-t	956	–	–
	C×R	1876	–	–
	M×R-t	1669	39.5	12.7
	C×M	2023	83.5	21.1
	C×M-t	1774	38.0	28.1
	R×C	2012	–	–
	R×C-t	1957	–	–
	R×M	2099	10.5	3.1
	R×M-t	2178	–	–

M, *Oncorhynchus masou*; C, *O. keta*; R, *O. mykiss*
– triploidization of hybrid

2. Electrophoresis for isozymes

In this study, intra- and interspecific distinctions and the tissue distributions of α-glycerophosphate dehydrogenase(αGPDH), isocitrate dehydrogenase(IDH), lactate dehydrogenase(LDH), malate dehydrogenase (MDH) and malic enzyme(ME) were analyzed electrophoretically in three salmonid species to determine the genetic bases of these enzymes and to survey the genetic markers useful for developmental studies. Isozyme patterns in parents and their hybrids were examined.

(1) Lactate dehydrogenase(LDH; EC,1. 1. 1. 27)

LDH isozymes in salmonids were encoded at five different loci formed by random association of two different subunits, each under the control of seperate genetic loci A and B (Okazaki, 1982; Wright *et al.*, 1970). As a result of gene duplication at the LDH-A and -B loci, the LDH-A_1 and A_2 loci and the LDH-B_1 and B_2 loci arose respectively.

There were some differencies at LDH loci among three salmonid fishes used as parents in this experiment. As shown in Figure 2 masu salmon exhibited the LDH variants at A_1 loci while rainbow trout exhibited it at A_2 loci. LDH patterns of chum salmon were observed at LDH-A_2 loci and its genotypes were *aa* or *ab* type.

(2) Malate dehydrogenase(MDH; EC,1. 1. 1. 37)

In all salmonid fishes two supernatant forms of this dimeric enzyme are known, described as MDH-A and MDH-B(Bailey *et al.*, 1970).

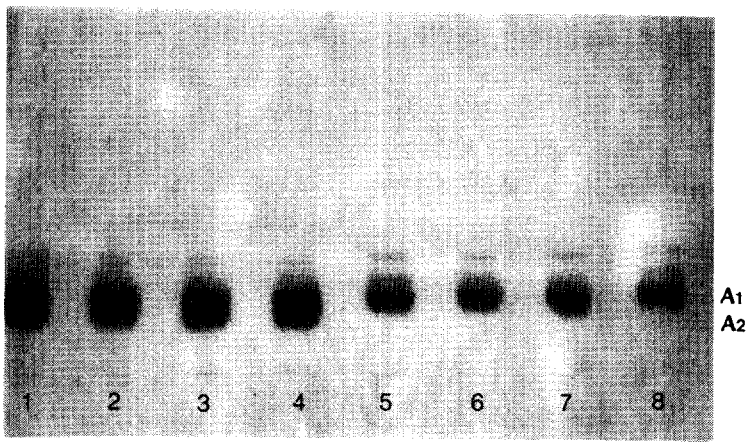

Figure 2. Starch gel patterns of LDH variants from muscle extracts in raibow trout (1-4) and masu salmon(5-6).

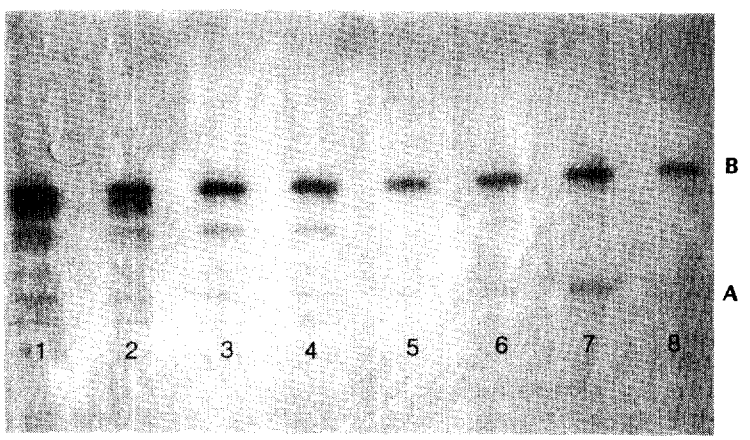

Figure 3. Starch gel patterns of MDH variants from muscle extracts in raibow trout (1-4) and masu salmon(5-6).

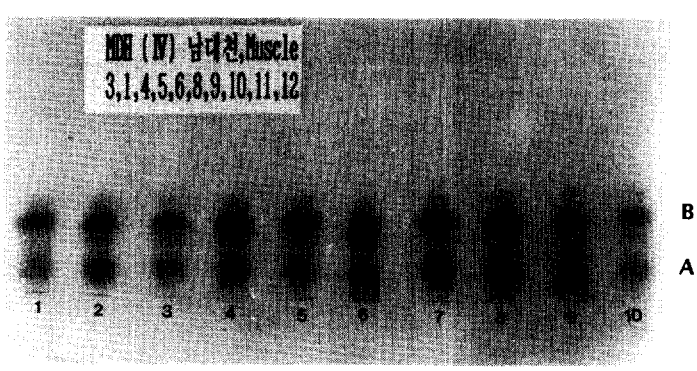

Figure 4. Starch gel patterns of MDH variants from muscle extracts in chum salmon.

MDH-A and -B are predominant in liver and skeletal muscle, respectively(Okazaki, 1982).

In this study, three allele polymorphism for muscle MDH-B has been identified in chum salmon and rainbow trout(Figure 3). However, MDH-B polymorphism was not found in parent masu salmon(Figure 4). Based on the relative intensities of bands in variant phenotypes there were no duplicated loci in rainbow trout and masu salmon. It appeared that there were to significant differences between masu salmon and rainbow trout on the tissue distribution of MDH isozymes among serum, skeletal muscle, liver, heart and gill(Figure 5).

The MDH patterns of chum salmon from heart muscle extracts were different from those of other two species. In chum salmon four distinct bands were appeared and only three bands were observed in other two species.

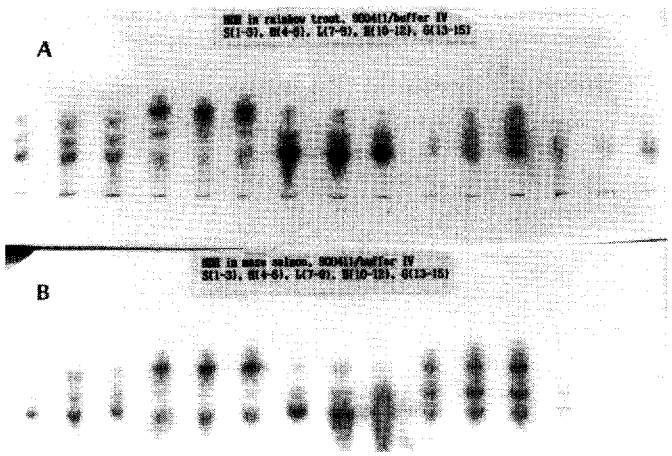

Figure 5. Tissue distribution of MDH variants in rainbow trout(A) and masu salmon(B).

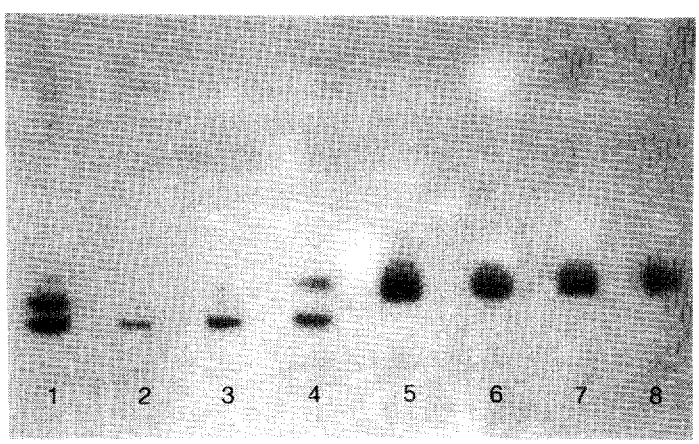

Figure 6. Starch gel patterns of IDH variants from muscle extracts in raibow trout(1-4) and masu salmon(5-6).

(3) Isocitrate dehydrogenase(IDH; EC,1. 1. 1. 42)

IDH variation is known to be controlled by two disomic loci(Allendorf, 1975; Okazaki, 1982). The IDH-3 loci are predominant in skeletal muscle. Phenotypes for this enzyme were different between rainbow trout and masu salmon. The result of this study indicated that the gene duplication for this enzyme in rainbow trout(Figure 6). In masu salmon all of the specimens examined didn't show any polymorphism for this enzyme from muscle extract.

(4) α-glycerophosphate dehydrogenase(α GPDH; EC,1. 1. 1. 8)

Utter *et al.*(1972) suggested that this enzyme from muscle extract had three phenotypes due to its dimeric inheritance. According to May *et al.*(1979), two alleles(100/111) were reported when the buffer system by Claton and Tretick(1972) was used.

In this study one distinct band appeared in both rainbow trout and masu salmon with significantly different staining intensity.

(5) Malic enzyme (ME; EC,1. 1. 1. 38 or 1. 1. 1. 39)

Two forms of this enzyme in rainbow trout were observed-one was predominant in muscle extract(ME-1) and the other in liver extract (ME-2). Both of these forms appeared as a single band(Alledorf,1975).

In this study, no difference existed for this enzyme common alleles(Figure 7).

DISCUSSION

For the purpose of genetic identification, several isozyme loci, LDH, MDH, IDH, α-GPDH and ME, from skeletal muscle extracts of three species were analyzed. Chum and masu salmon showed monomorphic patterns in most of loci, but rainbow trout were polymorphic at MDH-B, LDH and IDH loci. Especilly, there were significant differences at MDH-B loci among three species in gene frequency of b, and IDH patterns between masu salmon and rainbow trout were significantly different from each other. These loci would be utilized as efficient markers for the identification of hybrid's genotype and can be useful to enhance the efficiency on fish breeding.

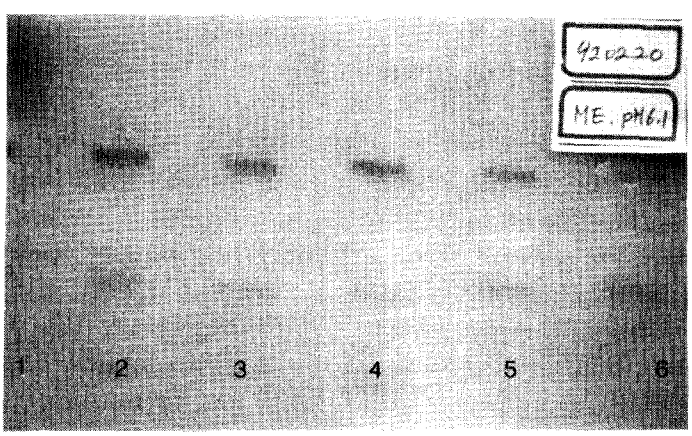

Figure 7. Starch gel patterns of ME from muscle extracts in raibow trout(1-4) and masu salmon(5-6).

The hybrid between rainbow trout(♀) and masu salmon(♂) is being analyzed at present, and the value of these loci will be measured.

SUMMARY

To test the possibility that isozyme patterns would be used as genetic markers in hybrid of salmonids, several isozyme loci in salmonids and their interspecific hybrids were analyzed.

Artificial interspecific hybridization and their reciprocal crosses were carried out using for three species of salmonids in Korea; rainbow trout, *Oncorhynchus mykiss*, masu salmon, *O. masou and* chum salmon, *O. keta*. Twelve experimental crosses including 6 allotriploidization groups together with three matings as controls were performed.

In reciprocal crosses among these species, the hybrids between *O. masou*(♀) *and O. keta*(♂) and between *O. mykiss*(♀) and *O. keta*(♂) were deceased before reaching the eyed stage. The hybrid between *O. keta*(♀) and *O. masou*(♂) showed the most effective result in the early developmental stage, and their allotriploid showed the highest hatching rate, 28.1%.

In the comparison of early growth rates between masu salmon and chum salmon, masu salmon exhibited higher growth rate than that of chum salmon. However, the reverse results were obtained for their hybrids.

For the purpose of genetic identification, several isozyme loci, LDH, MDH, IDH, α-GPDH and ME, from skeletal muscle extracts of three species were analyzed. Chum and masu salmon showed monomorphic patterns in most of loci, but rainbow trout were polymorphic at MDH-B, LDH and IDH loci. Especilly, there were significant differences at MDH-B loci among three species in gene frequency of b, and IDH patterns between masu salmon and rainbow trout were significantly different from each other. These loci would be utilized as efficient markers for the identification of hybrid's genotype and can be useful to enhance the efficiency on fish breeding. The hybrid between rainbow trout(♂) and masu salmon(♀) is being analyzed at present, and the value of these loci will be measured.

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