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Sub-lethal dose of *Bacillus sphaericus* suppresses vector competence of *Anopheles dirus* for *Plasmodium yoelii* by up-regulating the Imd signaling pathway

Sha-sha Yu[#], Jie Qin[#], Qiu-xia Li, Pan Wang, Jing Wang, Ting-ting Liu, Ying Wang[✉]

Department of Tropical Medicine, College of Military Preventive Medicine, Army Medical University, Chongqing, 400038, China

Objective: To investigate the effect of sublethal dose of *Bacillus sphaericus* (Bs) 2 362 strain on the malaria transmission ability of *Anopheles (An.) dirus* (Hainan strain) and its molecular mechanism.

Methods: Firstly, the fourth instar larvae of *An. dirus* was treated with sublethal dose of *Bacillus sphaericus*. The surviving larvae were then collected and placed into mosquito cages, where they were hatched into adult mosquitoes. These mosquitoes were recorded as Bs group. Meanwhile, the control group was set without any treatment. Then, for infection with *Plasmodium yoelii* BY265 RFP, 3- to 5-day old female adults were kept at 24 °C and fed on *Plasmodium yoelii* BY265 RFP-infected Kunming mice with a gametocytemia above 0.5%. On day 9-11 post infection, mosquitoes were dissected, and the oocysts on the midguts were examined under a fluorescence microscope. Thirdly, total RNA was extracted from mosquitoes of Bs group and the control group at different time-points respectively, and the cDNA were synthesized later. Finally, SYBR quantitative PCR was conducted to investigate the expression of Imd pathway anti-malaria molecules at different time-points, including TEPI and Rel2, in Bs and control group mosquitoes.

Results: Bs treatment remarkably reduced the infection rate of *Plasmodium* from 23.71% (124/523) to 16.23% (87/536) (*Chi-square* test, $P=0.002<0.05$), and the intensity of *Plasmodium* infection was greatly decreased in Bs group than that of the control group, either (Mann-Whitney Rank Sum Test, $P\leq 0.001$). However, the rate of melanization mosquitoes was 4.97% (26/523) in the control group and 5.78% (31/536) in Bs group, respectively; no significant difference was detected between them (*Chi-square* test, $P=0.558>0.05$). Additionally, the intensities of melanized oocysts were compared between the two groups, and no significant difference was found, either ($P=0.566>0.05$). Interestingly, compared with the control group, the expression levels of TEPI and Rel2 in Bs group were obviously up-regulated in larval, adult and infected mosquitoes. Especially in 3 dpi and 7 dpi, the expression level of TEPI in Bs group was nearly 4 times higher than that of the control group, while Rel2 reached to approximately 7 times.

Conclusions: We firstly found that the sublethal dose of Bs significantly suppressed the vector competence of *An. dirus* to malaria parasites, which revealed a new important role of Bs on the basis of killing mosquito larvae. Furthermore, the Imd signaling pathway might play an effective way in Bs impacting the vector competence of *An. dirus* through upregulating the expression of NF- κ B transcription factor Rel2, enhancing the expression of TEPI, which killed the *Plasmodium*, but not through melanization.

Keywords: *Bacillus sphaericus*; *Anopheles dirus*; Vector competence; Malaria; TEPI

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[#]These authors contributed equally to this work.

[✉]Corresponding author: Ying Wang, Ph.D., Department of Tropical Medicine, College of Military Preventive Medicine, Army Medical University, Chongqing, 400038, China.

Tel.: +086 02368771576

E-mail: wangyingtmmu2016@126.com

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