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A study on the possibility of identification of Phlebotomus caucasicus and Phlebotomus mongolensis, vectors of zoonotic cutaneous leishmaniasis, base on ITS2 rDNA by PCR-restriction enzyme

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P.caucasicus and P. mongolensis, subgenus of Paraphlebotomous, are secondary vectors of Zoonotic Cutaneous Leishmaniasis in Iran country. These are two closely related, morphologically similar sand fly species that we cannot find any differentiations between females of them by the standard identifiably keys. This study was carried out in order to verify identification and differentiation of P.caucasicus and P. mongolensis by PCR- restriction enzyme method. Genomic DNA was extracted from 12 specimens of P.caucasicus (male), 3 specimens of P.mongolensis (male) and 9 specimens of Cucasicus Group (female). We selected two primers from the species of sand flies and then ITS2 region of the rDNA gene was amplified by a Polymerase Chain Reaction (PCR). Primers selections were banded on alignment of ITS2 ribosomal DNA by using DNASIS software. By the PCR, 9 specimens of P.caucasicus (male), 2 specimens of P.mongolensis (male) and 6 specimens of Cucasicus Group (female) showed distinguish identical patterns with a visible fragment of about 500 bp in size. In RFLP, we had identical pattern with TasI used as restriction enzymes. For 5 specimens of three species of sand flies isolates, P.caucasicus P. mongolensis and P.papatasi, nucleotide sequencing was achieved, and comparison with other available sequences in GenBank was performed using Blast program. Two of them were the species of P.caucasicus (male), also two of another was P.mongolensis (male) and the fifth was P.papatasi. It is presumed that using PCR with specific primers could be useful for molecular analysis, diagnosis, epidemiological studies and differentiation between P.caucasicus and P. mongolensis.

Biography

Alireza Zahraei is a PhD research Scholar in Banaras Hindu University, Varanasi, India. He was graduated in two courses of Environmental Health and Medical Entomology and Vector Control in BSc and also postgraduate in Medical Entomology and Vector Control. He is a Lecturer in Department of Medical Entomology and vector control, School of Public Health, Tehran University of Medical Sciences, Iran. He has 36 published articles in different international journals and also 47 published in different congress around the world.

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Isolation, screening and optimization of production conditions for a novel hyperthermophillic lipase from a bacterial isolate STL-A59

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In the present study a hyperthermophillic lipase producing bacterial isolate has been screened by vigorous screening from the mud soil sample of a hot spring of Himachal Pradesh India. Lipase producing ability of 25 bacterial isolates was examined qualitatively, out of which 10 isolates showing maximum zone of hydrolysis on trybutyrin agar plate (0.5% v/v) has been selected for further study. The selected strain was found to be Gram-negetive, rod shaped, non-sporulating and forming round creamish white colonies with sharp edges. The physico-chemical parameters were studied to improve the production of lipase in the broth. A 12 h old seed culture (4 % v/v) was inoculated into 50 ml mineral based broth containing (g/l) $NaNO_3$ 3; K_2HPO_4 0.1; KCl 0.5; $MgSO_4.7H_2O$ 0.5; FeSO4.7H2O 0.01 and yeast extract (4%, w/v), pH 7.5. The broth/ medium autoclaved at 1.1 bar for 18 min at 121°C. The isolated strain was hyperthermophillic and a prominent growth was observed at 75°C. Initially total lipase activity of 103 U/ml was recorded for the hydrolysis of p-nitrophenylpalmitate at 65°C (7.5) after 18 h production under shaking at 150 rpm. Various reaction conditions buffer molarity, concentrations, buffer pH and were optimized at 65°C. The thermophilic lipase showed Km 0.166mM and Vmax 263.07 U/mg/min with specificity constant 231.68. The crude lipase was found to be stable at 65°C for 24 h and a 50% loss in the activity was observed after 11 h incubation at 65°C. The biochemical characterization of strain STL-A-59 showed Voges-proskeur positive, alkaline phosphatase negative, ONPG positive, urease negative and positive for sucrose and mannose utilization.

Keywords: Gram-negative hyperthermophillic, tributyrin, lipase, reaction conditions.

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