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Using infections to fight infections: paratransgenic fungi can block malaria transmission in mosquitoes

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Abstract

Paratransgenesis is the genetic manipulation of insect endosymbiotic microorganisms such as bacteria, viruses or fungi. Paratransgenesis has been proposed as a potential method to control vector-borne diseases such as malaria. In this article, Fang and colleagues have used genetic manipulation to insert multiple antimalaria effector genes into the entomopathogenic fungus *Metarhizium anisopliae*. When the modified fungus was used to infect *Anopheles* mosquitoes, it expressed the antimalaria effector molecules in the mosquito hemolymph. When several different effector molecules were coexpressed, malaria levels in the mosquito salivary glands were inhibited by up to 98% compared with controls. Significant inhibition could be initiated by as little as seven fungal spores and was very rapid and long lasting. These data suggest that recombinant entomopathogenic fungi could be deployed as part of a strategy to control malaria.

Keywords

Anopheles gambiae; effector molecule; entomopathogenic fungus; malaria; *Metarhizium anisopliae*; paratransgenesis; *Plasmodium falciparum*

Summary of methods & results

In this article, Fang and colleagues genetically modified a mosquito-specific entomopathogenic fungus to express anti-*Plasmodium* effector molecules. When mosquitoes were infected with the engineered fungus, it expressed the anti-*Plasmodium* genes, which blocked the ability of the mosquitoes to transmit the malaria parasites [1].

Malaria infects up to 500 million people per year, resulting in almost 3 million deaths [2]. The disease is caused by one of five parasites in the genus *Plasmodium* [3], which are transmitted to humans by the bite of infected *Anopheles* mosquitoes [4]. With no available vaccine, malaria control is currently reliant on control of the *Anopheles* mosquito vectors and drugs to kill the parasites [2,5]. These control measures are losing effectiveness, owing to the evolution of drug and insecticide resistance by parasites and mosquitoes, respectively.

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With the advent of modern molecular biology, genetic manipulation has been proposed as a new method to control the vector arthropods that transmit pathogens to humans and animals. While much work has focused on the direct genetic manipulation of vector insects such as mosquitoes [6,7], an alternative has been proposed. Organisms do not exist in isolation, but rather are associated with a diverse array of microbial agents. It is often more straightforward to engineer these microbes rather than the insect itself. Genetic manipulation of host microbial associates is termed 'Paratransgenesis' [8]. As originally formulated, the term paratransgenesis referred to engineering bacteria. However, the term has now been broadened to include other microbial agents such as viruses and fungi [9].

Entomopathogenic fungi are fungal pathogens of insects. They are ubiquitous in the environment, and can often be found in a free-living state. When spores of these fungi come into contact with an appropriate insect host, they attach to the surface of the insect's exoskeleton and germinate, producing fungal hyphae that secrete chitinase enzymes, which allow them to penetrate the insect cuticle and enter the hemocoel. Once inside the insect, they propagate in the insect body cavity, ultimately killing the insect. Fungal spores are released from the dead insect, completing the life cycle [10].

The entomopathogenic fungus *Metarhizium anisopliae* has been previously shown to be very effective at reducing malaria transmission risk in field trials [11]. Adult mosquitoes pick up the fungal spores when resting on treated surfaces. As the fungal infection progresses, the mosquitoes become increasingly sick and are eventually killed, but the process can take up to a week or more. The shortened lifespan of treated mosquitoes greatly contributes to the reduction in malaria transmission at the population level, because it reduces the proportion of mosquitoes that live through the extrinsic incubation period (the time period between when the mosquito acquires the malaria parasite by feeding on an infected human host and when it is able to transmit the parasite). However, at the individual mosquito level, some mosquitoes may not be killed in a timely enough manner and will become infectious.

Fang and colleagues addressed this critical shortcoming. Previously, antiparasitic effector molecules had been developed that blocked *Plasmodium* infection and/or transmission in mosquitoes. These include artificial small peptide genes such as salivary gland and midgut peptide 1 (SM1), single-chain antibodies raised against *Plasmodium* epitopes (such as PfNPNA-1) and the antimicrobial scorpion toxin scorpine (Table 1) [12–14]. The authors used transgenic manipulation to create recombinant *M. anisopliae* that expressed these effectors alone or in concert in the mosquito hemolymph.

All transgenic *M. anisopliae* strains significantly reduced *Plasmodium falciparum* sporozoite levels in the mosquito salivary glands. Significant *Plasmodium* inhibition was observed when mosquitoes were infected with as few as seven fungal spores. When the fungi expressed single effector genes, inhibition rates ranged from 70 to 90%. However, when the researchers expressed multiple effectors in concert, they saw a synergistic effect with much higher levels of sporozoite inhibition. When the fungus coexpressed scorpine in conjunction with a scorpine–SM1 fusion protein, *Plasmodium* levels in the mosquito salivary glands were suppressed by more than 98%. Most importantly, the fungus could be applied long after the mosquitoes imbibed infected blood (up to 11 days afterwards) and continued to suppress *Plasmodium* for the life of the mosquito [1].

Discussion

There are many potential advantages to using recombinant entomopathogenic fungi for control of vector-borne diseases such as malaria. These fungi can be readily mass produced

under field conditions using available technology. Indeed, *Metarhizium* is already used to control swarming locusts in Africa [15]. These fungi tend to be species specific, minimizing the risk of nontarget effects. They do not infect vertebrates, making them safe to apply near humans and domestic animals. *Anopheles gambiae* mosquitoes are adapted to live in the human environment, resting indoors on the walls and curtains of huts. This behavior makes exposing the mosquitoes to the fungus relatively easy – fungal spores can be applied on the walls, on hung black cloths or on curtains where the mosquitoes will become exposed [11]. Finally, since the recombinant fungi still exhibit the same slow killing effect of the unmodified fungus, there is a synergistic effect of fungal-induced mosquito life shortening and *Plasmodium* blocking that will result in a highly efficient reduction of malaria transmission at the population level [1].

Conclusion & future perspective

This study represents a major leap forward in the concept of paratransgenic control of vector arthropods. While the experiments outlined are proof-of-principle, the path forward to field application is much more straightforward for entomopathogenic fungi than for other potential paratransgenic agents such as mosquito midgut bacteria. Fungi are easy to genetically modify, can be cultured and mass produced in the endemic environment, can be deployed in the field and have already been used to control other arthropod pest species. This existing production pipeline means that the switch from wild-type fungal strains to genetically modified strains could potentially be relatively simple, if the regulatory hurdles of releasing genetically modified organisms can be overcome.

Executive summary

Objectives

- To create recombinant entomopathogenic fungal strains that express anti-*Plasmodium* effectors in *Anopheles* mosquitoes.
- Evaluate these strains in their ability to block *Plasmodium* transmission by *Anopheles* mosquitoes.

Methods

- Identification of anti-*Plasmodium* effector genes (scorpine, PNPNA-1, salivary gland and midgut peptide 1).
- Effectors placed under control of hemolymph-specific promoter sequences.
- *Agrobacterium*-mediated transformation of *Metarhizium anisopliae*.
- Sporozoite inhibition assays.

Results

- All effectors significantly inhibited *Plasmodium* sporozoite levels in the mosquito salivary glands.
- Multiple effector genes expressed simultaneously had synergistic effects.
- Inhibition was initiated with very few fungal spores and was rapid and long lasting.

Conclusion

- Recombinant strains of the entomopathogenic fungus *Metarhizium anisopliae* can be used to efficiently suppress *Plasmodium* transmission by *Anopheles* mosquitoes.

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Bibliography

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Fang W, Vega-Rodríguez J, Ghosh AK, et al. Development of transgenic fungi that kill human malaria parasites in mosquitoes. *Science*. 2011; 331(6020):1074–1077. [PubMed: 21350178]
2. Hay SI, Guerra CA, Tatem AJ, et al. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect. Dis.* 2004; 4(6):327–336. [PubMed: 15172341]
3. Kantele A, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite. *Plasmodium knowlesi*. *Clin. Infect. Dis.* 2011; 52(11):1356–1362.
4. Fontenille D, Simard F. Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. *Comp. Immunol. Microbiol. Infect. Dis.* 2004; 27(5):357–375. [PubMed: 15225985]
5. Snow RW, Guerra CA, Noor AM, et al. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 2005; 434(7030):214–217. [PubMed: 15759000]
6. Ito J, Ghosh A, Moreira LA, et al. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature*. 2002; 417(6887):452–455. [PubMed: 12024215] • First published report of genetically modified malaria-resistant *Anopheles* mosquito.
7. Corby-Harris V, Drexler A, Watkins de Jong L, et al. Activation of Akt signaling reduces the prevalence and intensity of malaria parasite infection and lifespan in *Anopheles stephensi* mosquitoes. *PLoS Pathog.* 2010; 6(7):e1001003. [PubMed: 20664791] •• First report of genetic modification that completely blocked *Plasmodium falciparum* transmission in *Anopheles* mosquitoes.
8. Coutinho-Abreu IV, Zhu KY, Ramalho-Ortigao M. Transgenesis and paratransgenesis to control insect-borne diseases: current status and future challenges. *Parasitol. Int.* 2010; 59(1):1–8. [PubMed: 19819346]
9. Ren X, Hoiczky E, Rasgon JL. Viral paratransgenesis in the malaria vector *Anopheles gambiae*. *PLoS Pathog.* 2008; 4(8):e1000135. [PubMed: 18725926] • First report of paratransgenic virus in *Anopheles gambiae*.
10. Kanzok SM, Jacobs-Lorena M. Entomopathogenic fungi as biological insecticides to control malaria. *Trends Parasitol.* 2006; 22(2):49–51. [PubMed: 16377249]
11. Scholte EJ, Ng'habi K, Kihonda J, et al. An entomopathogenic fungus for control of adult African malaria mosquitoes. *Science*. 2005; 308(5728):1641–1642. [PubMed: 15947190] • Successful demonstration that entomopathogenic fungus can be used to kill *Anopheles* mosquitoes in a field trial.
12. Ghosh AK, Ribolla PE, Jacobs-Lorena M. Targeting *Plasmodium* ligands on mosquito salivary glands and midgut with a phage display peptide library. *Proc. Natl Acad. Sci. USA*. 2001; 98(23):13278–13281. [PubMed: 11687659]
13. Carballar-Lejarazú R, Rodríguez MH, de la Cruz Hernández-Hernández F, et al. Recombinant scorpine: a multifunctional antimicrobial peptide with activity against different pathogens. *Cell. Mol. Life Sci.* 2008; 65(19):3081–3092. [PubMed: 18726072]

14. Chappel JA, Hollingdale MR, Kang AS. IgG(4) Pf NPNA-1 a human anti-*Plasmodium falciparum* sporozoite monoclonal antibody cloned from a protected individual inhibits parasite invasion of hepatocytes. Hum. Antibodies. 2004; 13(3):91–96. [PubMed: 15598989]
15. Lomer CJ, Bateman RP, Johnson DL, et al. Biological control of locusts and grasshoppers. Annu. Rev. Entomol. 2001; 46:667–702. [PubMed: 11112183]

Table 1

Anti-*Plasmodium* effector molecules used to block malaria transmission in *Metarhizium anisopliae*-infected *Anopheles* mosquitoes.

Effector	Type	Source	Mode of action
Scorpine	Protein	Scorpion (<i>Pandinus imperator</i>)	Unknown
SM1 ₍₈₎	Small peptide (8-mer)	Phage-display library screen	Salivary gland receptor competition
PFNPNA-1	Single-chain antibody	Human anti- <i>Plasmodium</i> immune response	Sporozoite agglutination