Treatment of *Wolbachia pipientis* Infection with Tetracycline Hydrochloride and the Change of Cytoplasmic Incompatibility in a Strain of *Culex pipiens quinquefasciatus* from Bangkok, Thailand

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Abstract: A wild type strain of *Culex pipiens quinquefasciatus,* which was originated from Bangkok, Thailand and maintained at an insectarium of 25℃ and 70% relative humidity, was cured by exposing the first instar larvae to 10%, 20%, and 20－10% water solutions of tetracycline hydrochloride for 24 hrs befor food was added. Two lines of the strain have been cured of the *Wolbachia* infection when it was treated by 20% solution, and when treated continuously by 20% and 10% solutions respectively. Although treated and aposymbiotic (or *Wolbachia* free) females produced viable progeny when they mated with aposymbiotic males, the females produced no progeny when they back crossed with the original males with symbiotes. The newly established aposymbiotic strain has been maintained for over 5 generations.

Key words: *Wolbachia pipientis,* Cytoplasmic incompatibility, *Culex pipiens quinquefasciatus,* Tetracycline hydrochloride

INTRODUCTION

In relation to the biological control of *Culex pipiens* complex, cytoplasmic incompatibility of this mosquito group has been studied by several workers and the results of the works showed that the crosses between members of the group from different geographical origins may be compatible, partially compatible, or incompatible in one direction or both directions (Laven, 1951; Barr, 1966; Sasa *et al.*, 1966; Subbarao *et al.*, 1977; Suenaga, 1982b). Laven (1957, 1967) showed that the factors causing incompatibility were maternally transmitted through the cytoplasm. Although the Expert Committee of the World Health Organization (WHO, 1964) has suggested that cytoplasmic incompatibility could be used to produce sterile males for eradication of *Cx. pipiens* by the steril male technique (Knipling, 1955), there has been no encouraging results in the field (Krishnamurthy *et al.*, 1962; Barr, 1970; Subbarao *et al.*, 1974). Yen and Barr (1971) proposed a new theory that one of the cytoplasmic factors responsible

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for incompatibility might be the presence of a rickettsia-like microorganism, *Wolbachia pipientis,* in the reproductive organs of the mosquitoes, and this hypothesis was confirmed by Yen and Barr (1973, 1974), Fine *et al.* (1977), and Suenaga (1982a). This paper presents results of curing *Wolbachia* infection in a Bangkok strain of *Cx. pipiens quinquefasciatus* by using tetracycline hydrochloride {Achromycin® V Capsules 50 mg, Lederle (Japan), LTD.}.

Materials and Methods

A wild type strain of *Cx. pipiens quinquefasciatus* which was originated from Bangkok, Thailand in 1984 and maintained at an insectarium of 25℃ and 70% relative humidity was used for the experiments.

As a pilot test, the first instar mosquito larvae which hatched within 12 hrs at 25℃ were treated by 2.5% and 5.0% tetracycline hydrochloride (TC) solutions for 24 hrs in the insectarium. The results of the test showed no effect on mosquitoes and wolbachiae. Therefore, in the following experiments, the larvae were treated by 10% and 20% solutions, and treated by 20% and then 10% solutions for 24 hrs, respectivery. After the treatment, the survived larvae were put into a polyethylene pan containing tap water and a piece of mouse-feed pellet as food, and aeration was started. Food was suitably added 3 or 4 days later. The pan was covered with plastic plate till the larvae become pupae. Pupae were transferred into a 90 ml plastic cup containing tap water in a rearing cage of 20×20×30 cm, and a cube sugar on a plastic plate was inserted in the cage as adult food. Some of the newly emerged female mosquitoes were examined for wolbachiae. About one week after their emergence, a mouse for blood feeding was exposed to mosquitoes for one night in the rearing cage. Engorged female mosquitoes laid egg rafts about 2 days after feeding, and hatched larvae were reared by the usual procedure to get the next generation.

Crossing experiments were conducted at the 2nd and the 5th generations after TC treatment. Two colonies, TC treated and original, of the strain were reared at the same time from early stage larvae. Pupae of each colony were separated to males and females individually under a stereomicroscope, and 50 females and 50 males of the 2 colonies were crossed and back crossed each other in a rearing cage. Some females of each colony were examined for wolbachiae in their ovaries by using Giemsa staining technique (Wright and Wang, 1980). About one week after their emergence, a mouse was exposed to the mosquitoes in the cage for blood feeding. Engorged females laid egg rafts on the surface of tap water in the cage a few days after feeding, and each egg raft was examined for developing or hatching conditions of eggs 2 or 3 days after oviposition under the stereomicroscope.

RESULTS AND DISCUSSION

Effects of TC solution on the mosquitoes and wolbachiae are shown in Table 1. When the mosquito larvae were treated by 10% TC solution, their survival rate to the pupal stage was 15.0%, and survived mosquitoes still had some wolbachiae in their ovaries. However, in the cases of 2 colonies treated by 20% and 20−10% TC solutions, 10.8% and 11.8% survived, respectively, and no wolbachiae were found in the mosquitoes of these 2 colonies.

The results of crossing experiments between the 2nd generation of aposymbiotic or *Wolbachia* free strain (KTap F2) and the 13th generation of original strain (KTF13) are shown in Table 2. Although the aposymbiotic females produced viable progeny when mated with treated males, the females produced no progeny when back crossed with the males of original strain with symbiotes. In contrast to aposymbiotic females, the females of original strain with wolbachiae produced viable progeny when crossed with both of the males of the same strain and the aposymbiotic strain.

Table 3 shows the results of crossing experiments between the 5th generation of aposymbiotic strain (KTapF5) and the 16th generation of original strain (KTF16). It is clear from this Table that among 4 crossing combinations, though each strain was compatible itself and also the females of original strain were compatible with the aposymbiotic males, only

Table 1. Effects of tetracycline hydrochloride solution on a Bangkok strain of *Culex pipiens quinquefasciatus* and *Wolbachia pipientis* at 25℃

Strain	Concentration of TC* (%)	Hours for treatment	No. of larvae		Survival rate (%)	Effect on *Wolbachia*
			treated	survived		
KTF6	10.0	24	5,000	750	15.0	No effect
KTF11	20.0	24	5,000	540	10.8	*Wolbachia* eliminated
KTF11	20.0 10.0	24 24	5,000	590	11.8	*Wolbachia* eliminated

*TC: tetracycline hydrochloride.

Table 2. Results of crossing tests in the 2nd generation of a Bangkok strain of *Culex pipiens quinquefasciatus* after tetracycline hydrochloride treatment

Cross (F × M)	No. of egg rafts examined	No. of eggs examined	% of eggs		
			hatched	developed	undeveloped
KTF13 × KTF13	10	1,508	99.1	0.4	0.5
KTF13 × KTapF2	10	1,383	99.2	0.2	0.6
KTapF2 × KTF13*	10	1,696	0.0	0.4	99.6
KTapF2 × KTapF2	10	1,487	99.0	0.5	0.5

ap: Aposymbiotic strain. *Incompatible cross.

Table 3. Results of crossing tests in the 5th generation of a Bangkok strain of *Culex pipiens quinquefasciatus* after tetracycline hydrochloride treatment

Cross (F × M)	No. of egg rafts examined	No. of eggs examined	% of eggs		
			hatched	developed	undeveloped
KTF16 × KTF16	10	1,925	98.7	0.4	0.9
KTF16 × KTapF5	10	1,872	99.0	0.4	0.6
KTapF5 × KTF16*	10	1,814	0.0	0.1	99.9
KTapF5 × KTapF5	10	1,855	97.9	0.5	1.6

ap: Aposymbiotic strain. *Incompatible cross.

aposymbiotic females produced no progeny when they back crossed with the males of original strain, and the results were almost the same as those of the former experiments with KTapF2.

The results of these two experiments indicate that the crossing type of the original strain with wolbachiae have changed after cured of the microorganism infection and the TC treatment rendered them aposymbiotic. All these results are fully consistent with those obtained by Yen and Barr (1973), Fine *et al.* (1977), and Suenaga (1982a). However, the successful concentrations of TC solution for curing *Wolbachia* infection in mosquitoes were much higher than that of the previous reports.

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