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酯酶同工酶在家鸡不同组织器官中的分布

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摘要 采用不连续的聚丙烯酰胺凝胶电泳法,结合组织特异性染色和酶谱区带活性扫描技术,对家鸡的心、肝、肾、脾、胸肌、睾丸、卵巢、脑、眼晶体共9种组织器官的酯酶同工进行了测定,发现不同组织器官酯酶同工酶均有分布,呈现广谱性,但谱带模式又各不相同,具有很明显的组织器官特异性。酯酶谱带活性与各组织器官所执行的功能相吻合,说明酯酶在调节组织器官代谢中发挥重要作用。

关键词 家鸡;酯酶同工酶;聚丙烯酰胺凝胶电泳;组织器官特异性

·新成果简介·

小麦蚜虫种群数量动态与防治决策研究

Study on the Population Dynamics and Control Desision-making of the Wheat Aphids

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小麦蚜虫种群数量动态与防治决策研究系"七五"国家攻关项目小麦病虫害综合防治研究的一个子专题。1993年获陕西省农业科学院科技进步一等奖;同年获陕西省科技进步一等奖。

首次在国内明确了麦管蚜、麦二叉蚜和禾谷缢蚜的发育起点温度和有效积温分别是3.4486℃、2.76℃、0.5730℃及140.2976、114.5、143.3291日度(全若虫期)。测定了不同虫龄的发育历期,制定了特定年龄和繁殖特征生命表。以Holling园盘方程为基础,测定了七星瓢虫、大灰食蚜蝇和三突花蛛对麦长管蚜的功能反应,提出每一个标准天敌单位控制105头麦长管蚜以及当天敌单位数与麦长管蚜的比例在1∶125以上时,控制麦长管蚜危害的量化指标。对小麦旗叶中不同氨基酸含量的高低与品种综合抗蚜性的关系作了分析。提出了小麦品种抗蚜性的综合抗性系数指标。依据越夏、秋苗及春季蚜虫数量动态,提出了秋苗期蚜量的预测指标及模型。以蚜虫—小麦—天敌—环境间各种亚耦联系,组建了小麦生长后期麦长管蚜种群动态的BOX CAR模型。以标准危害日概念为基础,提出了麦长管蚜在小麦生长后期危害小麦的动态经济阈值。提出了苗期以75% 3911处理种子,后期以抗蚜威4 000倍液或40%氧化乐果1 000倍液1次防治的最优方案。累计防治面积1 670万亩,挽回小麦损失26.72万t。

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DISTRIBUTION OF ESTERASE ISOZYMES IN THE DIFFERENT TISSUES AND ORGANS OF CHICKEN

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Abstract The discontinuous polyacrylamide gel electrophoresis (PAGE) with the tissue specifcity satining and zymogram band activity scanning techniques was used to detect the esterase isozymes of heart,liver,kideny,spleen,breaest,testis, ovary,brain and eyes of chicken. The results show that the esterase isozyme was in all the tissuse and organs studied and had the wide ranging distribution. However,the zymogram pattern of every tissue and organ was different,i. e. it had the obvious tissue and/or organ specificity. The activity of the esterase bands had a close correspondence with the function which the tissue and/or orgon had caried out, which demonstated that the esterases played an important role in the regulation of the metabolisms of tissues and organs

Key words Chicken;Esterase isozme;PAGE;Tissue and organ specificity

Esterase isozymes are groups of hydrolases which have a wide distrbution in the animal's various tissues and organs . In recent years they are widely concerned in the study fields of the genetic relationships of animals,breed classification,developmental genetics etc[1~3]. At the basis of having accomplished the study of esterase isozyme polymorphisms in chicken[4], we had investigated the esterase isozymes in the chicken's tissues and organs in order to detect their distribution characteristics and their relationhips with the functions of tissues and organs,further to seek the regulation of esterase isozymes to the tissue metabolism.

1 MATERIALS AND METHODS

Sample preparation 5 healthy adult chickens of Ross Brown (3♂,2♀) were selected and killed by exsanguination. About 1 g of samples of heart,liver,kidney,spleen,breast,testis,ovary,brain and eyelens was removed. Then the samples were washed in the 0～4℃ previously prepared physiological saline salution and the same weight of phosphate buffer (pH 7. 0) was added. They were grinded with glass grinder in the ice bath to prevent the enzyme decomposition. Then the grinded solution was transfered into 5 ml glass tube for centrifuge with 3000

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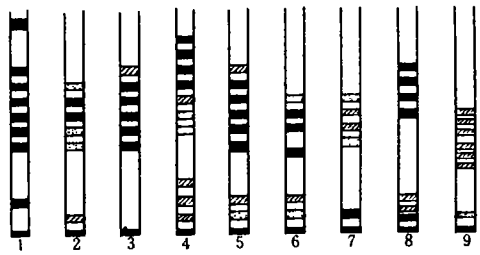
r/min for 30 min. The upper clear solution was stored at −20℃ in refrigerator before analysis.

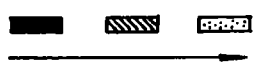
Electrophoresis the discontinuous buffer system was used with a buffer of tris-citric acid (pH 9.0)and a electrode buffer of boric-NaOH (pH 8.6)as described by Gahne[5]. The gel concentration T was 8% and C was 2.4%. The electrophoresis was conducted in refrigerator at the temperature of 0～4℃. The other conditions of electrophoresis were the same as the previous report[4].

Gel incubation and zymogram detection following the electrphoresis, the gel was immersed in 100 ml phosphate buffer (pH7.0) containing 60 mg fast blue B salt and 2 ml 1% α-nphthyl acetate acetone solution. The incubation and staining reaction were carried out in the permanant incubator at the constant temperature of 37℃ for about 30 minutes untill the enzyme band clearly presented.

Spectrophotometer The isozyme band was scanned with CS − 930 scanner (made in Japan) and scanning wave and relative area of every absorption peak were recorded.



1. liver 2. heart 3. kidney 4. spleen 5. testis 6. ovary 7. eyelens 8. brain 9. breast
1. 肝　2. 心脏　3. 肾　4. 脾　5. 睾丸　5. 卵巢　6. 眼晶状体　8. 脑　9. 胸肌



Show the isozyme activity decrease orderly
染色强度依次减弱

Fig 1　Zymogram of esterase bands in adult chicken
图 1　成年鸡组织器官酯酶同工酶模式图

2　RESULTS

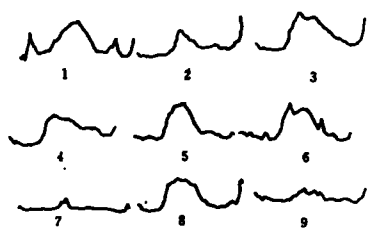
The pattern figure of isozyme band had been drawn (fig. 1) based on the result of electrophoresis. Because of the indetermination of genetic background and subunits composition in esterase, the traditional method[6] for the

Table 1　Distrbution of esterase isozyme band in tissues and organs of adult chicken
表 1　成年鸡各组织器官酯酶同工酶谱带分布

No. of band 谱带编号	Liver 肝	Heart 心	Kidney 肾	Spleen 脾	Testis 睾丸	Ovary 卵巢	Eyelens 眼晶状体	Brain 脑	Breast 胸肌
E−1	+								
E−2				+					
E−3				+					
E−4	+		+	+	+			+	
E−5	+	+	+	+	+			+	
E−6	+	+	+	+	+	+	+	+	
E−7	+	+	+	+	+	+	+	+	+
E−8	+	+	+	+	+	+	+		+
E−9	+	+	+		+		+		+
E−10						+			+
E−11									+
E−12									+
E−13				+					
E−14	+			+	+	+		+	
E−15								+	
E−16		+		+	+	+	+	+	+
Total band numbers 总带数	8	6	6	10	8	6	5	7	7

nomenclature of zymogran was used according to the band mobility from anode to cathoed.

It could be seen from table 1 that 16 strips of bands had been detected from adult chick's tissues and organs, and E−1 to E−16 been marked in order, in which liver presented E−1, E−4, E−5, E−6, E−7, E−8, E−9, E−14 and total 8 strips of bands; spleen had E−2, E−3, E−4, E−5, E−6, E−7, E−8, E−13, E−14, E−16 and total 10 strips of bands etc. Apart from the sex organs with numbers, patterns and enzyme activity of zymogram band (testis presents E−4, E−5, E−6, E−7, E−8, E−9, E−14, E−16, total 8 strips of bands, and E−6, E−7, E−8, E−10, E−14, E−16 in ovary), there are no differences in liver, heart, kidney, spleen, eyelens, brain and breaest between sex.



1. liver 2. heart 3. kidney 4. spleen 5. testis 6. ovary 7. eyelens 8. brain 9. breast
1. 肝脏 2. 心脏 3. 肾脏 4. 脾脏 5. 睾丸 6. 卵巢 7. 眼晶状体 8. 脑 9. 胸肌

Fig 2 Scanning wave of esterase zymogram band in tissues and organs of abult chicken

图 2 成年鸡不同组织器官酯酶同工酶扫描图

The result of zymogram activity scanning and the area under every scanning wave are shown in figure 2 and table 2. From which it can be seen that there had a great difference in scanning wave in tissues and organs of adult chicken and this illustrates the great difference of zymogram patterns among them. The difference under the wave area reflects the variation of enzyme activity.

Table 2 Relative activity of esterase zymogram bands in adult chicken's tissues and organs

表 2 成年鸡不同组织器官酯酶同工酶各区带相对活性

No. of band 谱带编号	Liver 肝	Heart 心	Kidney 肾	Spleen 脾	Testis 睾丸	Ovary 卵巢	Eyelens 眼晶状体	Brain 脑	Breast 胸肌
E−1	23.5	0	0	0	0	0	0	0	0
E−2	0	0	0	10.7	0	0	0	0	0
E−3	0	0	0	3.0	0	0	0	0	0
E−4	8.7	0	23.2	3.4	13.4	0	0	9.5	0
E−5	10.5	24.0	11.1	4.9	8.7	0	0	8.2	0
E−6	11.1	11.2	7.5	17.3	6.5	18.9	7.9	22.5	0
E−7	8.4	13.4	10.6	16.3	5.0	14.3	4.0	14.2	6.4
E−8	11.4	22.9	11.2	3.3	12.1	15.1	4.2	0	5.9
E−9	10.3	16.4	36.4	0	8.4	0	4.0	0	4.7
E−10	0	0	0	0	0	13.7	0	0	5.8
E−11	0	0	0	0	0	0	0	0	6.8
E−12	0	0	0	0	0	0	0	0	4.4
E−13	0	0	0	8.0	0	0	0	0	0
E−14	16.1	0	0	10.8	17.2	12.9	0	10.6	0
E−15	0	0	0	0	0	0	0	15.7	0
E−16	0	12.1	0	22.3	28.7	25.1	79.9	19.3	66.0

3 DISCUTION

The analysis for esterase isozymes in chicken's 9 kinds of tissues and organs has been

carried out and the distribution of the isozyme in all the tissues and organs has been observed. These results have the correspondence with the previous studies to the tissues and organs with the same isozyme of mosquite[7], domestic silkworm[8] and fish[2]. It is suggested that esterase isozymes have a wide ranging distribution among the animal species and various tissues and organs in the same species. On one hand, the wide ranging distribution of esterase isozymes illustrates that they play an important role in the metabolic regulation, on the other hand, it is very useful to the gengetic detection of various animals as a genetic marker in the future.

The distribution of esterase isozymes presents not only the wide ranging characteristics, but also the tissue and organ specificity. For example, E−1 is the feature band in liver and E−2, E−3 in spleen, E−10 in ovary, E−11, E−12 in breast and so on. The difference of the spectrophotometric wave in figure 2 and zymogram staining activity in table 2 is another exhibition of tissue and organ specificity.

Esterase is the direct product of gene and esterase gene regulates the metabolism of various tissues and organs through the systemetic expression and inhibition. The wide ranging distribution of esterase isozymes illustrates the very important role of the isozymes in the regulation of the tissue and organ metabolism, wherease, the characteristics of stage development and tissue and organ specificity reflect the particularity of fatty metabolism in the different stage and different tissues and organs, and the delicate regulation of isozyme gene in time and space. The enzyme activity is also useful to illustrate this problem. Liver is the most important organ in fat metabolism. The synthesis, decomposition and transformation of fat are all accomplished through this"medium station", therefore, reflected in the zymogram band of esterase, apart from the feature band of E−1, the anzyme activity of the other bands are all powerful (see sample 1 in figure 1). Fat metabolism in skeletal muscle is relatively weak and the zymogram bands are all weakly stained (sample 9 in figure 1). The esterase gene regulutes the specific metabolic types of various tissues and organs through the number of zymogram bands (i.e. the difference of molecular characteristics) and the difference of enzyme activity in order to establish the metabolic coodination in the body of chicken.

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