Development of an enzyme-linked immunosorbent assay for detection of the organophosphate pesticide, chlorpyrifos

Chlorpyrifos [*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate] (Figure 1) is one of the most commonly used organophosphate pesticides, due to its low cost and broad-spectrum activity.

However, injudicious and indiscriminate use of the pesticide has inadvertently led to several long-lasting consequences on plant and animal health, soil and environmental pollution, ecological imbalances and development of pesticide resistance in pests. Even small quantities of chlorpyrifos have adverse effects on the health of individuals exposed to it. Hence, it becomes essential to monitor the contamination of chlorpyrifos at minute levels. Conventional methods used for detection of chlorpyrifos give low sensitivity limits, viz. 0.5 ng with liquid chromatography[1], 25.3 ng using coupled-column liquid chromatography/electrospray ionization tandem mass spectrometry[2] and 0.029 μg/g using HPLC. Moreover, these require skilled labour besides being cost-intensive and laboratory-oriented. Hence it becomes necessary to look for alternate methods which overcome these limitations. Immunoassays, especially enzyme-linked immunosorbent assay (ELISA), are best suited being rapid, cost-effective as well as sensitive and specific. They have shown great promise in a number of studies related to pesticide residue analysis, viz. detection of bromophos[3], cyanophos[4], triazophos[5], fenthion[6] and chlorpyrifos-methyl[7]. In the present study, an attempt has been made to develop ELISA for the detection of chlorpyrifos. Though ELISA has been developed earlier for the detection of chlorpyrifos, a sensitivity of only 70 pg has been reported[8]. The present study was undertaken with an aim to enhance the sensitivity of the assay.

Anti-chlorpyrifos antibodies were produced by artificial immunization of rabbits, using chlorpyrifos–BSA conjugate synthesized in the laboratory according to the method described by Manclus *et al.*[9]. These anti-chlorpyrifos antibodies were subsequently employed for the development of competitive inhibition ELISA for quantitative detection of chlorpyrifos (Figure 2).

Competitive inhibition ELISA is based on the competition between solid phase-bound and free antigen for limited antibodies. With increase in concentration of free chlorpyrifos added for competition, lesser amount of antibodies is available for binding with the bound antigen and hence there is a decrease in absorbance. Percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = \frac{A_{492\,\max} - A_{492}}{A_{492}} \times 100,$$

where $A_{492\max}$ is the absorbance in the absence of inhibitor (maximum absorbance) and A_{492} is the absorbance at any given concentration.

A sigmoidal standard curve (Figure 3) was obtained for detection of chlorpyrifos by competitive inhibition ELISA depicting percentage inhibition versus concentration of antigen. Antiserum was tested at dilutions of 5 K, 10 K and 20 K. In all the above assays, at a concentration of 100 ng or above, no colour developed, thereby indicating 100% inhibition and the sensitivity remained 10 fg. The sensi-

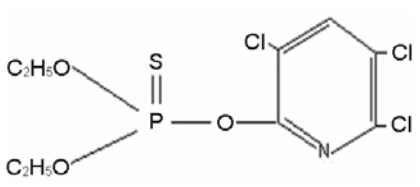

Figure 1. Structure of chlorpyrifos.

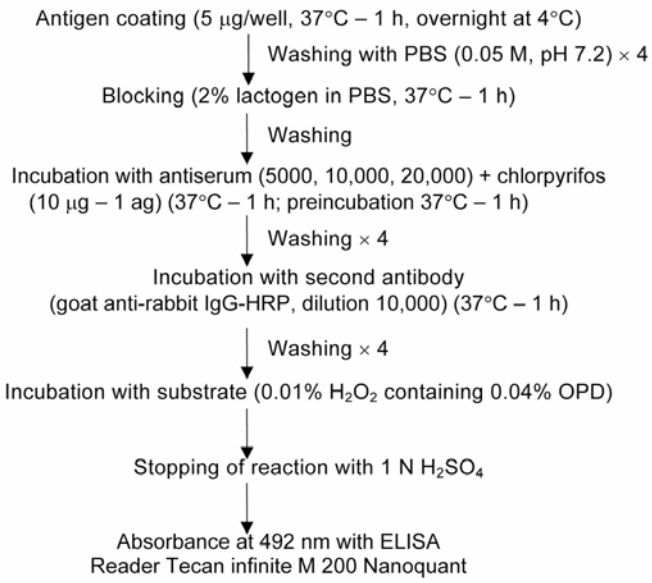

Figure 2. Development of competitive inhibition ELISA for detection of chlorpyrifos.

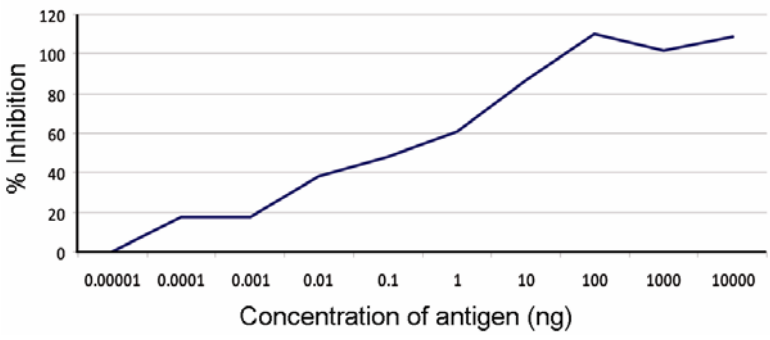

Figure 3. Percentage of inhibition versus concentration of chlorpyrifos.

tivity of the assay was found to be independent of antiserum concentration used.

The competitive inhibition ELISA developed showed a sensitivity limit as low as 10 fg. This can be further adopted as a routine test for the detection of chlorpyrifos as a rapid and cost-effective tool for onsite mass screening of a large number of samples simultaneously.

1. Salm, P., Taylor, P. J., Roberts, D. and de Silva, J., *J. Chromatogr. B*, 2009, **877**, 568–574.
2. Williamson, L. N., Terry Jr, A. V. and Bartlett, M. G., *Rapid Commun. Mass Spectrum.*, 2006, **20**, 2689–2695.
3. Park, W. C., Cho, Y. A., Kim, Y. G., Hammock, B. D., Lee, Y. T. and Lee, H., *Bull. Korean Chem. Soc.*, 2002, **23**, 1399–1403.
4. Park, J. H., Park, W. C., Kim, Y. J. and Lee, Y. T., *Bull. Korean Chem. Soc.*, 2002, **23**, 605–609.
5. Gui, W. J., Jin, R. Y., Chen, Z. L., Cheng, J. L. and Zhu, G. N., *Anal. Biochem.*, 2006, **357**, 9–14.
6. Zhang, Q. *et al.*, *Food Chem.*, 2008, **106**, 1278–1284.
7. Qian, G., Wang, L., Wu, Y., Zhang, Q., Sun, Q., Liu, Y. and Liu, F., *Food Chem.*, 2009, **117**, 364–370.
8. Brun, E. M., Garces-Garcia, M., Puchades, R. and Maquieira, A., *J. Agric. Food Chem.*, 2005, **53**, 9352–9360.
9. Manclus, J. J., Primo, J. and Montoya, A., *J. Agric. Food Chem.*, 1994, **42**, 1257–1260.

Received 9 May 2012; revised accepted 11 September 2012

P. GOEL
J. KAPUR-GHAI*

Department of Zoology,
Punjab Agricultural University,
Ludhiana 141 004, India
**For correspondence.*
e-mail: kapurghaij@yahoo.com

Ice nucleating bacteria in hailstones during a hailstorm over Karad, India

Hailstones are a form of solid water formed within thunderstorms produced by cumulonimbus clouds. They are formed when warm air with high moisture rises up rapidly and meets the cold front of clouds in the atmosphere. Spontaneous crystallization of supercooled water is known to occur only at –40°C, the homogenous nucleation temperature. For crystallization to occur at higher temperatures, other heterogenous ice nucleating agents are required. Bacteria are known to produce special membrane-associated proteins called 'ice nucleation proteins' (INP) that are known to crystallize water at –2 to –14°C and serve as templates for ice crystals[1]. Bacteria are also known to form ice on leaf surfaces and have been discovered in clouds as well[2–5], but their role in the formation of hailstones is little known. Michaud *et al.*[6] reported the occurrence of bacteria in hailstones in 2011 and postulated on their possible role in the hailstone formation as well as in bioprecipitation. Here we report two bacterial species obtained from hailstones that show a marked ability to catalyse crystallization of supercooled water.

Four hailstones around 3 cm in diameter were collected during a thunderstorm in March 2011 on the campus of Yashwantrao Chavan College of Science, Karad, Maharashtra, India, in a sterile polypropylene beaker of 500 ml capacity. The largest hailstone having three layers was allowed to melt layer by layer in a sterile watch glass under aseptic conditions in a horizontal Laminar Air Flow Workbench (Yorco Sales Pvt Ltd, Model No. HL-42) at ambient temperature. The melted water of each layer was transferred to sterile tubes by means of sterile pipettes and streaked onto sterile nutrient agar plates that were incubated at 28°C till bacterial colonies appeared (around 48–72 h). The isolates were analysed for their phenotypic characters and their partial 16s rRNA sequences were determined using universal Eubacteria-specific primers[7]. They were analysed for sequence matching using the web-based SeqMatch of RDP-II and submitted to the database of DDBJ (available in DDBJ/EMBL/GenBank under accession numbers AB716761 and AB716762). Their evolutionary history was inferred by the neighbour joining method using MEGA 4 (refs 8 and 9).

The ice nucleation (IN) potential was studied by the 'tube nucleation test' method described by Hirano and Baker[10]. Glass test tubes of 18 × 150 mm size

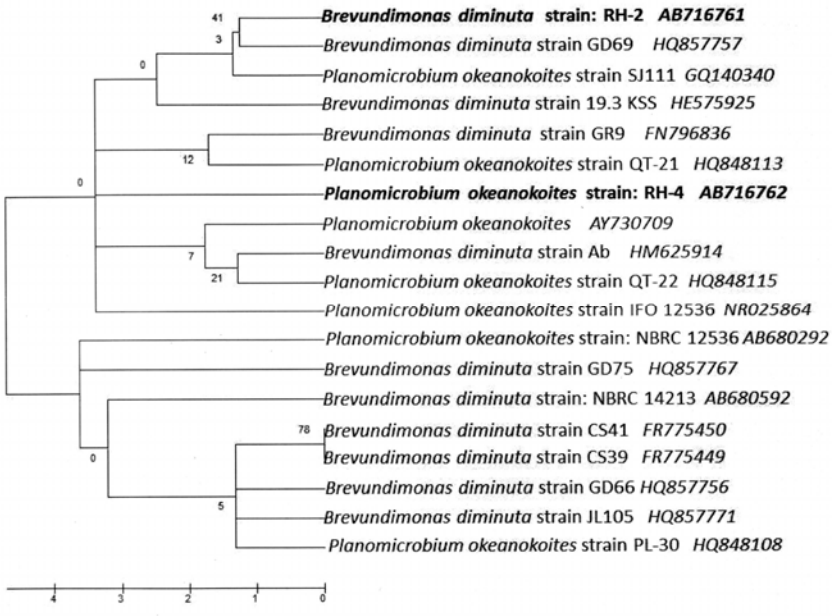

Figure 1. Neighbour joining tree of isolates RH-2 and RH-4 using bootstrap method.