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Additional Property of *Xenorhabdus stockiae* for Inhibiting Cow Mastitis-causing Bacteria

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***Xenorhabdus*bacteria isolated from entomopathogenic nematodes have been found to produceseveral antimicrobial agents for inhibiting pathogenic bacteria. In this study,*X. stockiae* strains PB09, SS7 andUV58 (UV-mutant of PB09)were evaluated for antibacterial activities against cow mastitis-causing isolates of *Bacillus subtilis, Staphylococcus aureus, Staphylococcus intermedius, Streptococcus agalactiae, Enterococcus faecalis, Klebsiella pneumoniae*, and *Escherichia coli*. The paper disc diffusion showed thatcell-free supernatants of all *X. stockiae* strains cultivated for 48, 72 and 96 h had anti-mastitis activities, but 72- and 96-h supernatants were more effective andtended to inhibitgram-positive bacteria. The maximal activities were found when *X. stockiae* PB09 were evaluated against *S. agalactiae*(12.00±2.16mm), *X. stockiae* SS7and*X. stockiae* UV58 against *S. aureus*(11.75±1.71 mmand 12.50±0.58 mm, respectively).The overlay assay also showed that*X. stockiae* colonies were more likely to inhibit gram-positive bacteria.Also, live*X. stockiae* UV58 colonies were more effective than *X. stockiae* PB09 and *X. stockiae* SS7 colonies for suppressing *S. intermedius,S. agalactiae*and *E. coli*(31.33±1.53, 22.67±1.53, 18.33±0.58 mm, respectively). This study may suggest that *X. stockiae* can be used for future effective suppression of mastitis infection in dairy cows.**

Key words: *Xenorhabdus stockiae,* cow mastitis bacteria, paper disc diffusion assay, overlay assay.

Bovine mastitisis the inflammation of the lactating cows' mammary glands. This disease can cause both clinical and subclinical symptoms, resulting in decrease of yield and quality of milk as well as alteration of milk biochemical property[1].Because of this devastating disease, the dairy farmers also have to experience economic loss due to high treatment cost,inevitable animal culling and incidence of resistance against common antimicrobial agents[2,3]. The common pathogens that caused cow mastitis are coagulase-negative staphylococci, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*[4], *Enterococcus faecalis*[5] and*Klebsiella pneumoniae*[6,7,8]. Hence, the economic burden of bovine mastitis on dairy industry has prompted research into the development of safe, effective treatments. Some studies have focused on using alternative approaches for treatment of mastitis due to the rising prevalence of drug resistance, for example, the use of medicinal plants[9], endophytic fungi[10] and lactic acid bacteria[11]. In particular, the secondary metabolites derived from bacteria with broad antibiotic property were found to have anti-mastitis activity. For instance, bacteriocin-like

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substances produced by lactic acid bacteria were shown to suppress mastitis-causing isolates of *Listeria innocua, L. monocytogenes* Scott A and *Streptococcus dysgalactiae*[11]. Moreover, there are some approaches that using *Xenorhabdus* bacteria isolated from entomopathogenic nematodes as sources of antibiotic substances. These Xenorhabdusbacteria are symbiotic to the nematodes of the family Steinernematidae which have been used as biological control agents for a number of agricultural insect pests. When the nematodes infect the insect hosts, Xenorhabdus bacteria are released to insect's haemocoel and resulting in the death of insect hosts. After that, Xenorhabdus bacteria can produce the secondary metabolites that control the growth of a variety of surrounding bacterial or fungal competitors[12]. Therefore, it is interesting to apply the various substances of Xenorhabdus sp. for controlling the clinical isolates of mastitis-causing bacteria. Xenorhabdus bacteria, including *Xenorhabdusnematophila, X. szentirmaii*and*X. budapestensis,* were found to inhibit mastitis-causing isolates of*S. aureus, E. coli* and *K. pneumoniae,* whereby the anti-matitis activity was highest against *S. aureus*and lowest against *K. pneumoniae*[13]. A number of substances were isolated from *Xenorhabdus* bacteria and investigated for their potentials to be used asantimicrobial agents, including benzylideneacetone (trans-4-phenyl-3-buten-2-one) derived from *X. nematophila*[14],xenematide from *X. nematophila*[15], xenocoumacins 1 and 2which were derivatives of benzopyran-1-onefrom*X. nematophila*[16], xenoxides from*X. bovienii*[17], and peptides(GP-19 and EP-20) from *X. budapestensis* NMC-10[18]. In this study, the wild-type*X. stockiae* PB09and*X. stockiae* SS7 isolated from the entomopathogenic nematodes (*Steinernemasiamkayai*) and the mutant*X. stockiae* UV58 (obtained by UV treatment of *X. stockiae* PB09)were investigated for their activity against mastitis-causing bacterial isolates, including *Bacillus subtilis, Staphylococcus aureus, Staphylococcus intermedius, Streptococcus agalactiae, Enterococcus faecalis, Klebsiella pneumoniae,* and*Escherichia coli.* The knowledge from this study could be beneficial for future development of safe and effective approach for treatment of bacterial mastitis infection.

MATERIALS AND METHODS

Culture of *Xenorhabdus stockiae* bacteria

X. stockiae PB09and*X. stockiae* SS7were wild type isolates derived from the entomopathogenic nematodes (*Steinernemasiamkayai*) according to Kaya and Stock (1997)[19]. The nematodes were kindly provided by the Department of Agriculture, Ministry of Agriculture. *X. stockiae* PB09 was further mutated by UV treatment that resulting in *X. stockiae* UV58. All bacterial strains were maintained on Luria Bertani (LB) agar slants at 28°C and subcultured every month. *X. stockiae* bacteria that were used in the further experiments must be in the phase I which could be confirmed by growing their colonies on Nutrient Bromothymal blueTriphenyltetrazolium chloride agar(NBTA) at 28°C in the dark for 48 h. The bacterial colonies in the phase I could be detected by their absorption of bromothymol blue to produce the blue colonies that were surrounded by clear zones on NBTA. A single blue colony was inoculated to 100 ml of LB in 250 ml-flask, then incubated in the dark at 28°C and 200 rpm for 16-24 h, and used as seed culture in the further experiments.

Culture of mastitis-causing bacteria

The clinical strains of mastitis-causing bacteria, including *Bacillus subtilis, Staphylococcus aureus, Staphylococcus intermedius, Streptococcus agalactiae, Enterococcus faecalis, Klebsiella pneumoniae,* and *Escherichia coli*were isolated and maintained on Mueller Hinton (M-H) agar slants at 4°C. To prepare each bacteria as seed culture, a loopful of bacterial colonies was inoculated into 15 ml of M-H broth in 25 ml-flask,then incubated at 37°C and 150 rpm for 16-24 h, and finally adjusted to have the concentration of approximately 10^7-10^8cfu/ml.

Evaluation of antibacterial activity by paper disc diffusion assay

The antibacterial activities of *X. stockiae* PB09, *X. stockiae* SS7 and *X. stockiae* UV58 against 7 mastitis bacteria were determined by paperdisc diffusion assay by using the method modified fromWang *et al.* (2008)[20]. Briefly, 1 ml of *X. stockiae*seed culture at the concentration of 10^8cfu/ml was added to 99 ml of tryptic soy broth (TSB) and incubated at 28°C and 200 rpm for 48, 72 and 96 h. Each culture was then centrifuged at

10,000 rpm at 4°C for 5 min and filtered through 0.22 µm-filter kit to obtain cell-free supernatant[21]. Twenty µl of each cell-free supernatant of *X. stockiae*were applied to 6-mm filter paper disc (Whatman no.1) and let to dry. In the meantime, 100 µl of each mastitis bacterial culture at the concentration of approximately 10^7-10^8cfu/ml in M-H broth were spread onto M-H agar and incubated at room temperature for 2 h. Each dried filter paper containing cell-free supernatant of *X. stockiae*was carefully placed onto M-H agar plate which had been spread with mastitis bacteria. The M-H plateswere incubated at 37°C for 24 h. The antibacterial activity was determined by the size of clear zone surrounded the 6 mm-filter paper. The control groups were M-H broth and streptomycin at the concentration of 1 mg/ml.

Measurement of antibacterial activity by overlay bioassay

The overlay assay was modified from the method previously described by Furgani*et al.* (2007)[13]. This method was used to measure the antibacterial interaction between the live cells of *X. stockiae* and the tested mastitis bacteria. Briefly,*X. stockiae* was cultured in M-H broth at 28°C and 150 rpm overnight in the dark. After that, 5 µl of *X. stockiae* culture were dropped at the center of M-H agar plate and let to dry, followed by incubation at 28°C for 5 days. In the meantime, a single colony of each of the mastitis bacteria, including *B. subtilis, S. aureus, S. intermedius, S. agalactiae, E. faecalis, K. pneumoniae*, and*E. coli*, was separately grown in 15 ml of M-H broth for 24 h, and 100 µl of each culturewere individually mixed with 3.5 ml of50°C-melting, M-H agar (containing agar at 0.6% v/w). Each mastitis bacteria-melting agar mixture was poured on M-H plate that had grown *X. stockiae* for 5 days. After the plates became solidified, they were incubated at 37°C for 48 h, then measured for the sizes of clear zone surrounded *X. stockiae* colony, which representing the activity of live *X. stockiae* cells against mastitis bacteria.

Data analysis

The obtained data from four replicates were analyzed and compared by One-way Analysis of Variance (One-Way ANOVA) and LSD (Fisher's Least Significant Difference) test at *p*d" 0.05 using SAS program (1990).

RESULTS

The cell-free supernatants of *X. stockiae* isolates (PB09, SS7 and UV58) which were cultured for different durations (48, 72 and 96 hours) were evaluated against the mastitis-causing bacteria by paper disc diffusion assay. The results showed that cell-free supernatants of *X. stockiae* PB09 could inhibit all mastitis bacteria, but they were more likely to inhibit gram-positive ones (Table 1). In addition, cell-free supernatants of *X. stockiae* that were cultivated for 48, 72 and 96 hours could inhibit eachmastitis bacteria at similar levels. However, for inhibiting*S. agalactiae,* the72- and 96-h supernatants werefound to be significantly more effective than 48-h supernatant. The highest antibacterial activity of *X. stockiae* PB09 was found in 72-h supernatant when being used against*S. agalactiae*(12.00±2.16mm), followed by 96-h supernatant when also being used against *S. agalactiae*(11.75±1.50 mm); however, both of them were not significantly different(Table 1).

The cell-free supernatant of *X. stockiae* SS7 (Table 2) was also found to inhibit all mastitis bacteria withsimilar antibacterial activitiesto*X. stockiae*PB09 (Table 1). Also, 48-, 72-, and 96-h cell-free supernatants of *X. stockiae* SS7 were not significantly different at suppressing each mastitis bacteria, except that 72- and 96-h supernatants that were significantly more effective than 48-h supernatant at inhibiting*S. intermedius*. The maximum antibacterial activity of *X. stockiae* SS7 against mastitis bacteria was found in 96-h supernatant when being used against *S. aureus*(11.75±1.71 mm), followed by 72-h and 48-h supernatantswhen also being used against *S. aureus*(11.25±2.06 and11.25±1.71 mm, respectively), but all of them were not significantly different(Table 2).

The antibacterialactivity of *X. stockiae* UV58 (Table 3), a mutant of *X. stockiae* PB09 by UV treatment, was similar to its wild type (Table 1).For *X. stockiae* UV58, the supernatants of 48-,72- and 96-h supernatants had similar activities against mastitis bacteria, with the exception of 96-h supernatant that was more effective than 48- and 72-h supernatant s when being used against*S. aureus*. The highest antibacterial activities of *X. stockiae* UV58 were found in 96-h supernatant when being used against *S. aureus*(12.50±0.58 mm),

Table 1. Antibacterial activities of cell-free supernatant of *X. stockiae* PB09 cultivated for 48, 72 and 96 hours against mastitis-causing bacteria by disc diffusion assay

Cultivation period (hours)	Size of inhibition zone (mm; mean±SD)						
	B. subtilis	*S. aureus*	*S. intermedius*	*S. agalactiae*	*E. faecalis*	*K. pneumoniae*	*E. coli*
48	10.50 ± 2.38^{bA}	10.00 ± 1.41^{bA}	9.50 ± 1.73^{bA}	8.50 ± 1.73^{cA}	10.50 ± 1.91^{bA}	9.00 ± 0.82^{bA}	9.00 ± 1.41^{bA}
72	11.25 ± 1.71^{bAB}	10.00 ± 1.83^{bAB}	10.00 ± 2.16^{bAB}	12.00 ± 2.16^{bA}	11.25 ± 1.26^{bAB}	10.00 ± 0.82^{bAB}	9.50 ± 1.29^{bB}
96	10.75 ± 1.26^{bABC}	10.00 ± 1.83^{bABC}	11.25 ± 0.50^{bAB}	11.75 ± 1.50^{bA}	11.25 ± 1.26^{bAB}	9.75 ± 0.96^{bBC}	9.25 ± 1.26^{bC}
TSB	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dA}	0.00 ± 0.0^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
Strep.	24.00 ± 1.83^{aA}	24.00 ± 1.83^{aA}	18.00 ± 2.16^{aBC}	19.75 ± 1.71^{aB}	18.00 ± 0.82^{aBC}	16.00 ± 1.15^{aCD}	14.50 ± 1.29^{aD}

Note:Data followed by the different upper-case letters ($^{A,B,C\cdots}$) in the same row were significantly different ($p\leq0.05$) as compared by LSD
Data followed by the different lower-case letters ($^{a,b,c\cdots}$) in the same column were significantly different (p0.05) as compared by LSD
TSB = tryptic soy broth
Strep. = streptomycin at the concentration of1 mg/ml
Diameter of paper disc = 6 mm

Table 2. Antibacterial activities of cell-free supernatant of X. stockiae SS7 cultivated for 48, 72 and 96 hours against mastitis-causing bacteria by disc diffusion assay

Cultivation period (hours)	Size of inhibition zone (mm; mean±SD)						
	B. subtilis	*S. aureus*	*S. intermedius*	*S. agalactiae*	*E. faecalis*	*K. pneumoniae*	*E. coli*
48	10.25 ± 0.96^{bAB}	11.25 ± 1.71^{bAB}	9.00 ± 0.00^{cD}	9.50 ± 1.00^{bCD}	10.25 ± 1.50^{bBCD}	9.75 ± 0.96^{bBCD}	11.00 ± 0.00^{bABC}
72	10.50 ± 1.29^{bA}	11.25 ± 2.06^{bA}	10.00 ± 0.96^{bA}	10.50 ± 1.29^{bA}	11.00 ± 1.63^{bA}	10.00 ± 0.82^{bA}	11.00 ± 0.82^{bA}
96	10.00 ± 0.82^{bB}	11.75 ± 1.71^{bA}	10.75 ± 0.96^{bAB}	11.00 ± 0.82^{bAB}	10.75 ± 1.71^{bAB}	10.00 ± 0.82^{bB}	10.50 ± 0.58^{bAB}
TSB	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{cA}	0.00 ± 0.0^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
Strep.	24.00 ± 1.83^{aA}	24.00 ± 1.83^{aA}	18.00 ± 2.16^{aBC}	19.75 ± 1.71^{aB}	18.00 ± 0.82^{aBC}	16.00 ± 1.15^{aCD}	14.50 ± 1.29^{aD}

Note:Data followed by the different upper-case letters ($^{A,B,C\cdots}$) in the same row were significantly different ($p\leq0.05$) as compared by LSD
Data followed by the different lower-case letters ($^{a,b,c\cdots}$) in the same column were significantly different (p0.05) as compared by LSD
TSB = tryptic soy broth
Strep. = streptomycin at the concentration of1 mg/ml
Diameter of paper disc = 6 mm

Table 3. Antibacterial activities of cell-free supernatant of *X. stockiae* UV58 cultivated for 48, 72 and 96 hours against mastitis-causing bacteria by disc diffusion assay

Cultivation period (hours)	Size of inhibition zone (mm; mean±SD)						
	B. subtilis	*S. aureus*	*S. intermedius*	*S. agalactiae*	*E. faecalis*	*K. pneumoniae*	*E. coli*
48	11.00 ± 0.82^{bAB}	10.50 ± 0.58^{cABC}	10.25 ± 1.50^{bABC}	9.75 ± 0.96^{bBC}	11.50 ± 1.29^{bA}	9.25 ± 0.50^{bC}	9.50 ± 0.58^{bC}
72	10.75 ± 0.50^{bAB}	10.50 ± 0.58^{cABC}	11.00 ± 0.82^{bA}	10.75 ± 0.50^{bAB}	10.50 ± 0.58^{bABC}	9.75 ± 0.96^{bBC}	9.50 ± 1.00^{bC}
96	11.50 ± 0.58^{bAB}	12.50 ± 0.58^{bA}	11.50 ± 1.00^{bAB}	11.00 ± 1.15^{bB}	10.50 ± 0.58^{bBC}	9.50 ± 1.00^{bC}	10.25 ± 0.96^{bBC}
TSB	0.00 ± 0.00^{cA}	0.00 ± 0.00^{dA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.0^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
Strep.	24.00 ± 1.83^{aA}	24.00 ± 1.83^{aA}	18.00 ± 2.16^{aBC}	19.75 ± 1.71^{aB}	18.00 ± 0.82^{aBC}	16.00 ± 1.15^{aCD}	14.50 ± 1.29^{aD}

Note:Data followed by the different upper-case letters ($^{A,B,C\ ...}$) in the same row were significantly different ($p\leq0.05$) as compared by LSD
Data followed by the different lower-case letters ($^{a,b,c...}$) in the same column were significantly different ($p0.05$) as compared by LSD
TSB = tryptic soy broth
Strep. = streptomycin at the concentration of1 mg/ml
Diameter of paper disc = 6 mm

followed by 48-h supernatant when being used against *E. faecalis*(11.50±1.29 mm) (Table 3).

The live colonies of *X. stockiae* PB09, *X. stockiae* SS7 and *X. stockiae* UV58 were overlaid by mastitis bacteria to determine the antibacterial activities that live colonies could produce (Table 4).*X. stockiae* UV58 colonies were found to be significantly more effective than *X. stockiae* PB09 and *X. stockiae* SS7 colonies for suppressing *S. intermedius, S. agalactiae*and *E. coli*. For *X. stockiae* UV58 colonies, the largest inhibition zone was found when they were overlaid by*S. intermedius* (31.33±1.53 mm), followed by*B. subtilis* (23.67±1.53 mm) and*S. agalactiae* (22.67±1.53 mm). Moreover, *X. stockiae* PB09 and SS7 colonies were found to be most effective for inhibiting *B. subtilis*(22.33±1.53 and 21.00±1.00 mm, respectively), but both were not different to *X. stockiae* UV58 (23.67±1.53 mm). However, both gram-negative mastitis bacteria (*K. pneumoniae* and *E. coli*) were found to be resistant to *X. stockiae* colonies, with the exception that *X. stockiae* UV58 colonies when being evaluated against *E. coli* (18.33±0.58 mm) (Table 4).

DISCUSSION

The cell-free supernatants of*X. stockiae*PB09, SS7 and UV58 were shown to be effective at inhibiting the clinical isolates of both gram-positive and gram-negative mastitis-causing bacteria, particularly*S. agalactiae*and *S. aureus,*as shown by paper disc diffusion assay, and*S. intermedius,B. subtilis* and *S. agalactiae,*as shown by overlay bioassay.The activities of *X. stockiae*strains against mastitis-causing bacteria were found to slightly increase with*X. stockiae* cultivation time. Moreover, type of cultivation media (TSB, LB and M-H broths) was found to have no effect on the anti-mastitis activities of *X. stockiae* isolates (data not shown). This may suggest that,in the enriched media, the secondary metabolites withanti-mastitis properties of *X. stockiae*strains could be produced, regardless of cultivation time. There are a number of reports that showing the abilities of *Xenorhabdus*sp. to produce antibiotics against mastitis-causing bacteria. Several species of *Xenorhabdus* bacteria, including *X. budapestensis, X. szentirmaii, X. innexi, X. nematophila, X. cabanillassii*, were

Table 4. Inhibitory activities of *X. stockiae* colonies against mastitis-causing bacteria by overlay bioassay

Mastitis-causing bacteria	Size of inhibition zone(mm; mean ± SD)		
	X. stockiae PB09	*X. stockiae* SS7	*X. stockiae* UV58
B. subtilis	22.33 ± 1.53^{aA}	21.00 ± 1.00^{aA}	23.67 ± 1.53^{bA}
S. aureus	17.00 ± 2.00^{bA}	17.67 ± 0.58^{bA}	16.33 ± 1.53^{dA}
S. intermedius	16.67 ± 1.53^{bB}	17.67 ± 0.58^{bB}	31.33 ± 1.53^{aA}
S. agalactiae	18.33 ± 2.08^{bB}	17.67 ± 2.08^{bB}	22.67 ± 1.53^{bA}
E. faecalis	17.67 ± 1.53^{bA}	18.00 ± 1.00^{bA}	19.33 ± 1.53^{cA}
K. pneumoniae	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{eA}
E. coli	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cB}	18.33 ± 0.58^{cdA}

Note: Diameter of *X. stockiae* ≈ 15 mm
Data followed by the different upper-case letters ($^{A,B,C\ \cdots}$) in the same row were significantly different ($p\leq0.05$) as compared by LSD
Data followed by the different lower-case letters ($^{a,b,c\cdots}$) in the same column were significantly different ($p0.05$) as compared by LSD

shown to have antibacterial activities against *S.aureus, E. coli* and*K. pneumoniae*[22]. However, in this study, the cell-free supernatants and live colonies of *X. stockiae* PB09, SS7 and UV58 were found to have antibacterial activities that were more likely to be specific to gram-positive bacteria, especially*S. intermedius*and *S.aureus*, than gram-negative ones. This was similar to the studies of *X. bovienii*and*X. nematophila* which could produce Xenorhabdins that inhibited gram-positive mastitis bacteria(*Micrococcus luteus, B. subtilis, S. pyogenes* and *S. aureus*) more effectively than gram-negative ones[23].In addition, althoughboth cell-free supernatants and live colonies of*X. nematophila, X. budapestensis**X. szentirmaii*were found to have antibacterial activities against mastitis-causing isolates of *S.aureus, E. coli* and*K.pneumoniae*, but the antibacterial activities were highest against *S. aureus* and lowest against *K. pneumoniae*[13].

The activities of *Xenorhabdus* sp. against mastitis-causing bacteria may be due to their production of a variety of antibiotic derivatives. Interestingly, *X. nematophila* was one of the symbiotic bacteria that capable of generating several antibacterial metabolites with broad spectrum activities. For example, *X. nematophila* could produce Indolecompounds which capable of inhibiting both gram-positive and gram-negative bacteria[24,25].It was also found to generate Xenocoumacins 1 and 2 that effectively inhibiting gram-positive bacteria, particularly Streptococci andStaphylococci groups, and also gram-negative bacteria, including some strains of *E. coli*[16].In addition, its Nematophin compounds were shown to inhibit *Staphylococci* bacteria[26]. Even though several reports have suggested that metabolites of*X. nematophila* were more likely to be effective against gram-positive bacteria, there were a number of gram-negativeplant pathogenic bacteria that were found to be suppressed by its Benzylideneacetone compounds, including *Agrobacterium vitis*, *Pectobacteriumcarotovorum* subsp. *atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *Pseudomonas syringae*pv. *tabaci*, and*Ralstonia solanacearum*[14].Furthermore, both *X. nematophila* and *X. bovienii*were found to produce Xenorhabdins, the compounds that could effectively inhibit several gram-positive bacteria, including *Micrococcus luteus, B. subtilis, S. pyogenes*and*S. aureus*, but they hadrather low efficacy specific to gram-negative bacteria[13]. Also,*X. bovienii*was also found to generate Xenoxides, the compounds that could inhibit several pathogenic strains of gram-positive bacteria, including*B.subtilis, M. luteus*and *S. aureus*[17]. Similarly, in this study, all three strains of *X. stockiae* were shown to suppress mastitis bacteria, especially the gram-positive ones. This requires further analysis of their secondary metabolites that are capable of effectively inhibiting the mastitis bacteria and can be developed into practical commercial products for dairy farming.

CONCLUSION

The cell-free supernatants and live colonies of three *X. stockiae* strains(PB09, SS7, UV58) were evaluated against mastitis-causing isolates of *B. subtilis, S. aureus, S. intermedius, S. agalactiae*and*E. faecalis, K. pneumoniae* and*E. coli*. The anti-mastitis activities of *X. stockiae*strains were higher against gram-positive bacteria rather than gram-negative ones and found to increase with their cultivation time. This may suggest that theanti-mastitis efficacy of *X. stockiae* strains was more likely to be due totheir secondary metabolites. Further studies are required to determine which metabolites of *X. stockiae* that have anti-mastitis property and whether mutation of *X. stockiae*could result in increase of its anti-mastitis activity.

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