The involvement of microsomal oxidases in pyrethroid resistance in *Helicoverpa armigera* from Asia

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Helicoverpa armigera (Hübner)

-------One of the most important agricultural pests in Asia, Africa, Australia and southern Europe.

-------This pest has developed serious resistance to many classes of insecticides, especially to pyrethroids.

Mechanisms of resistance to pyrethroid in *H. armigera*

- Delayed penetration
- Nerve insensitivity
- Metabolic detoxification

Metabolic mechanisms of pyrethroid resistance in *H. armigera*

------- a degree of contradiction

(A.R. McCaffery,1998)

------- the subject of controversy

(J.G. Scott, 1999)

- Most evidence from the published papers that implicates the role of P450s in pyrethroid resistance in *H. armigera* is circumstantial and indirect.
- In the present study, we confirmed the role of P450s in pyrethroid resistance in Asian *H. armigera* populations from China, India and Pakistan by:

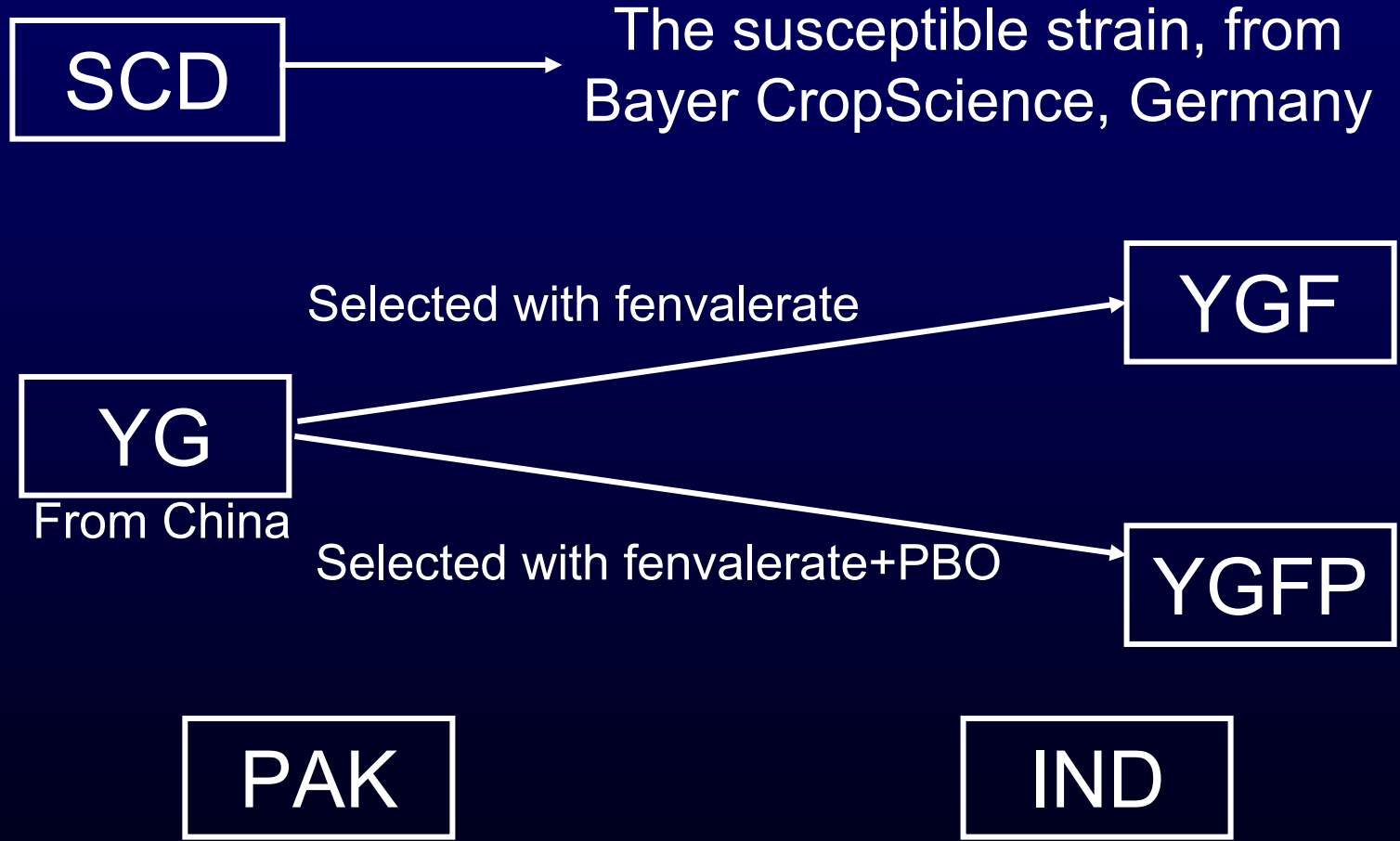
    1. Synergist bioassay

    2. Metabolic enzyme assay

    3. Pyrethroid metabolic study

    4. P450 gene sequencing

Insect strains used in the study



1. Synergist bioassay

Topical toxicity of pyrethroid alone and with synergist against *H. armigera* (1)

Strain	Test	RR [a]	SR [b]
SCD	Fenvalerate	1	
	Fenvalerate+PBO	1.3	0.8
	Cypermethrin	1	
	Deltamethrin	1	
IND [c]	Cypermethrin	11200	
	Cypermethrin+PBO	4	2700
PAK	Fenvalerate	2320	
	Fenvalerate+PBO	5	450
	Cypermethrin	4100	
	Cypermethrin+PBO	4	950

a RR (resistance ratio) = LD_{50} of resistant strain ÷ LD_{50} of SCD strain

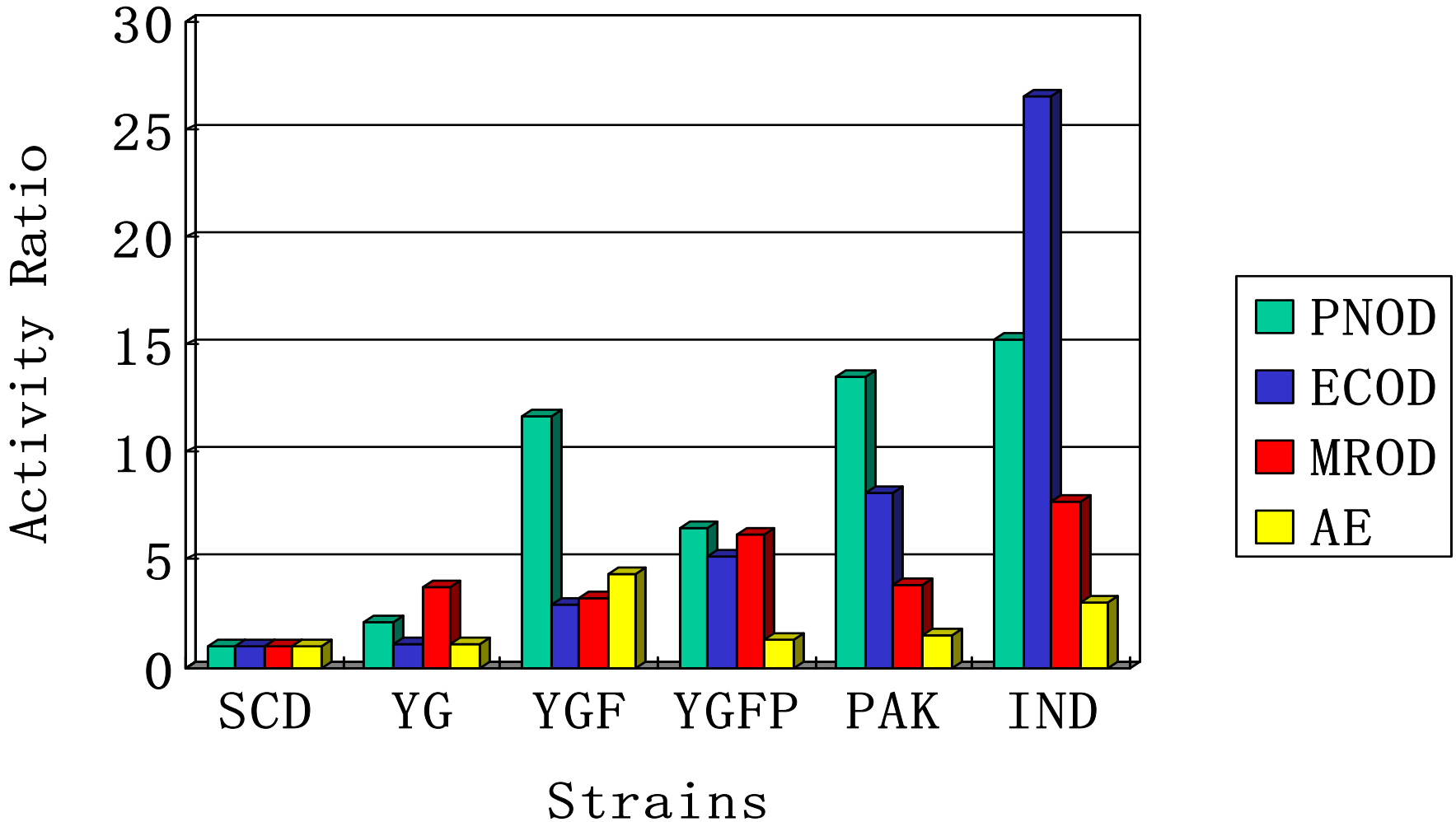
b SR (synergism ratio) = LD_{50} of insecticide alone ÷ LD_{50} of insecticide+PBO

c Data was kindly supplied by Dr Keshav Kranthi, Central Institute for cotton Research, Nagpur, India

Topical toxicity of pyrethroid alone and with synergist against *H. armigera* (2)

Strain	Test	RR [a]	SR [b]
YGF	Fenvalerate	1690	
	Fenvalerate+PBO	4	462
	Fenvalerate+DEF	400	4
	Cypermethrin	540	
	Cypermethrin+PBO	10	54
	Cypermethrin+DEF	190	3
YGFP	Fenvalerate	2510	
	Fenvalerate+PBO	211	12
	Fenvalerate+DEF	226	11
	Cypermethrin	2920	
	Cypermethrin+PBO	63	46
	Cypermethrin+DEF	1330	2
YG	Fenvalerate	7	
	Fenvalerate+PBO	1.5	5
	Cypermethrin	14	

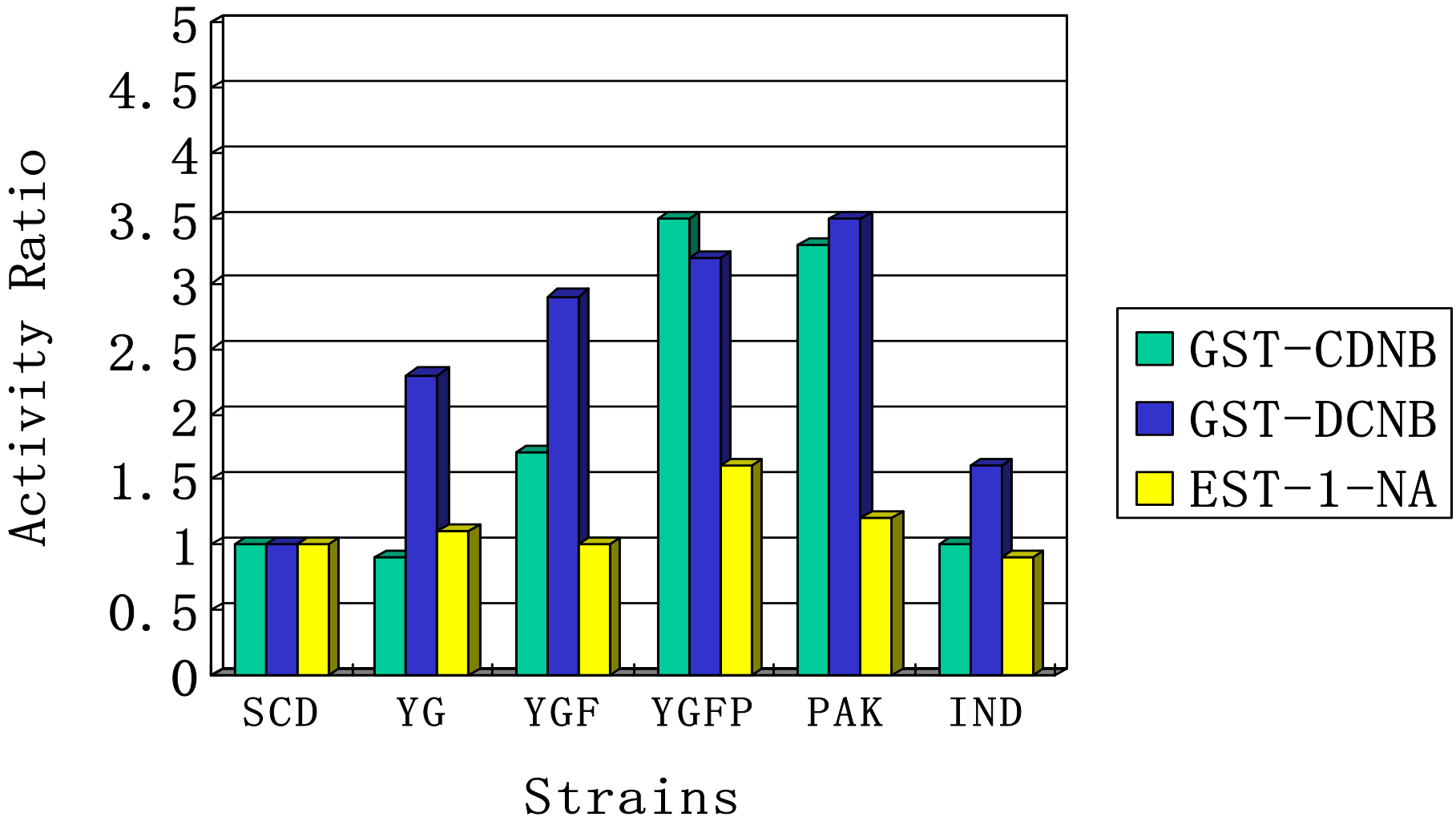
2. Metabolic enzyme assay



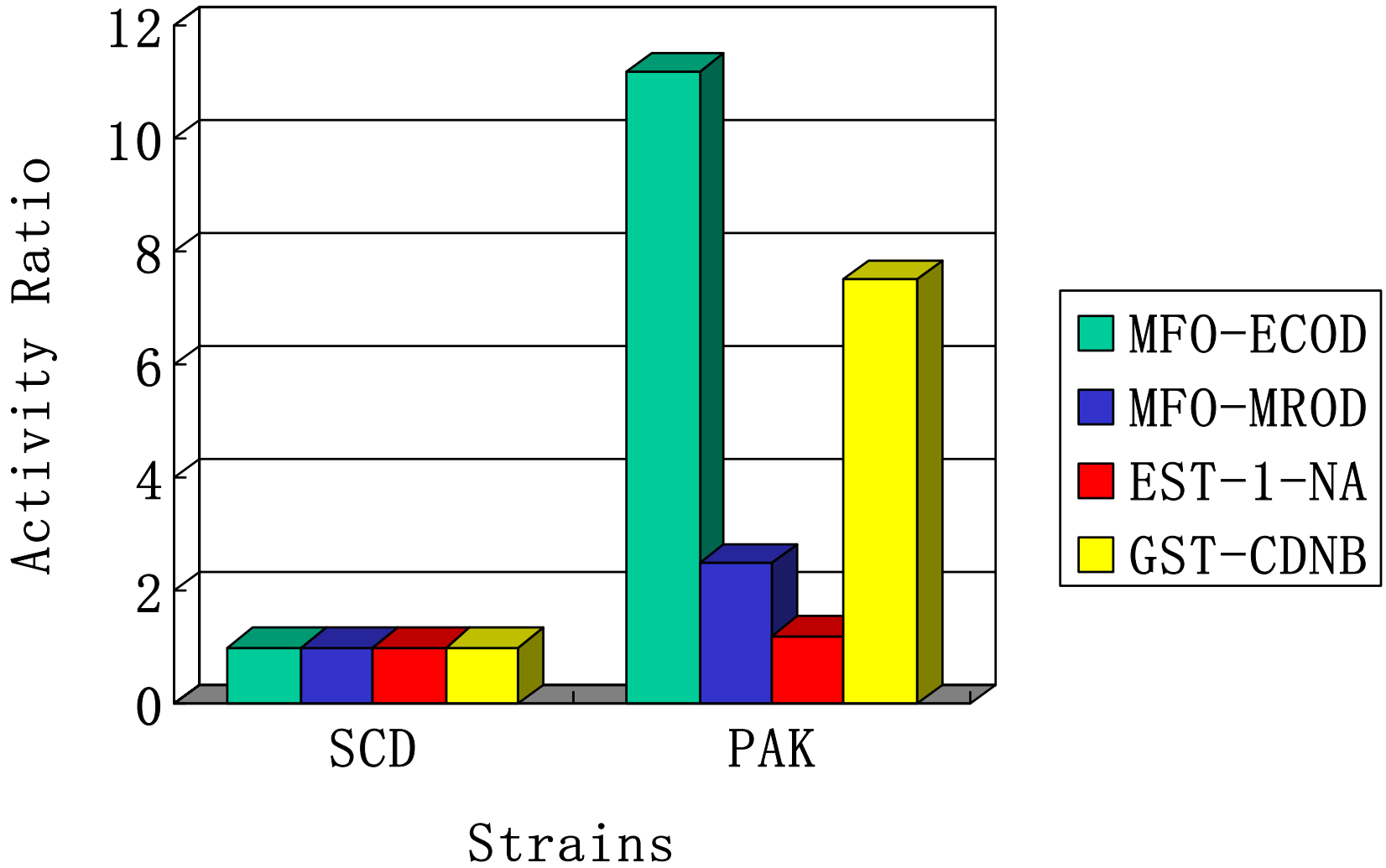
Cytochrome P450 activities of midgut toward different substrates

PNOD: *p*-nitroanisole O-demethylation; ECOD: ethoxycoumarin O-deethylation;

MROD: methoxyresorufin O-demethylation; AE: aldrin epoxidation

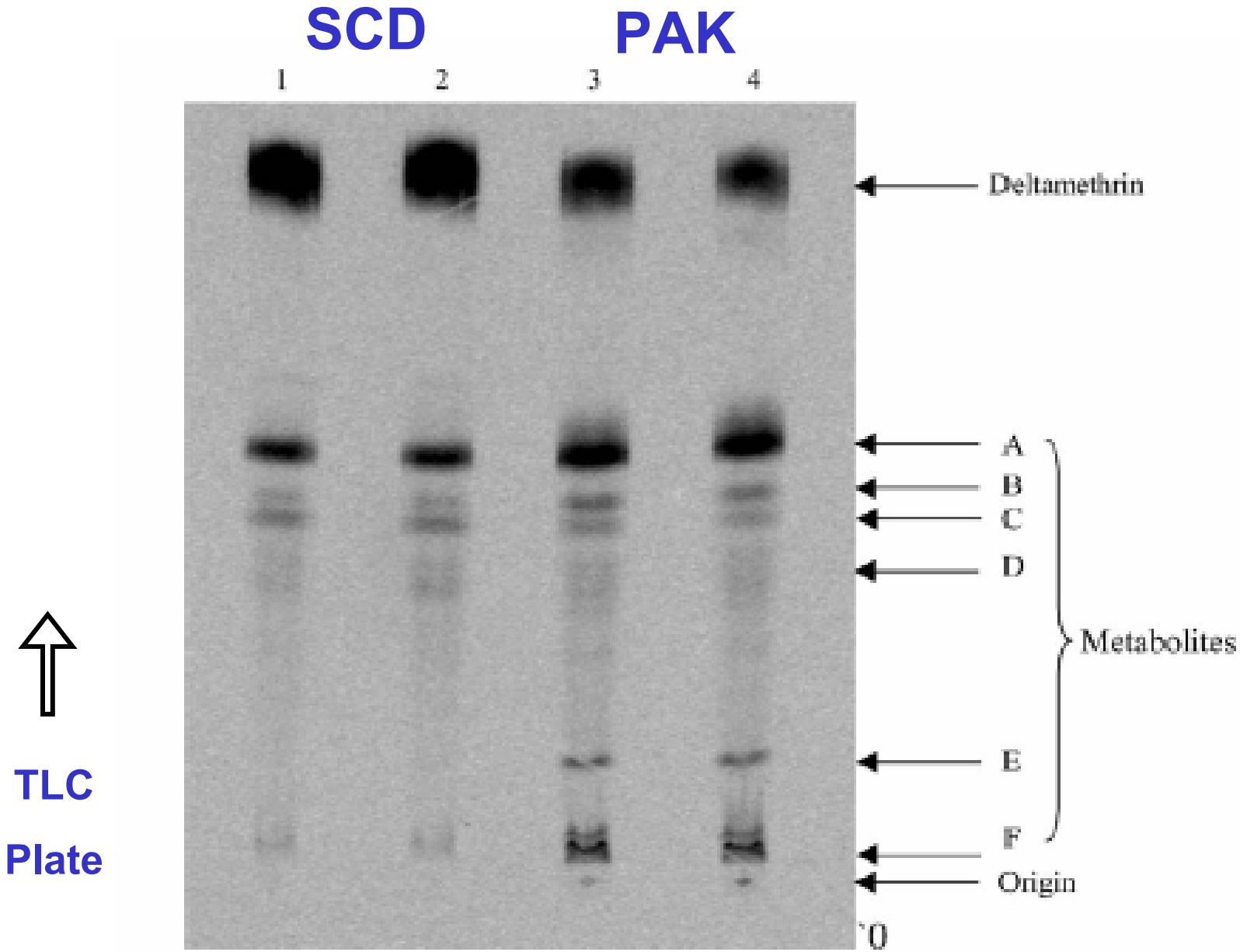


Glutathione S-transferase and esterase activities of midgut



Metabolic enzyme activities of microsomes of midguts in *H. armigera*

3. Pyrethroid metabolic study



Phospher image of ^{14}C-deltamethrin metabolism by microsomes

In Vitro Metabolism of ^{14}C-deltamethrin by microsomes of midguts of *Helicoverpa armigera*

Strain	NADPH	Via	H_2O fraction	Organosoluble fraction	
				Metabolites	Deltamethrin
PAK	+	12%	16.5%	31.4%	15.7%
	-	7.6%	12.4%	7.6%	48.6%
SCD	+	8.1%	10.4%	16.1%	41.3%
	-	7.3%	5%	9.8%	54.5%
Control	+	11%	0.8%	1.6%	63.7%

4. P450 gene sequencing

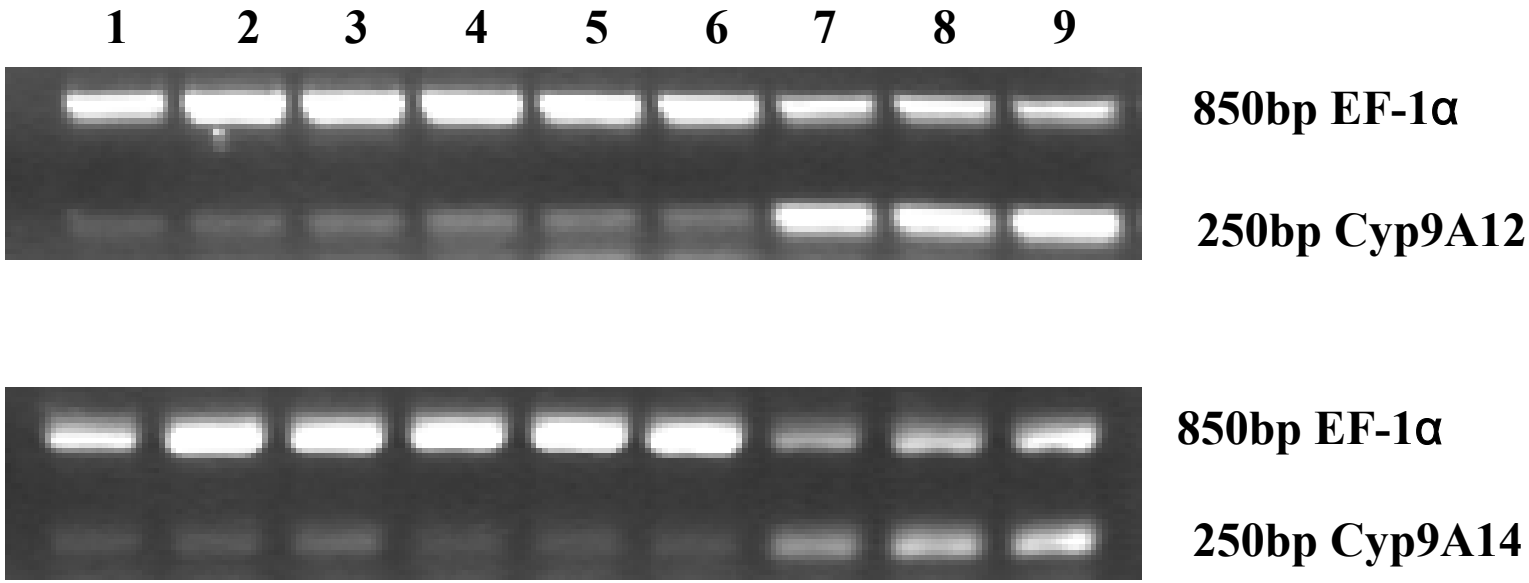
- **Sequencing and functional expression of cytochrome P450 genes associated with pyrethroid resistance in *H. armigera***

- ***Two new P450 genes were cloned.***

  Cyp9A12 (GenBank No. AY371318)

  Cyp9A14 (GenBank No. AY487948)

Semi-quantitative PCR



Lane 1-3: YG control strain (No selection)

Lane 4-6: German susceptible strain

Lane 7-9: YG-Fen strain (selected with fenvalerate)

EF1-α : elongation factor gene as control

Cyp9A12 and Cyp9A14

Functional expressing is ongoing.

(Yeast expression system)

Expressed P450 proteins have MROD activities.

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