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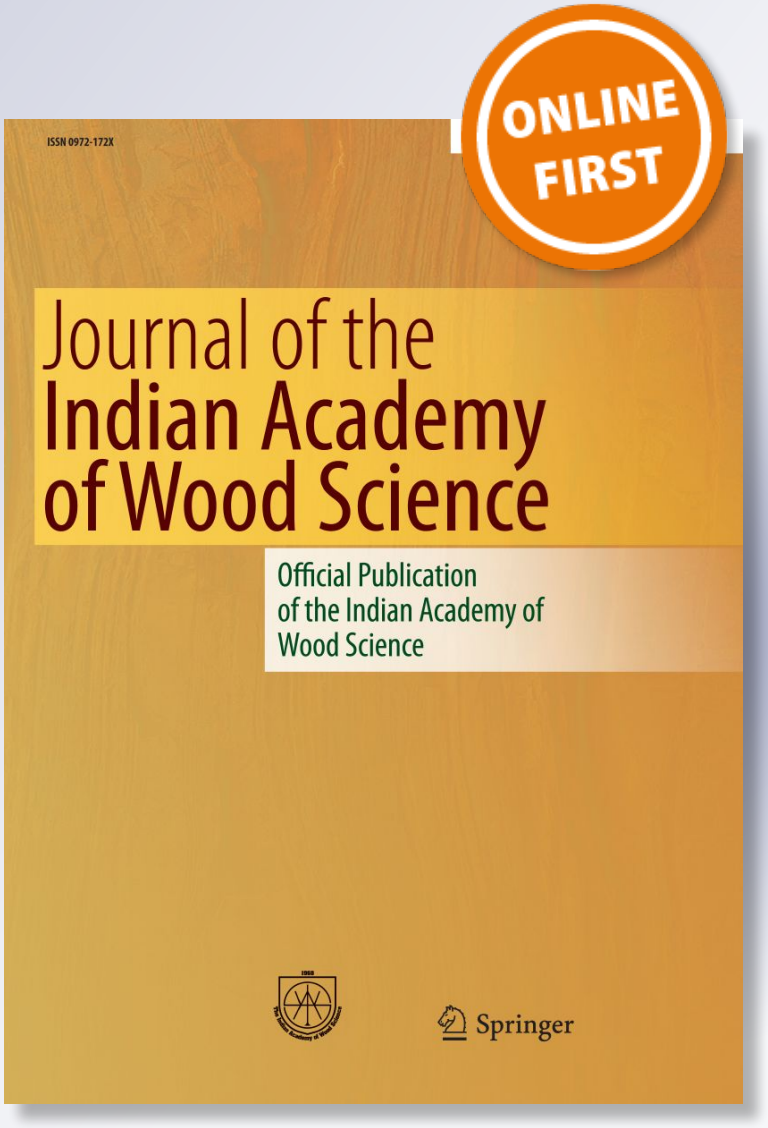

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ORIGINAL ARTICLE

Evaluation of antibacterial susceptibility pattern of cellulolytic bacteria isolated from *Coptotermes curvignathus* gut to heavy metals, disinfectants and common antibiotics for termite control

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Abstract The study aimed to isolate cellulolytic bacteria from the gut of subterranean termite (*Coptotermes acinaciformis*) and carry out antibacterial studies for termite control. The cellulase enzyme activity was determined by qualitative and quantitative techniques. The antibiotics and their combinations on isolated bacteria as well as heavy metals and disinfectants were performed by using disc diffusion method. The effective antibacterial agents were used as termiticide. Antibacterial study revealed that the isolates were 100% sensitive to rifampicin, tetracycline, gentamycin and neomycin antibiotics, cadmium and mercury as heavy metals and lactic acid, formalin and hydrogen peroxide as disinfectants. In addition, 17 out of 36 antibiotic combinations showed synergistic effect, while eight combinations showed antagonistic effect on isolates. The heavy metals and disinfectants that showed 100% effectiveness as well as 22 antibiotic combinations that showed synergistic effect were used for termite control. Among the 27 selected antibacterial agents, mercury, lactic acid, formalin and hydrogen peroxide were found to be the most effective termiticide. Therefore, we conclude that these effective antibacterial agents possess a great potential to be a new application to control the termites.

Keywords Termiticides · Antibacterial · *Coptotermes acinaciformis* · Disinfectant

Introduction

Termites possess unique evolutionary adaptions and interacting biological features such as eusocial lifestyles, feeding habits, symbiosis and pest status. Termites have received great attention from basic and applied science especially from termite-targeted biotechnology for pest control (Scharf 2015). Termites are group of eusocial insects classified at the taxonomic rank of the order *Isoptera*. Termites normally live in a large colony and their diet depends solely on plant materials, mostly dead wood (Verma et al. 2009). They play an important role in terrestrial ecosystem by turnover and mineralization of complex biopolymers, such as wood and other cellulose or hemicellulose containing materials (Wenzel et al. 2002; Upadhyaya et al. 2012). It has been reported that termites have ability to assimilate 74–99% of cellulose and 65–87% of hemicellulose constituents of their digested lignocellulose (Ohkuma 2003). The capacity of termites to breakdown cellulose relies on coalition of gut cellulolytic microflora or on mutual symbionts. In nature, this special symbiotic association is only found in termites and wood-feeding cockroaches of the genes *Cryptocercus* (Yamin 1979). The cellulolytic symbionts play an important role in both lignocellulose or cellulose digestion and termite nutrient (Matsui et al. 2009). The presence of cellulolytic

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symbionts in the gut of termites is responsible for their cellulosic feed digestion (Saxena et al. 1993; Dillon and Dillon 2004). Cellulolytic bacteria from the termite's gut have been frequently isolated and studied. Aerobic and facultatively anaerobic cellulolytic bacteria have been isolated from the gut of the termite *Zootermopsis angusticollis* (Wenzel et al. 2002). The cellulolytic bacteria are reported in the termite gut of *Coptotermes formosanus* including *Acinetobacter*, *Bacteroides thetaiotaomicron*, *Escherichia coli*, and *Caulobacter* (Mathew et al. 2011). However, the impacts of termite gut symbionts is little known except the knowledge of their role in cellulose digestion and host nutrition (Rosengaus et al. 2011). Termites are pests that invade urban, agricultural and forest environment worldwide including Malaysia. They are economically important owing to the damage they cause to wood, wood products, building materials, agricultural products and forests (Edwards and Mill 1986; Logan et al. 1990). Estimated cost of termite control and damage-repair is around 8–10 million US dollars (Lee 2002). Effective termite control methods have to be found to solve the problem permanently. The feeding activity can be controlled by antimicrobial agents. With the idea of disrupting the mutual symbiotic association, better management practices may attain without environmental and ecological drawbacks (Rosengaus et al. 2011). In the present study, cellulolytic microorganisms have been isolated from gut of termites and screened for antibacterial activities.

Materials and methods

Collection of termite samples and isolation

Subterranean Termite (*Coptotermes acinaciformis*) were collected from rubber plantation of Bahau, Negeri Sembilan, Malaysia and transferred to laboratory of Universiti Malaysia Pahang (UMP). The collected samples were maintained as described by Matsui et al. (2009). Mature worker termites were used throughout the experiment. Isolation of bacteria from the gut of termites was performed by Husseneder et al. (2009) method with modification under sterile conditions. Termite was sterilized using 70% ethanol. The heads of termites were removed from the abdomen and bodies were crushed with glass rods to form the paste which was cultured onto nutrient agar and incubated at 37 °C for 24 h. Bacterial colonies with different morphological characteristics were purified by using streak plate method. All purified bacterial isolates were subjected to Gram reaction.

Screening of cellulolytic bacteria

In screening stage, the Congo Red agar medium was prepared according to Gupta et al. (2012). The isolated bacteria were streaked onto the agar medium and incubated at 37 °C for 48 h. Bacterial colonies with positive cellulase activity were selected for further studies. About 10 ml of basal media supplemented with 1% carboxymethyl cellulose (CMC) was inoculated with purified bacterial isolates and incubated at 37 °C for 24 h in an incubator shaker at 80 rpm (Ngangi et al. 2013). Then, 10 ml of cultures were centrifuged at 8000 rpm for 10 min. Pellets were discharged while supernatants were collected and stored at 4 °C. About 1% CMC was added into 0.1 M sodium acetate buffer pH 5.0. The prepared solutions were incubated together with 1 ml of the crude enzyme (cell free filtrate CFF) obtained from previous steps for 30 min at 63 °C (Abo-State et al. 2010). The cellulose degrading activity was measured by the resulted reducing sugars using Dinitrosalicylic acid (DNS) reagent (Miller 1959).

Antibacterial activity

Antibiotics sensitivity test

Antibacterial activity on purified cellulolytic bacteria was screened by Disc diffusion method. Purified colonies were inoculated and incubated overnight at 37 °C. The concentration of broth cultures was adjusted to 0.5 McFraland standard. The suspension was swabbed onto Muller Hinton agar (MHA) medium evenly by a sterile cotton swab. Commercialized antimicrobial susceptible test disc was placed gently on the MHA medium. The petri dishes were incubated for 24 h at 37 °C. The inhibition zones were measured and recorded (Matuschek et al. 2014). A negative control disc was included by using distilled water. Antibiotic study was carried out by using different concentrations of commercialized antibiotic discs of Oxoid Ltd., Basingstoke, UK, including Penicillin G (10 µg), Ampicillin (10 µg), Rifampicin (5 µg), Tetracycline (30 µg), Gentamycin (120 µg), Amoxillin (10 µg), Chloramphenicol (10 µg), Neomycin (10 µg) and Streptomycin (10 µg).

Heavy metals sensitivity test

The heavy metals activities test was carried out as mentioned above using different heavy metal's standard solutions with 100 mg/l concentration such as iron (Fe), copper (Cu), aluminum (Al), antimony (Sb), nickel (Ni), lead (Pb), silver (Ag), chromium (Cr), cadmium (Cd) and mercury (Hg). Sterilized standard filter paper discs with 6 mm

diameter were soaked in heavy metal solutions. The filter paper discs were left to dry before applying onto MHA medium.

Disinfectants sensitivity test

Disinfectant activity test was carried out using different concentrations of lactic acid (88.0%), acetic acid (99.80%), formalin (37.0%), hydrogen peroxide (30.0%), 1-propanol (99.50%), crystal violet (0.1%), iodine (0.1 N), mouthwash, dish soap, liquid laundry detergent (LLD), sodium hydroxide (1 N and 0.1 N), benzoic acid (0.1 N) and toothpastes. LLD was prepared according to company suggestion which is 1 cup of LLD for 4.5 l water and also diluted with water in ratio of 1:1. Dish soap and toothpaste was also prepared by diluted with water in ratio of 1:1. Diffusion discs (6 mm diameter) were prepared and soaked into different disinfectant solutions and left to dry before applying onto MHA medium.

Combined action of antibiotics

Antibiotic combinations were studied by double disc diffusion method. Inoculum was first prepared according to Matuschek et al. (2014). A sterile cotton swab was dipped into the suspensions and swabbed evenly on MHA. Two different antibiotic discs were placed on MHA in a distance of 20 mm from each other and placed in refrigerator for about 15 min before incubation for 24 h at 37 °C (Mayer and Nagy 1999).

Antibacterial treatment for termite biocontrol

Heavy metals and disinfectants that showed 100% total bacteria sensitivity as well as antibiotic combinations that showed synergistic effect were used to proceed to the next step. Filter papers were impregnated with these antibacterial agents. The soaked filter papers were fed to five termite workers by placing them in a petri dish with antibacterial agents each. Sterilized distilled water was used as a negative control. Five replicates of each plate were prepared and filter papers were replaced every 2 days. The petri dishes were placed at room temperature with dark condition. Numbers of surviving termites were recorded every day continuously for a week.

Results and discussion

Screening of cellulolytic bacteria

A total of 24 purified bacterial isolates were obtained and the screening for potential cellulose degrading activity was performed as shown in Table 1. Positive results with formation of inhibition zone were observed in all isolates by Congo red assay indicate the cellulolytic bacteria. For CMCase assay, B1 isolate showed highest enzyme activity with 0.906 ± 0.012 IU/ml while B2 isolate can be considered as poor cellulose degrading isolates with lowest degradation activity at 0.124 ± 0.013 IU/ml. Upadhyaya et al. (2012) determined the cellulolytic activity according to the diameter of halo zones. Larger diameter indicates the higher cellulose degradation activity. Gupta et al. (2012) found the highest extracellular activity of 0.196 IU/ml and maximum activity of endoglucanase assay of 0.400 IU/ml.

Antibacterial sensitivity

The sensitivity of 24 isolates to 9 antibiotics was evaluated. Figure 1 shows that isolates have the least percentage of sensitivity (0%) to penicillin G (PCG) and streptomycin (Strep). *Enteric* bacteria isolated from hingut of termite were resistance to penicillin (Adams and Boopathy 2005). PCG belongs to β-lactams antibiotic family. Usually PCG is effective against Gram-positive bacteria rather than Gram-negative bacteria by inhibiting their cell wall synthesis and murine assembly. Streptomycin inhibits the protein synthesis of bacteria (Dosaj et al. 2013). Both Ampicillin (Amp) and amoxillin (AMX) only inhibit the growth of three isolates with 12.50% of sensitivity were same due to their similar mode of action. They belong to same antibiotic family, which is semisynthetic β-lactams and effective against both Gram-positive and Gram-negative bacteria by inhibited steps in cell wall synthesis and murein assembly (Dosaj et al. 2013). Meanwhile, chloramphenicol (CHL) has medium inhibition effects with 79.17% of sensitivities isolates. 100% of isolated bacteria were sensitive to rifampicin (RMP), tetracycline (TCN), gentamycin (CN) and neomycin (Neo). Monserrate et al. (2001) proposed that growth of nitrogen-fixing cellulolytic bacterium isolated from soil was inhibited by TCN, Amp, kanamycin, Neo and CHL. Amp, Neo and CN are able to inhibit the growth of actinomycete strain isolated from gut of *Speculitermes* sp. (Sinma et al. 2011). RMP can cause a permanent reduction of bacteria in the termite gut (Rosengaus et al. 2011) and able to inhibit transcription of bacterial RNA polymerase while TCN and CHL inhibit the protein synthesis in bacterial cell (Dosaj et al. 2013). The effect of antibiotics on isolates showed the following order: RMP = TCN = CN = Neo (100%) > CHL (71.19%) > Amp = AMX (12.50%) > PCG = Strep (0%). The four antibiotics, including RMP, TCN, CN and Neo have absolute suppression effects on bacterial growth. These four antibiotics may have more tendencies to use as a termiticide.

Table 1 Determination of Congo red and CMCase assay for bacterial isolates

No.	Isolate code	Congo red (clear zone)	CMCase activity (IU/ml)	Activity (%)
1	B1	+	0.906 ± 0.004	100
2	B2	+	0.124 ± 0.005	14
3	B3	+	0.331 ± 0.001	36.5
4	B4	+	0.353 ± 0.001	39
5	B5	+	0.714 ± 0.002	79
6	B6	+	0.360 ± 0.004	40
7	B7	+	0.827 ± 0.002	91
8	B8	+	0.135 ± 0.003	15
9	B9	+	0.679 ± 0.004	75
10	B10	+	0.532 ± 0.000	59
11	B11	+	0.177 ± 0.002	19.5
12	B12	+	0.188 ± 0.002	21
13	B13	+	0.138 ± 0.001	15
14	B1A	+	0.183 ± 0.003	20
15	B3A	+	0.343 ± 0.006	38
16	B3B	+	0.224 ± 0.003	25
17	B6A	+	0.868 ± 0.003	96
18	B6B	+	0.379 ± 0.003	42
19	B6C	+	0.362 ± 0.002	40
20	B6D	+	0.406 ± 0.005	45
21	B6E	+	0.824 ± 0.000	91
22	B7A	+	0.827 ± 0.002	91
23	B8A	+	0.477 ± 0.006	53
24	B8B	+	0.441 ± 0.002	49

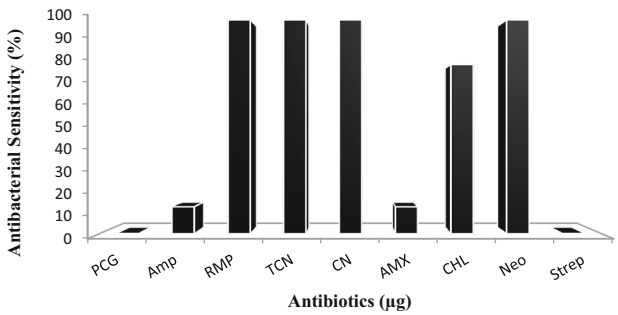

Fig. 1 Antibacterial sensitivity (%) of 24 bacterial isolates against nine different antibiotics (µg)

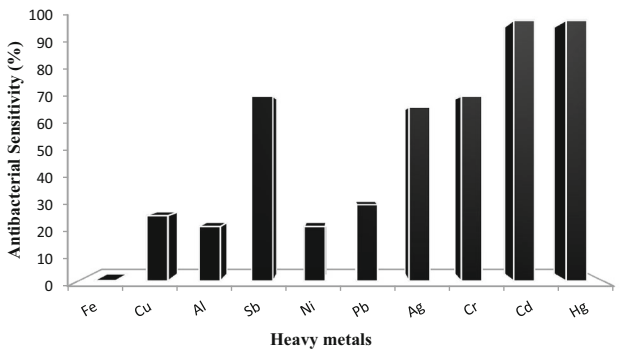

Fig. 2 Antibacterial sensitivity (%) of 24 bacterial isolates to 10 different heavy metals

Sensitivity of bacteria to heavy metals

The sensitivity of 24 isolates to 10 heavy metals was evaluated by disc diffusion test. As data represented in Fig. 2, isolates showed 0% of sensitivity to Fe and low percentage of sensitivity to Cu (25.00%), Al (20.83%), Ni (20.83%) and Pb (29.17%). More than 60% of isolates were sensitive to Sb (70.83%), Ag (66.67%) and Cr (70.83%). Cd and Hg were able to inhibit all isolates with 100% sensitivity. Heavy metals such as Fe, Zn, Mn, Cu, Ni and Mo are essential micronutrients for bacteria. These heavy metals are incorporated into bacterial enzymes as cofactors. However, high quantities of heavy metals are toxic when they bind to bacterial enzymes and DNA (Lopez-Maury et al. 2002). However, study has been found that high level of accumulated iron(II) in the gut of dampwood termite was reduced at a rapid initial rate and *Clostridium* sp. and a *Desulfovibrio* sp. were found to be able to reduce iron(III) (Vu et al. 2004). Another study on soil-feeding termites reported that the reduction of iron existing in parent soil and nest material upon the passage through the gut (Kappler 2002). Lugauskas et al. (2005)

studied the impact of Cu, zinc and Pb acetates on microorganisms in soil and showed that cellulose degrading bacteria isolated from soil has highest tolerance towards metal acetates. This result is similar to our study where isolates were less sensitive with both Cu and Pb. Most of the wood preservatives contain heavy metal components such as mercury, lead, tin, copper, zinc, cadmium, arsenic, antimony and silver (Stutz 2014) which have biocide effects. The effect of heavy metals on isolates showed the following order: Cd=Hg (100%) > Sb= Cr (70.83%) > Ag (66.67%) > Pb (29.17%) > Cu (25.00%) > Al=Ni (20.83%) > Fe (0%). Since isolates have 100% sensitivity to Cd and Hg, these heavy metals were subjected to further study.

Sensitivity of bacteria to disinfectants

The sensitivity of 24 isolates to 15 different types and concentrations of disinfectants was evaluated. According to data presented in Fig. 3, isolates showed 0% of sensitivity to crystal violet, LLD-i, 0.1 N NaOH and 0.1 N benzoic acid and low percentage of sensitivity to acetic acid (8.33%), 1-propanol (29.17%), mouthwash (20.83%), LLD-ii (16.67%) and 1 N NaOH (8.33%). By comparing LLD-i and LLD-ii, the lower concentration has lower inhibitory effects. Same result was found on 1 N NaOH, it has a better inhibition effect than 0.1 N NaOH. Higher concentration of disinfectants may increase the sensitivity against isolates. Isolates exhibited moderate percentage sensitivity to toothpaste (50.00%), iodine (83.33%) dish soap (91.67%) but high sensitivity (100%) to lactic acid, formalin and hydrogen peroxide. This result agrees with the study of Saha et al. (2009) formalin and hydrogen peroxide were found to be highly effective while a saturated solution of iodine has low effect to pathogenic bacteria. Lactic acid can create a low pH environment to the isolates. Cellulolytic bacteria are unable to grow in low pH environment. However, in this study, the isolates were resistant to acetic acid. Long term application of acetic acid in rubber plantation for coagulated latex (Bac et al. 1991) may cause resistant of isolates. LLD contains benzalkonium chloride (BZK) which is generally used as disinfectant. Bacteria are found to be less sensitive to BZK but show higher sensitivity to pyridinium and quinolinium stilbene benzensulfonates (Chanawanno et al. 2010). In this study, the effect of disinfectants on isolates showed the following order: lactic acid = formalin = hydrogen peroxide (100%) > dish soap (91.67%) > iodine (83.33%) > toothpaste (50.00%) > 1-propanol (29.17%) > mouthwash (20.83%) > LLD-i (16.67%) > acetic acid = 1 N NaOH (8.33%) > crystal violet = LLD-ii = 0.1 N NaOH = benzoic acid (0%). Since isolates have 100% sensitivity to lactic acid, formalin and hydrogen peroxide, these disinfectants were subjected to further study on their chances to be used as a termiticide (Fig. 4).

Antibiotic combined action effect

From the data represented in Fig. 5, about 17 out of 36 antibiotic combinations showed higher synergistic effect while 8 of the combinations have higher antagonistic effect. The PCG–TCN combination was the most synergistic (58.33%) against the isolates compared to the other combinations. The antagonistic effect of TCN–CN combination was highest among the antibiotic combinations that showed antagonism by having 79.17% of antagonism. According to Olajuyigbe and Afolayan (2012), differentiating synergistic from antagonistic interactions is a key to develop improved strategies for microbial management. Antibiotic combination is desired to provide a broader spectrum activity than the individual regimes and to prevent or minimize the occurrence of drug-resistant bacteria. Antimicrobial agents can interact to produce synergism

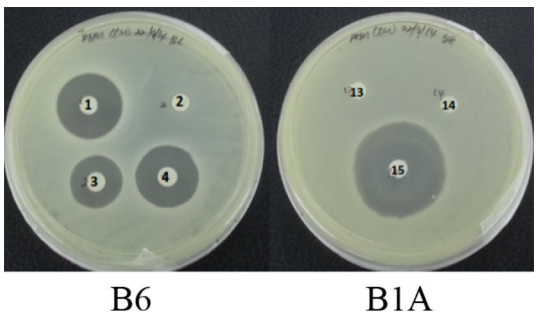

Fig. 3 Antibacterial sensitivity Percentage (%) of 24 bacterial isolates to 15 different disinfectants. (i: 1cup of LLD for 4.5 kg of water; ii: 1:1 dilution ratio; iii: 1 N concentration; iv: 0.1 N concentration)

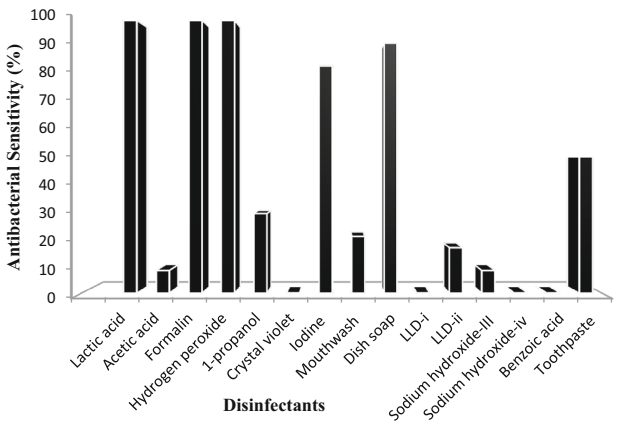

Fig. 4 The photo of antibacterial activity of lactic acid (1), acetic acid (2), formalin (3) and hydrogen peroxide (4) against isolate B6 and the activity of 0.1 N sodium hydroxide (13), benzoic acid (14) and toothpaste (15) against B1A isolate

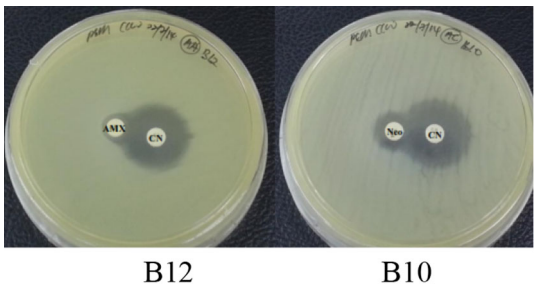

Fig. 5 Percentage of sensitive isolates (%) subjected to synergism and antagonism inhibitory effects by 36 different antibiotic combinations

inhibitory activity with different mechanism. Although the sensitivity of gut bacteria to various antibiotics has been widely studied (Treves and Martin 1994; Rosengaus et al. 2011), little information is available on the synergism and antagonism effects of antibiotic combinations on termite's gut bacteria (Figs. 6 and 7).

Termites biocontrol

Filter papers impregnated with 27 antibacterial agents (2 heavy metals, 3 disinfectants and 22 antibiotic combinations) were fed to termites. The number of survived termites observed and recorded for 1 week. According to Table 2, only 12 out of 27 selected antibacterial agents were able to kill all termites. The effective antibacterial agents include PCG–TCN, PCG–CN, PCG–CHL, AMP–CHL, RMP–TCN, RMP–CN, AMX–CHL, Cd, Hg, lactic acid, formalin and hydrogen peroxide. Only one heavy metal (Hg) and three disinfectants (lactic acid, formalin and hydrogen peroxide) were most effective against termites where all of the termites killed within 1 day. Cd, PCG–CHL and AMP–CHL have a moderate termiticide effect which killed all the termites within 3–5 days. The other five antibiotic combinations were only able to kill all the termites after 6 days treatment. Overall, antibiotic combinations with significant synergistic effects together with no or little antagonistic effects were more effective. For example, PCG–TCN, PCG–CN, PCG–CHL, AMP–TCN and AMX–CHL have only synergism effect on isolates were then able to kill all the termites after 1 week observation. 4–4.5 termites (80–90%) survived after 1 week observation and were tested against CN–CHL and CHL–Neo respectively. Moreover, the larger the inhibition zone formed, the more effective the antibacterial agent. Our result show that Cd was less effective when compared with other heavy metals and disinfectants. RMP antibiotic treatment administered to *Nasutitermes takasagoensis* had significantly reduced cellulose degradation activity in the hingut (Tokuda and Watanabe 2007). RMP not only permanently reduces the diversity of gut bacteria, but also reduces oviposition rates of queens and have severe long term fitness effects on termites *Z. angusticollis* (Rosengaus et al. 2011). Saha et al. (2009) proposed that hydrogen peroxide is a better choice than formalin since formalin is corrosive for human beings. Moreover, the use of antibiotics and/or other antimicrobial agents has potential applicability for biological control of termites. The antibiotic treatment permanently reduced the diversity of the gut microbiota. By disrupting the mutualistic interaction between termite hosts and their symbionts, better management practices for termite control may be achieved

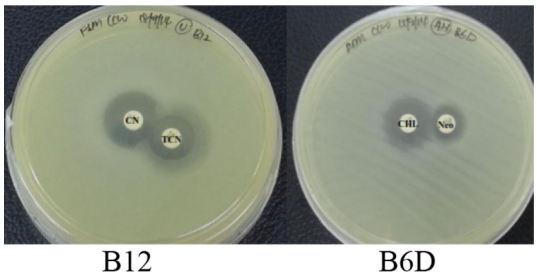

Fig. 6 The photo of synergism inhibitory effects of CN–AMX and CN–Neo against B12 and B10 isolates respectively

Fig. 7 The photo of antagonism inhibitory effects of TCN–CN and CHL–Neo against B12 and B6D isolates respectively

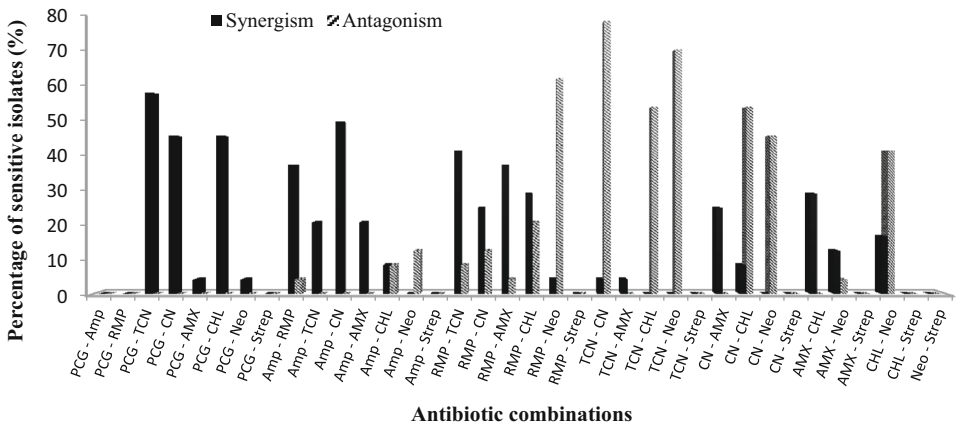

Table 2 Number of survived termites that fed with effective antibacterial agents from 0 to 7th day

Antibacterial agents	Number of survived termites (mean ± SD)								F ratio	*P* value	NDKT[a]
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			
PCG–TCN	5.00 ± 0.00	3.50 ± 0.71	3.50 ± 0.71	3.50 ± 0.71	2.50 ± 0.71	1.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	16.18	0.001	6
PCG–CN	5.00 ± 0.00	4.50 ± 0.71	4.50 ± 0.71	4.00 ± 0.00	2.50 ± 0.71	2.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	9.07	0.009	6
PCG–CHL	5.00 ± 0.00	2.00 ± 0.00	1.50 ± 0.71	0.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.34	0.000	4
Amp–CHL	5.00 ± 0.00	4.50 ± 0.71	4.00 ± 0.00	4.00 ± 0.00	1.00 ± 1.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.40	0.004	5
RMP–TCN	5.00 ± 0.00	3.00 ± 0.00	2.50 ± 0.71	2.50 ± 0.71	2.50 ± 0.71	2.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	22.89	0.000	6
RMP–CN	5.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	2.50 ± 0.71	2.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	11.34	0.004	6
AMX–CHL	5.00 ± 0.00	4.50 ± 0.71	3.50 ± 0.71	3.50 ± 0.71	2.00 ± 0.00	0.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	13.36	0.002	6
Cd	5.00 ± 0.00	4.00 ± 0.00	1.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	25.84	0.000	3
Hg	5.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	49.00	0.000	1
Lactic acid	5.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	49.00	0.000	1
Formalin	5.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	49.00	0.000	1
H_2O_2	5.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	49.00	0.000	1

Each value represents the mean number of the replicates survived termites that fed with effective antibacterial agents using the sampling methods of direct count within 7 days. Rows of means compared according to different treatments showed in 1st column using Holm–Sidak

P value = significant at $P < 0.05$

[a]NDKT: No. of day(s) to kill all termites (n = 5)

without the environmental and ecological problems typically associated with the use of other toxic chemicals.

Conclusions

In conclusion, antibacterial investigations were carried out against 24 bacterial isolates and overall exhibited 100% sensitive against 44.44% of total antibiotics, 20% of total heavy metals and 21.43% of total disinfectants. About 47.22% of antibiotic combinations showed synergistic effect, while 22.22% showed antagonistic effects. All were selected for termite biocontrol treatment. Among the 27 selected antibacterial agents, 12 of them were found to be effective to kill all the termites within 1–6 days. Hg, lactic acid, formalin and hydrogen peroxide were found to be the most effective against the termites where all the termites were only killed in 1 day. These effective antibacterial agents have potential to be a new application to control the termite pest species. Overall, disinfectants are recommended due to their better antibacterial activity, low cost and availability.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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