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YEAST-GENERATED CO_2 AS A CONVENIENT SOURCE OF CARBON DIOXIDE FOR ADULT MOSQUITO SAMPLING

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ABSTRACT. A new, convenient method was developed to supply CO_2 for mosquito sampling by using yeast, which converts sugar into CO_2 and ethyl alcohol. The system could, at average, generate 32.4 ml/min of CO_2 for at least 27 h. The total weight of the CO_2 generated was estimated to be 94 g. The efficacy of yeast-generated CO_2 as attractant for mosquitoes was significant, and the following 6 mosquito species were collected using yeast-generated CO_2 traps from July to September 2003 in a residential area of southern and northern Yokohama City, Japan: *Aedes albopictus* (Skuse), *Armigeres subalbatus* (Coquillett), *Culex halifaxii* Theobald, *Cx. pipiens pallens* Coquillett, *Ochlerotatus japonicus* (Theobald), and *Tripteroides bambusa* (Yamada). Besides mosquitoes, various other insects were collected in the trap. Species compositions of insects collected in yeast-generated CO_2 traps and dry-ice-baited traps were compared.

KEY WORDS CO_2, yeast, attractant, yeast-generated CO_2

INTRODUCTION

Carbon dioxide is a mosquito attractant (Gillies 1980, Clements 1999) and has been used in various traps (Service 1993). In most of the previous studies, dry ice has been used as a source of CO_2. As an alternative CO_2 source, Hoy (1970) used CO_2 and CO fumes generated by an engine adapted to operate on liquid propane gas, and recently, some commercially available traps using that system have been developed (Burkett et al. 2001). However, CO_2 cylinders or generators are heavy and expensive, and thus have limitations, especially when trying to cover a wide area for mosquito surveillance. Dry ice is cheap and light, but in certain areas, like tropical countries, it is sometimes difficult to obtain.

We developed an alternative convenient method to supply CO_2 by using yeast, which converts sugar into CO_2 and ethyl alcohol. The idea of yeast-generated CO_2 as a source of carbon dioxide was first used in aquatic plant cultivation (Narten 1994). In aquatic plant cultivation, the length of the CO_2 supply period is most important, while for mosquito sampling, amount of CO_2 as well as the length of supply period are relevant. We conducted laboratory experiments to find a cheap and convenient method that would produce enough CO_2 for a long enough time to be used for mosquito collection. The efficacy of yeast-generated CO_2 as an attractant for mosquitoes was evaluated in field collections.

MATERIALS AND METHODS

Carbon dioxide production by yeast: Figure 1 is a schematic picture of the yeast-generated CO_2 trap. Two plastic bottles (2-liter volume) were used to hold water solutions of sugar and yeast. The 2 bottles contained different concentrations of dry yeast and sugar: bottle A, 150 g of sugar + 12 g of dry yeast, and water added to total a volume of 1,500 ml; in bottle B, 100 g of sugar + 6 g of dry yeast, and water added to total a volume of 1,750 ml. Because bottle A contains a larger amount of dry yeast, the output rate of CO_2 is higher and the length of supply period is shorter than in bottle B. By using the 2-bottle system, we could achieve the high output rate of CO_2 as well as the long supply period. The bottles were connected to each other with polypropylene tubing and to a small (500-ml-volume) plastic bottle holding the overflowed water solution. Generated CO_2 was released from a 5-mm hole on the outer wall of the small bottle. For easy preparation of the water solution as well as cleaning, there were 3 joints in the connection tubing (Fig. 1). The small bottle was hung close to the opening of a suction trap, similar in design to the CDC-light trap (Service 1993). It was made of 14-cm-long acrylic tubing with an inside diameter of 8.5 cm attached to a 3.0-V motor driving a three-bladed plastic fan powered by four 1.5-V dry batteries.

Measurement of yeast-generated CO_2: The amount of CO_2 gas generated by the dry yeast was measured in the laboratory. The CO_2 gas released from the connection tube was accumulated into a bottle filled with water and the volume of CO_2 gas was measured every 3 h for 28.5 h. Because it took about 1–1.5 h to stabilize the output rate of CO_2 gas from the bottles, the measurement started 1.5 h after the initiation of the experiment. The experiment was replicated 5 times. Temperature condition during the experiment ranged between 25 and 27°C.

Field evaluation of efficacy of yeast-generated CO_2 in mosquito collections: Mosquito collections were conducted from July to September 2003 in residential areas of southern and northern Yokohama City, Japan. The efficacy of yeast-generated

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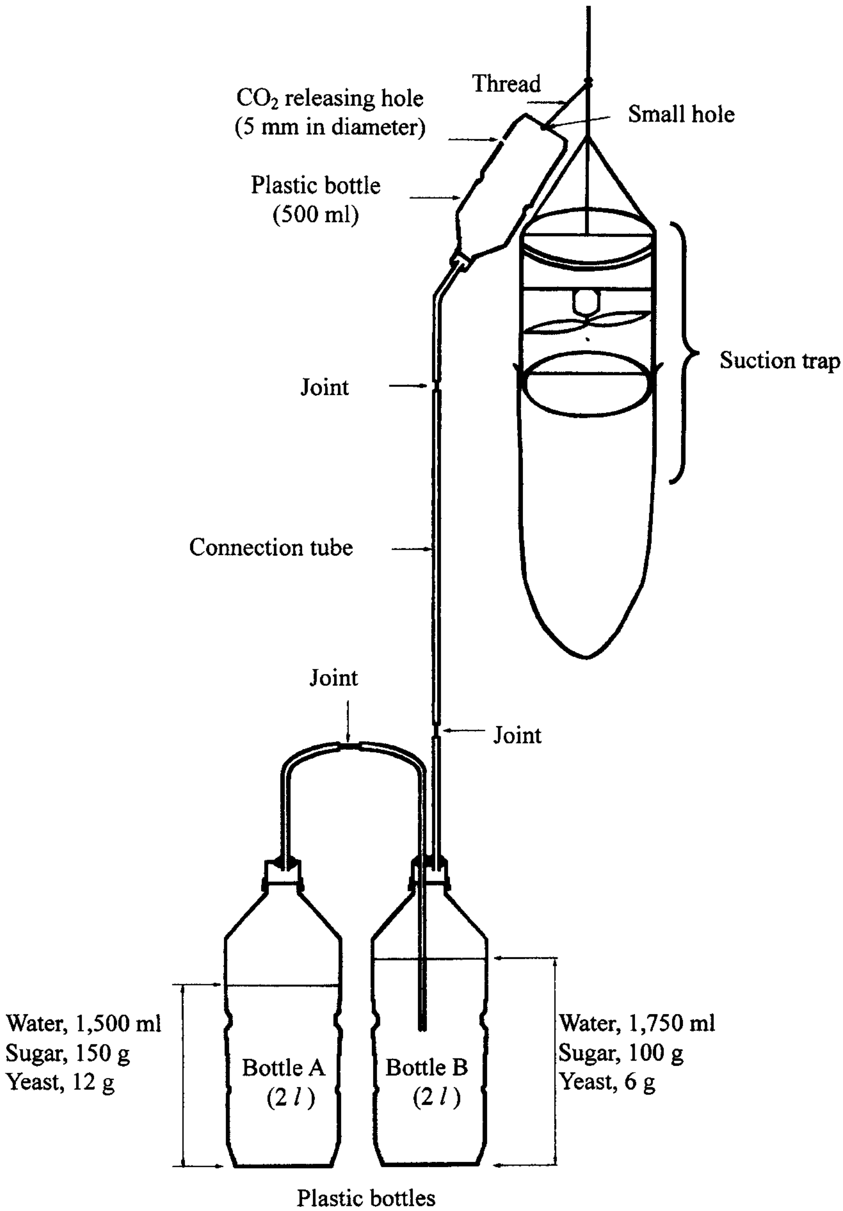

Fig. 1. Yeast-generated CO_2 trap. Carbon dioxide gas was generated inside plastic bottles A and B, with different concentrations of dry yeast and sugar, and released from the top of a suction trap through connection tubing and a small plastic bottle, which holds the overflowed water solution.

CO_2 was evaluated by 1) a comparison between suction-trap collections with and without yeast-generated CO_2 and 2) a comparison of trap collection between yeast-generated CO_2 trap and a dry-ice-baited (1 kg) trap. The first experiment was conducted 5 times in southern Yokohama City in August 2003. Two suction traps were operated for 24 h. The traps were placed 1.7 m apart, one of them enhanced with yeast-generated CO_2 and the other without CO_2. Mosquitoes collected were counted and species compared. The second experiment was conducted 4 times in northern Yokohama City, August–September 2003. The yeast-generated CO_2 trap and a dry-ice-baited trap were placed 4–5 m apart and operated for 24 h. The dry ice was wrapped with paper and placed in a styrofoam-box. A piece of dry ice always remained in the box after 24 h of collection. Collected insects were killed and counted, mosquitoes were identified to species, and other dipterans were identified to family.

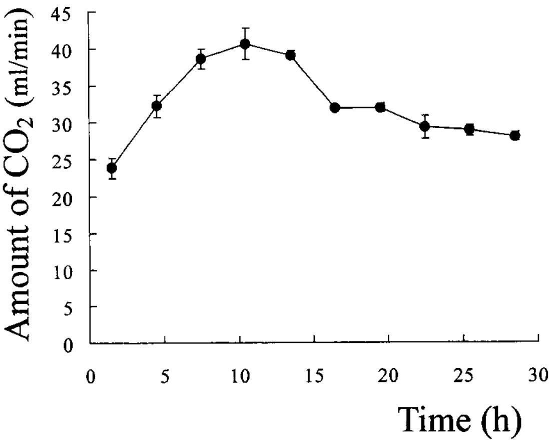

Fig. 2. Temporal changes in amount of CO_2 (mean ± standard deviation [SD]) generated by yeast under temperature conditions of 25–27°C.

RESULTS AND DISCUSSION

The amount of CO_2 generated by the yeast increased during the first 10.5 h, reached a maximum output rate of 40.6 ± 2.1 ml/min, gradually decreasing (Fig. 2). At the end of the 28.5-h experiment, the output rate of CO_2 was 28.0 ± 0.6 ml/min. The mean output rate of CO_2 during the experiment (27 h) was 32.4 ml/min, nearly equal to the 30–40 ml/min CO_2 output rate of a chicken (Clements 1999). The total amount of CO_2 generated during the experimental period was about 52 liters. Assuming 1 atm and a mean temperature of 26°C during the experiment, the estimated weight of CO_2 generated was 94 g. In this study, tap water was used in the experiments. Preliminary observations showed that water collected from a pond (chemical oxygen demand = 4 ppm, pH = 7.3) and a river (COD = 2 ppm, pH = 6.6) could be used instead of tap water.

The collection was repeated 5 times and the number of mosquitoes collected in yeast-generated CO_2 traps was always larger (mean number = 18.0) than the number collected in traps without CO_2 (mean number = 1.0). Therefore, the efficacy of yeast-generated CO_2 as an attractant for mosquitoes was significant (sign test, $P = 0.031$).

The following 6 mosquito species were collected in yeast-generated CO_2 traps (Table 1): *Aedes albopictus, Armigeres subalbatus, Culex halifaxii, Cx. pipiens pallens, Ochlerotatus japonicus,* and *Tripteroides bambusa.* Both diurnal as well as nocturnal species were collected (Tanaka et al. 1979). The dominant species was *Cx. pipiens pallens* (253 ♀) followed by *Ae. albopictus* (56 ♀).

Besides these mosquitoes, various other insects were collected in the traps. The species composition of insects collected in yeast-generated CO_2 traps and dry-ice-baited traps is summarized in Table 2. The dominant mosquito species were the same in both yeast-generated CO_2 traps and dry-ice-baited traps: *Cx. pipiens pallens* and *Ae. albopictus.* The number of mosquitoes collected in yeast-generated CO_2 traps was smaller than in dry-ice-baited traps: 63 versus 103 *Cx. pipiens pallens* and 13 versus 24 of *Ae. albopictus* in yeast-generated CO_2 traps versus dry-ice-baited traps. Because the average output rate of CO_2 from 1 kg of dry ice was calculated as 387 ml/min, 12 times more than from yeast-generated CO_2, the difference in mosquito numbers may be largely ascribed to the difference in output rate between the yeast method and dry ice. Some differences in species composition were found, especially in the orders of Lepidoptera and Thysanoptera, between yeast-generated CO_2 traps and dry-ice-baited traps. Lorenzo et al. (1998) found that *Triatoma infestans* can be captured by yeast-baited traps. Because yeast converts sugar into CO_2 and ethyl alcohol, a certain amount of ethyl alcohol gas may also be released. Additional comparative experiments will be required to clarify the effects of the CO_2 and ethyl alcohol mixture on the species composition of the insects collected.

In this study, we used 2 plastic bottles (A and B) to hold the yeast and sugar solutions. For field surveys, it may be more convenient to use only 1 bottle. We conducted an additional experiment to compare the CO_2 output rate of the 2-bottle system with a 1-bottle system, in which the same amount of sugar (250 g) and dry yeast (18 g) as in the 2-bottle system was now kept in one 4-liter bottle. Three different amounts of water, 2.5 liter, 2.7 liter, and 2.9 liter, were used in the 1-bottle system and the mean CO_2 output rates during the first 7–24 h were calculated to be 33.6, 32.3, and 30.0 ml/min, respectively. There were no significant differences in CO_2 output rate between the 2-bottle system and the 1-bottle system (ANOVA, $F = 2.44$, $P = 0.26$). Therefore, the 1-bottle system can be expected to be as effective in attracting mosquitoes as the 2-bottle system at least for the first 24 h.

The amount of CO_2 generated by yeast depends on temperature, and thus, is affected greatly by seasonal changes, especially in temperate areas. To

Table 1. List of mosquito species collected by a suction trap enhanced with yeast-generated CO_2 from 19 July to 19 August 2003, in southern Yokohama City, Japan.[1]

Species	Female	Male
Aedes albopictus	56	7
Armigeres subalbatus	4	0
Culex halifaxii	1	
Cx. pipiens pallens	253	6
Ochlerotatus japonicus	1	0
Tripteroides bambusa	11	1
Total	326	14

[1] Trap collection was conducted 12 times during the study period.

Table 2. List of insects collected by suction trap enhanced with yeast-generated CO_2 or dry ice (1 kg) in northern Yokohama City, Japan, August–September 2003.[1]

Order	Family	Species	Yeast	Dry ice
Diptera	Culicidae	*Ae. albopictus*	13	24
		Cx. pipiens pallens	63	103
		Cx. bitaeniorhynchus	0	1
	Cecidomyidae		23	12
	Ceratopogonidae		9	4
	Chironomidae		6	3
	Chloropidae		1	0
	Phoridae		0	1
	Psychodidae		20	8
	Sciaridae		6	4
	Tipulidae		6	1
Coleoptera			1	2
Hemiptera			6	10
Hymenoptera			25	11
Lepidoptera			59	0
Psocoptera			4	3
Thysanoptera			0	36
Total			242	223

[1] The trap was operated for 24 h. Trap collection was made 4 times during the study period.

achieve a constant output rate of yeast-generated CO_2 gas throughout the year, a temperature-control system will be necessary in temperate areas. However, in tropical countries, temperature conditions are rather constant, so that our system will work well throughout the year.

Although the effect of CO_2 gas on the number of mosquitoes collected in suction traps is clear, it is usually difficult to obtain gas cylinders or dry ice in tropical areas, where mosquito-borne diseases are serious. The yeast-generated CO_2 trap developed in this study is convenient and cheap, and all the materials necessary are locally available. Our system might be valuable for *Ae. aegypti* surveillance in dengue-epidemic areas and malaria mosquito surveillance.

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