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The screening of bacteria with antimalarial activity, a novel alternative to drugs

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Abstract

Malaria remains one of the important human diseases in the world with 1-2 million deaths annually. An increasing in drug resistance of malaria parasites particularly, *P. falciparum*, to available drugs has led to a novel drug development. Antimalarial drug development can follow several strategies, ranging from modifications of existing agents to the design of novel agents that act against new targets. Several compounds isolated from natural products including plant and microorganisms such as bacteria and fungi will be a rich source many bioactive substances with diverse structures for optimization to obtain better therapeutic agents. The objective of this work is to find a new compound from bacterial origin to combat against drug resistant malaria parasite, *P. falciparum*. The bacterial extract prepared from two isolated bacteria with anti-MRSA activity (strain SOPB1 and WARY7-4) show inhibitory activity toward chloroquine resistant malaria strain K1 as shown by a decrease up to 50% in the parasite number at every concentration used. Moreover, these two substances also inhibit the parasite development and reinfection as shown by pyknotic formation and died parasite at the end of incubation. To our knowledge, this work is the first report on the antimalarial activity of bacterial extract. These two bacterial extracts are promising target used for treatment of drug resistance malaria *P. falciparum* and might be exploited as a novel drug for mono- or combination therapy for malaria in the future.

Keywords: bacteria, antimalarial activity, screening

Introduction

Malaria remains one of the most important diseases of man with over half the world's population at risk of infection and 1-2 million deaths annually. There are four different species of human malaria parasites namely, *Plasmodium falciparum*, *P. vivax*, *P. malaria*, and *P. ovale*. *P. falciparum* is the most dangerous malaria species as it often leads to the death and can be fatal within 2-3 hours of the first symptom (Hyde *et al.*, 2002). A major contributor to malarial morbidity and mortality is certainly the increasing resistance of malaria parasites to available drugs (Olliaro and Bloland, 2001). Resistance is primarily seen in *Plasmodium falciparum*, the most virulent human malaria parasite. Considering increasing resistance to available agents, there is broad consensus that there is a need to develop new antimalarial drugs (Ridley, 2002). Antimalarial drug development can follow several strategies, ranging from modifications of existing agents to the design of novel agents that act against new targets (Rosenthal 2003). Nature remains a rich source for compounds for medical applications. Several compounds isolated from nature including plant and microorganisms such as bacteria and fungi will be a rich source many bioactive substances with diverse structures for optimization to obtain better therapeutic agents.

The objective of this work is to find a new compound from bacterial origin to combat against drug resistant malaria parasite, *P. falciparum*.

Methods

Sensitivities of *P. falciparum* strain K1 used in this study to chloroquine, quinine, mefloquine and artesunate were investigated by an *in vitro* drug sensitivity assay based on the incorporation of [^{3}H] hypoxanthine into parasite nucleic acids or radioisotopic technique (Desjardins *et al.*, 1979). Two selected isolated bacteria (strain SOPB1 and WARY7-4) with anti-MRSA activity isolated from many areas of Thailand (Aunpad and Na-Bangchang 2007) was used as a source for preparation of bacterial extract to test the activity against chloroquine resistant malaria standard K1 strain. Synthesis of the bacterial extract from isolated bacteria was carried out in Tryptic soy broth (TSB medium, Difco, USA). A 200 ml flask was inoculated with 1% (10^6 CFU/ml) of an overnight culture of each isolate. The cultures were incubated at 37^{o}C for 16-18 hours with shaking at 200 rpm (New Bruchwics, England). The cell culture were collected in a 50-ml falcon tube and stored –20^{o}C for freezing. In order to obtain highest efficiency, the freeze culture cell was concentrated by lyophilization until dryness and this preparation was designated as bacterial extract and tested for antimalarial activity. In order to examine the effect of bacterial extract on malaria, the malaria was cultured with various concentrations of bacterial extracts for 48 hours. The culture were taken at 0, 6, 12, 24 and 48 hour for parasite number counting as a count parasite/10000RBC under microscope and the morphology of malaria was also observed under microscope.

Results

The level of chloroquine and artesunate susceptibility was determined by using the criteria of Pickard and colleague, (Pickard *et al.,* 2003). There was no evidence indicating artesunate resistant so far therefore artesunate susceptibility was not classified. The result shows that strain K1 has mean IC_{50} against chloroquine, quinine, mefloquine and artesunate at 100.50 nM, 314.982 nM, 10.940 nM and 0.424 nM, respectively. The strain K1 was therefore sensitive to quinine, artesunate and mefloquine and resist to chloroquine. The parasite number was decreased from 1 to 0.5 count parasite/10000RBC at 12 hour of incubation at every concentration of bacterial extract from strain SOPB1 (Table 1). The morphology of standard K1 strain grown with bacterial extract strain SOPB1 at 48 hour was different when compared to those of control without bacterial extract as shown in Fig. 1. At high concentration (10 mg/ml and 5mg/ml), the parasite can not develop into trophozoite stage. The parasites developed into early trophozoite stage at lower concentration of bacterial extract (2.5 mg/ml) however they can not reinfect the red blood cell (Fig. 1). At the concentration of 1.25 mg/ml of bacterial extract, the parasite developed into mid trophozoite however they can not reinfect the red blood cell. Interestingly, the parasite grown with bacterial extract strain SOPB1 developed slowly when compare to that of control.

The effect of bacterial extract prepared from strain WARY7-4 toward standard K1 strain was shown in Table 2. At high concentration (10 mg/ml), the parasite developed to ring stage after 6 hour of incubation which was the same as control group however the parasite formed pyknotic at 12 hour of incubation and stopped growing at 24 hour of incubation. At lower concentration (5mg/ml), the parasite developed slowly and died at 24 hour after incubation (Fig. 2).

Discussion

Two isolated bacterial extract strain SOPB1 and WARY7-4 show inhibitory activity toward chloroquine resistant malaria strain K1 as shown by a decreased up to 50% in the parasite number at every concentration used. Moreover, these substances also inhibit the parasite development and reinfection as shown by pyknotic formation and died parasite at the end of incubation.

Table 1 Effect of bacterial extract strain SOPB1 to strain K1

Substance concentration	**Count Parasite/10000RBC**				
	0 hour	**6 hour**	**12 hour**	**24 hour**	**48 hour**
Control	1.0	1.0	1.0	1.0	4.0
10 mg/ml	1.0	1.0	0.5	0.02	not found
5.0 mg/ml	1.0	0.5	0.5	0.03	not found
2.5 mg/ml	1.0	1.0	0.5	0.05	0.02
1.25 mg/ml	1.0	1.0	0.5	0.05	0.02

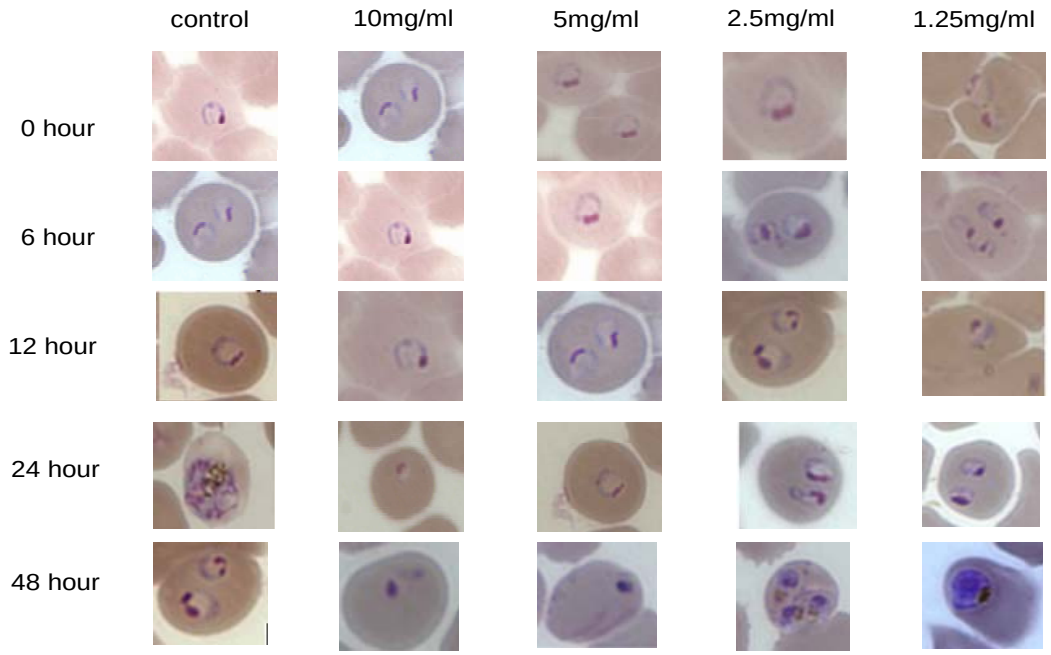

Figure 1 The morphology of standard K1 strain grown with various concentration of bacterial extract strain SOPB1 at different time (0-48 hour).

Table 2 Effect of bacterial extract strain WARY7-4 to strain K1**.**

Substance concentration	**Count Parasite/10000RBC**				
	0 hour	**6 hour**	**12 hour**	**24 hour**	**48 hour**
Control	1.0	1.0	1.0	1.0	4.0
10 mg/ml	1.0	1.0	0.5	0.02	not found
5.0 mg/ml	1.0	0.5	0.5	0.03	not found
2.5 mg/ml	1.0	1.0	0.5	0.7	0.5
1.25 mg/ml	1.0	1.0	0.5	0.6	0.5

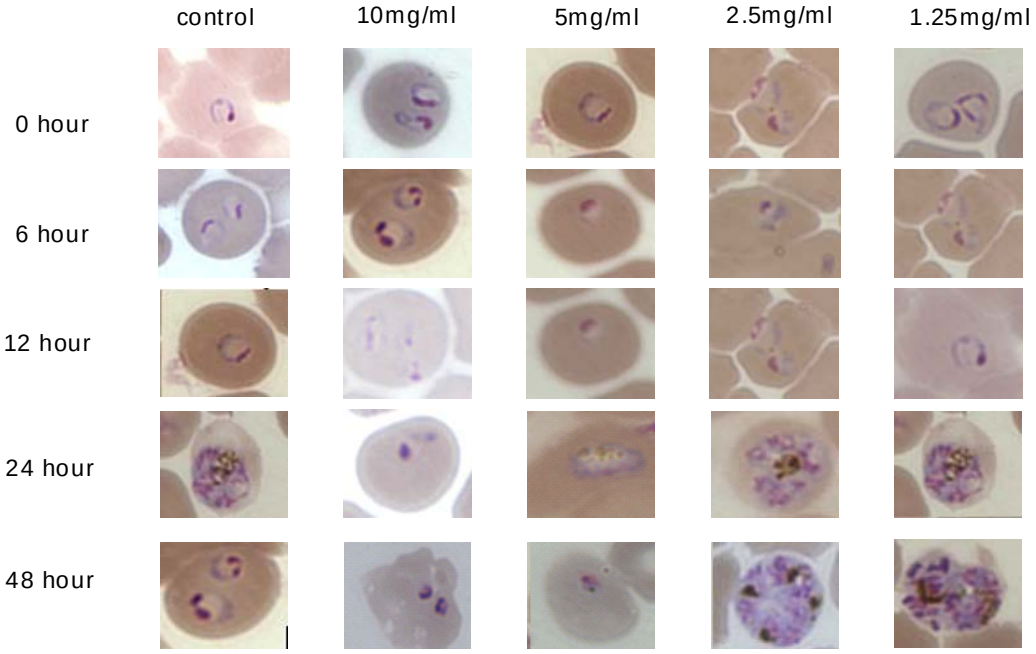

Figure 2 The morphology of standard K1 strain grown with various concentration of bacterial extract strain WARY7-4 at different time (0-48 hour).

Conclusion

It is to our knowledge; this work is the first report on the antimalarial activity of bacterial extract. Those two bacterial extracts are promising target used for treatment of drug resistance malaria *P. falciparum* and might be exploited as a novel drug for mono- or combination therapy for malaria in the future.

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References

1. Aunpad R and Na-Bangchang K. Pumilicin 4, A Novel Bacteriocin with Anti-MRSA and anti-VRE activity produced by newly isolated bacteria *Bacillus pumilus* strain WAPB4. Curr. Micobiol 2007; 55: 308-13.
2. Desjardins RE, Canfield CJ, Haynes JD and Chulay JD. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents. Chemother 1979; 16: 710-18.
3. Hyde JE. Mechanisms of resistance of *Plasmodium falciparum* to antimalarial drugs. Micro. Inf. 2002; 4: 165-74.
4. Olliaro PL and Bloland PB. Clinical and public health implications of antimalarial drug. In: P.J. Rosenthal, Editor, Drug Discovery, Humana Press, Totowa, NJ, 2001; pp. 65–83.
5. Pickard AL, Wongsrichanalai C, Purfield A, Kamwendo D, Emery K, Zalewski C, Kawamoto F, Miller RS and Meshnick SR. Resistance to antimarials in southeast asia and genetic polymorpisms in *pfmdr1*. Antimicrob.Agent.Chemoth. 2003; 47: 2418-23.
6. Ridley RG. Malaria: To kill a parasite. Nature 2002; 424: 887-9.
7. Rosenthal PJ. Antimalarial drug discovery: old and new approaches. J Experiment Biol. 2003; 206: 3735-44.