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Nonspecific Esterase Patterns of *Rattus norvegicus* (Berkenhout, 1769) in Western Turkey

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Abstract: Nonspecific esterases in the muscle, heart, kidney and liver tissues of wild rats (*Rattus norvegicus*) were examined by horizontal starch gel electrophoresis. Zymogram bands corresponding to Es-10 and Es-4 in the muscle, heart, kidney and liver homogenates, Es-3 and Es-17 in the kidney and liver, Es-18 in the liver tissue, Es-15 in kidney and liver, Es-2 in the muscle and heart, Es-16 in the heart tissue were detected. These findings showed that nonspecific esterases are very polymorphic and tissue-specific, and are not of taxonomic importance for the populations of *Rattus norvegicus*.

Key Words: Nonspecific esterase, horizontal starch gel electrophoresis, *Rattus norvegicus*, Turkey.

Batı Türkiye *Rattus norvegicus* (Berkenhout, 1769)'larının Nonspesifik Esteraz Özellikleri

Özet: Sıçanların kas, kalp, böbrek ve karaciğerinde nonspesifik esteraz enzimi horizontal nişasta jel elektroforezi ile incelendi. Zimogram bantlarına karşılık gelen Es-10 ve Es-4 kas, kalp, böbrek ve karaciğerden; Es-3 ve Es-17 böbrek ve karaciğer homojenatlarından; Es-18 karaciğerden; Es-15 böbrek ve karaciğerden; Es-2 kas ve kalpten ve Es-16 kalpten tespit edildi. Bu bulgular nonspesifik esterazların çok polimorfik, dokuya özel olduğunu ve *Rattus norvegicus* populasyonları için taksonomik önemde olmadığını göstermiştir.

Anahtar Sözcükler: Nonspesifik esterazlar, horizontal nişasta jel elektroforezi, *Rattus norvegicus*, Türkiye.

Introduction

The wild rat, *Rattus norvegicus*, is present throughout the world (1-4). In the Palearctic region, Ellerman and Morrison-Scott (1) identified three subspecies for *R. norvegicus*. The distribution and taxonomic status of *R. norvegicus* from Turkey and areas neighboring Turkey have been studied by Misonne (2), Bodenheimer (3), Hatt (4), Ondrias (5), Lay (6) and Yiğit et al. (7). Recent taxonomic studies on the order Rodentia have been focused on enzyme electrophoresis. Esterases have been intensively studied in the laboratory races of *R. norvegicus*, and are polymorphic enzymes (8-14). Many esterase polymorphisms have been described by von Deimling et al. (12, 13), Matsumoto (11), Womack (8), Kluge et al. (14). The classification of nonspecific esterases is extremely difficult, and their occurrence in various tissues is affected by many internal and external factors (12, 13, 15-19). In this study, nonspecific esterase, i.e.,

carboxylesterase or B-esterase (EC 3.1.1.1) of wild rats "*R. norvegicus*" was studied in order to provide comparative data on their taxonomy. We briefly described nonspecific esterase alleles revealed by electrophoretic separation from different tissues such as muscle, heart, kidney and liver. Thus, we aimed to provide in sights from the comparative data among wild rat populations by the characterization of esterases.

Materials and Methods

Specimens (5, 4) were collected from three localities in western Turkey (Fig. 1). Specimens were caught with Sherman live traps and transferred to the laboratory. Muscle, heart, kidney and liver were taken after killing the animals. Tissue samples were stored at -70° C until homogenized. The samples were homogenized in approximately 500 µl distilled water with a glass homogenizer. Homogenates were centrifuged at 12100 rpm for 5 min and the supernatants stored at - 20° C before electrophoresis. These homogenates were absorbed to Whatman No. 3 filter papers. Samples were inserted into the gel slab composed of 11% hydrolyzed potato starch (Sigma Chemical Co., St. Louis, Mo) in a gel buffer (76 mM Tris, 5 mM citric acid) of pH 8.65 (20). Samples were examined using a discontinuous buffer system (electrode buffer: 300 mM boric acid and 60 mM NaOH, pH 8.2) as described by Poulik (20). Horizontal starch gel electrophoresis was carried out at 8 V/cm for 5 h in a refrigerator at 4° C. Following electrophoresis, the gels were soaked in 0.5 M boric acid and then the gels were stained at room temperature for up to 2 h with a staining solution composed of 3 ml 1 % α-naphthyl acetate solution (1:1 water: acetone), 50 mg Fast Blue BB salt in 50 ml 0.2 M Tris-HCl buffer pH 7.0 (21).

Results

Nonspecific esterase zymograms of four tissues (muscle, heart, kidney, liver) examined in 9 specimens from three localities revealed very different electrophoretic patterns. Zymograms indicated that the intensity and the number of the nonspecific esterase bands are very variable. The number of bands were found to be lower in muscle and heart than in kidney and liver. The first bands in the samples of muscle, heart, kidney and liver are the most likely corresponding to the Es-10. Es-10 with one or two alleles was seen in all rat tissues examined, and also Es-4 with one or two alleles migrated towards the front of the Es-10 bands. Wild rats from three different localities in Turkey possessed the Es-4. Es-3 was only detected in kidney and liver homogenates but was not found in muscle and heart homogenates. Es-18, Es-15 and Es-16 were suspected in some tissues examined (Fig. 2a, b, c, d).

Muscle: Nonspecific esterases were observed with one or four bands in the zymograms of rat muscles. We examined five specimens from Ankara (Fig.1.1, Fig. 2a, slots: 1-5). Specimens 1, 4 and 5 showed the same nonspecific esterase pattern with four bands, other specimens had two or three nonspecific esterase bands (Fig. 2a, slots: 1, 5). Both specimens from Adana had four bands (Fig. 1.2, Fig. 2a, slots: 6, 7). Two specimens were examined from Samsun. As the first one (slot 8) had two nonspecific esterase bands, the latter had a weak and fast nonspecific

esterase band (Fig. 1.3, Fig. 2a, slots: 8, 9). Nonspecific esterase 10 was detected in muscle tissue. Zymograms corresponding to Es-4 were seen in all specimens as monomeric or dimeric except in slot 3. Es-2 was observed in all muscle tissues examined. A band under the Es-2 zone was seen in slots 6 and 7 (Fig. 2a).

Heart: Nonspecific esterase zymograms in specimens from Ankara varied between 3 and 4 bands with three different patterns (Fig. 2b, slots: 1-5). Slots 1, 3 and 5 had four nonspecific esterase bands in same pattern, slots 2 and 4 had three nonspecific esterase zymograms in different patterns. Specimens from Adana had two-three bands (Fig. 2b, slots: 6, 7). In two specimens from Samsun, slot 8 had a weak band, the latter had five well marked nonspecific esterase bands (Fig. 2b, slots: 8, 9). The first band in heart tissues corresponding to Es-10 was common in all specimens, with respect to slot 8. The second band in the zymogram corresponding to Es-4 was found to be monomeric and dimeric in six out of nine specimens. The products of Es-4 were not observed in heart tissues of slots 6, 7 and 8. The bands moving to the most anodal region in the samples 1-9, except for slots 7 and 9, were defined as Es-2, and it was constantly seen in all heart tissues examined. The fast moving bands in the zymogram of heart were defined as Es-16 in slots 7 and 9 (Fig. 2b).

Kidney: Nonspecific esterase bands in specimens examined varied from 2 to 5. We determined five different nonspecific esterase patterns in the zymogram of Ankara specimens (Fig. 2c, slots: 1-5). Specimens from Adana had five nonspecific esterase bands (Fig. 2c, slots: 6, 7). Two specimens from Samsun, No. 8 and No. 9 had five and four bands, respectively. The fourth weak band in No.8 were found to be absent in No. 9 (Fig. 2c, slots: 8, 9). Es-10 with two different phenotypes and Es-4 as monomeric and dimeric appeared in the kidney tissues of some specimens. Es-3, Es-17 and Es-15 were tentatively detected in the kidney tissues (Fig. 2c).

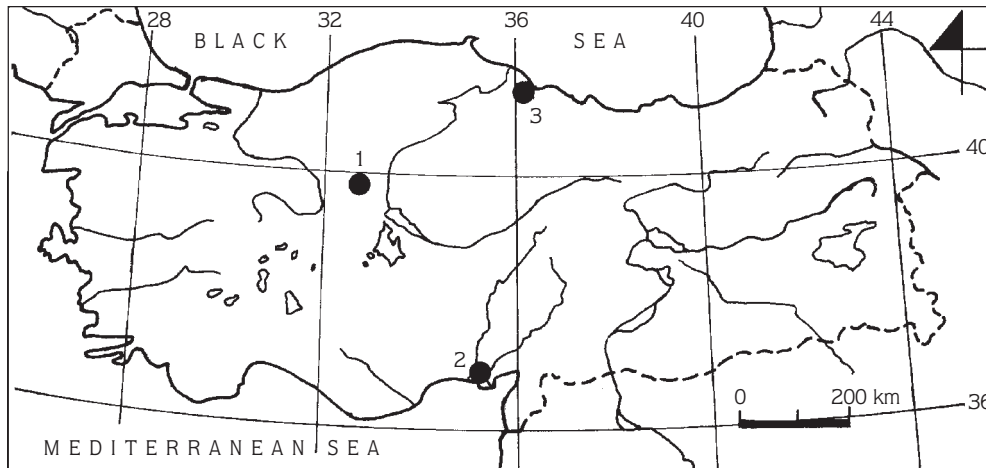


Figure 1. The localities of the specimens examined. 1. Ankara, 2. Adana, 3. Samsun.

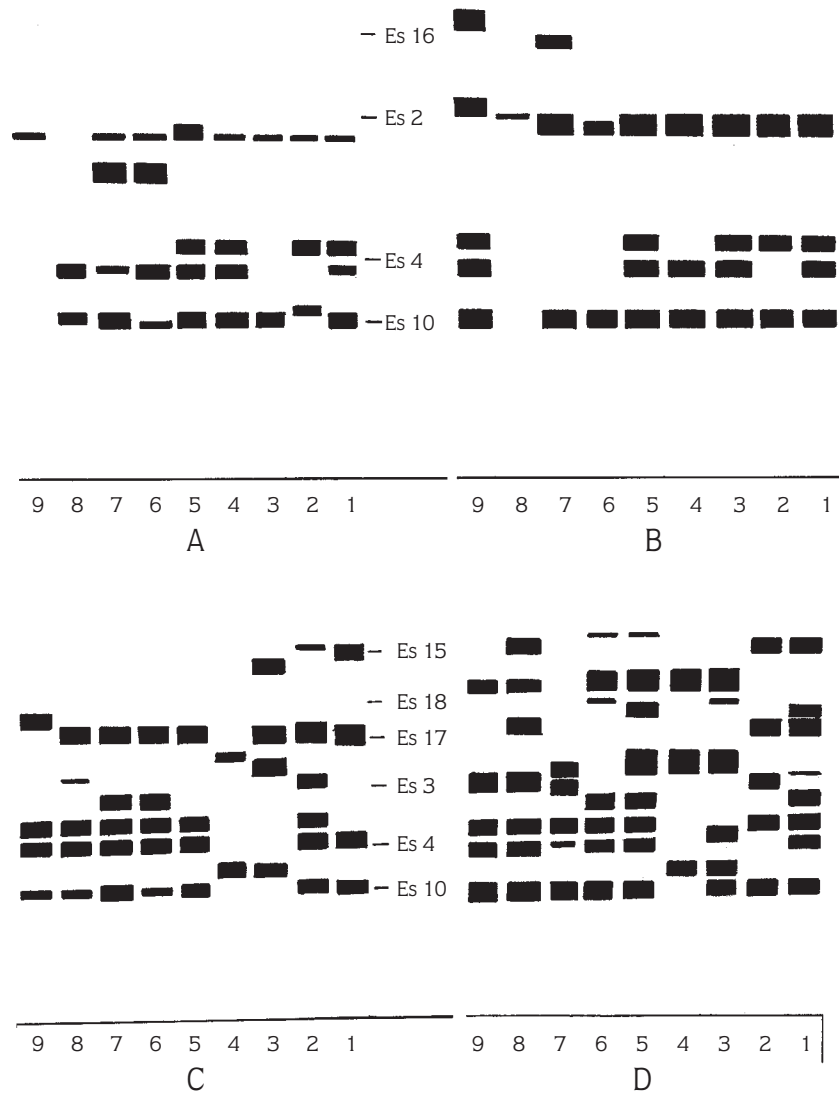


Figure 2. Zymograms of rat muscle (a), heart (b), kidney (c), and liver (d). Slots 1-5 Ankara, 6-7 Adana, 8-9 Samsun.

Liver: The numbers of nonspecific esterase bands in the liver zymogram varied from 3 to 8. In specimens from Ankara, we detected five different nonspecific esterase bands patterns in the zymogram (Fig. 2d, slots: 1-5). Two specimens from Adana had two different patterns with 5 and 7 bands (Fig. 2d, slots: 6, 7). Two specimens from Samsun showed two different nonspecific esterase patterns with 5-7 bands (Fig. 2d, slots: 8, 9). Es-10 was consistently found

to be as monomeric and dimeric as in other tissues examined. Es-4 was detected in all liver tissues examined except for samples from No. 4. Es-3, Es-17, Es-18, and Es-15 were defined in the zymogram from liver homogenates (Fig. 2d).

According to these findings, nonspecific esterases of wild rats in Turkey are very polymorphic and tissue-specific. Tissues samples of animals from three different localities showed that nonspecific esterase patterns might give different bands in some specimens even in the same locality.

Discussion

Es-10 was previously reported in heart tissues by von Deimling et al. (12). It was similarly found in heart tissues and in muscle, kidney and liver. Womack (9) found polymorphism in a cathodal group of kidney esterase. Researchers also revealed three different zymograms phenotype for Es-4 in kidney tissues of their rat colony. In addition, Womack and Sharp (10) also detected Es-4 in kidney and liver tissues of *R. norvegicus*. Es-4 in the liver tissues of four rat strains and kidney tissues of wild rats were reported by Mouier et al. (23) and Yamada et al. (24) respectively. van Zutphen (22) also detected Es-4 in kidney tissues of rats. Es-4 in some of Turkish wild rats was observed with two different phenotypes. The bands from kidney tissues corresponding to Es-4 were similarly observed in the some tissues of our specimens. But these were found to be absent in some samples (Fig. 2a, b, c, d). Womack (8, 9) and van Zutphen et al. (22) only detected Es-3 in the small intestine but not in kidney homogenates. Womack (9) also stated that heavy multiple banding in the liver tissues prohibited the exclusion of this tissue as the source of Es-3 product. However, Moutier et al. (23) detected Es-3 in liver tissues of rats. Jimenez-Marin and Dessauer (25) suggested that Es-3 occurred only in females, and was detected in liver, muscle, kidney, and heart. In contrast Kluge et al. (14) detected Es-3 in the liver of male rats. We defined Es-3 in kidney and liver in both male and females, but not in muscle or heart homogenates. Yomori and Okamoto (17) revealed that zymogram analyses of nonspecific esterase varied from tissue to tissue. According to their findings, esterase zymograms from the renal cortex and liver showed different patterns among spontaneously hypertensive rats and control normative rats. They also detected 10 bands in two different patterns in liver. Our findings from wild rats gave completely different nonspecific esterase patterns compared with those of Yomori and Okamoto (17). Kluge et al. (14) detected Es-18 in kidney, jejunum, heart, lung, tongue, testis, brown and white fat, erythrocytes and serum. They also stated that Es-18 is specific to the liver. Our results are consistent with those given by Kluge et al. (14).

Es-15 was detected in liver homogenates by Kluge et al. (14). Bands in liver homogenates of Turkish wild rats were defined as Es-15 in slots 1, 2, 5, 6, 8 (Fig. 2d). Moreover, bands from kidney zymogram corresponding to Es-15 in liver were also tentatively defined as Es-15 in slots 1, 2, 3 (Fig 2c).

Von Diemling et al. (13) reported that Es-16 was defined in lung and heart. We similarly detected Es-16 in slots 7 and 9 of heart tissues from Adana and Samsun specimens. In contrast

to our findings, Jimenez-Marin, and Dessauer (25) described Es-16 migrated to the cathode from homogenates of pancreas. According to these findings, nonspecific esterases are very polymorphic, tissue-specific, and variable in populations of *Rattus norvegicus*, there is also very poor information on nonspecific esterases of wild populations.

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