## ORIGINAL PAPER

# Different mosquito species host *Wickerhamomyces anomalus* (*Pichia anomala*): perspectives on vector-borne diseases symbiotic control

Irene Ricci · Michela Mosca · Matteo Valzano · Claudia Damiani ·
Patrizia Scuppa · Paolo Rossi · Elena Crotti · Alessia Cappelli · Ulisse Ulissi ·
Aida Capone · Fulvio Esposito · Alberto Alma · Mauro Mandrioli ·
Luciano Sacchi · Claudio Bandi · Daniele Daffonchio · Guido Favia

**Abstract** The genetic manipulation of the microbial community associated with hematophagus insects is particularly relevant for public health applications. Within mosquito populations, this relationship has been overlooked until recently. New advances in molecular biotechnology propose the genetic manipulation of mosquito symbionts to prevent the transmission of pathogens to humans by interfering with the obligatory life cycle stages within the insect through the use of effector molecules. This approach, defined as 'paratransgenesis', has opened the way for the investigation and characterization of microbes residing in the mosquito body, particularly those localised within the gut. Some interesting bacteria have been identified as candidates for genetic modification, however, endosymbiotic yeasts remain largely unexplored with little information on the symbiotic relationships to date. Here we review the recent report of symbiotic relationship between *Wickerhamomyces anomalus* (*Pichia anomala*) and several mosquito vector species as promising methods to implement control of mosquito-borne diseases.

We refer to 'symbiotic relationship' as de Bary (1879) defined it and used the term 'endosymbiont' to mean an internal associate of the insect including the gut.

I. Ricci · M. Mosca · M. Valzano · C. Damiani ·
P. Scuppa · P. Rossi · A. Cappelli · U. Ulissi ·
A. Capone · F. Esposito · G. Favia (✉)
Scuola di Bioscienze e Biotecnologie, Università degli
Studi di Camerino, 62032 Camerino, Italy
e-mail: guido.favia@unicam.it

E. Crotti · D. Daffonchio
Dipartimento di Scienze e Tecnologie Alimentari e
Microbiologiche, Università degli Studi di Milano,
20133 Milan, Italy

A. Alma
Dipartimento di Valorizzazione e Protezione delle Risorse
Agroforestali, Università degli Studi di Torino,
10095 Turin, Italy

M. Mandrioli
Dipartimento di Biologia, Università degli Studi
di Modena e Reggio Emilia, 41125 Modena, Italy

L. Sacchi
Dipartimento di Biologia Animale, Università degli Studi
di Pavia, 27100 Pavia, Italy

C. Bandi
Dipartimento di Patologia Animale, Igiene e Sanità
Pubblica Veterinaria, Università degli Studi di Milano,
20133 Milan, Italy

## Introduction

Many invertebrate animals, particularly insects, have evolved with symbiotic microorganisms to expand their ecological niches within harsh environments. Symbiotic relationships in insects are widespread, with many of these having fungal associations. It is often suggested that such associations are mutualistic, with the fungus presumably providing nutritional supplements and the insect vector providing a habitat for the fungus (Vega and Dowd 2005). Yeast symbionts have been found in association with a broad range of insects including lacewings, wasps, bees and beetles (Gibson and Hunter 2005, 2009; Rosa et al. 2003; Torto et al. 2007; Benda et al. 2008). Invertebrates rely on microbes for various metabolic functions, including synthesis of amino acids, vitamins, lipids, sterols and pheromones, degradation of nutritional substrates, and detoxification of compounds (Suh et al. 2003). Well-known examples include lacewings that supplement the amino acids missing from their diet by co-existing with yeasts residing in crops, termites that use cultivated fungi to efficiently degrade dead plants, passalid beetles that acquire fungal enzymes to degrade woody plant parts and anobiid beetles that live in tobacco detoxified by yeast-like fungi (Gibson and Hunter 2005; Kudo 2009; Suh et al. 2003; Dowd 1989, 1991).

Little is known about yeast-symbionts in mosquitoes because most of the information concerns bacterial microorganisms such as *Wolbachia*, an endosymbiont able to interfere with host functions (Saridaki and Bourtzis 2010). In the last 10 years, the microbiota associated within the mosquito gut is under investigation largely because there is the possibility that these microorganisms might interfere with the capacity of disease transmission by insects (Riehle and Jacobs-Lorena 2005). Paratransgenic control of mosquito borne diseases intends to use safety microbes stably associated to the insect gut for genetic transformation aimed to interfere with the pathogens hosted in the mosquito body. This approach is considered more applicable than the already proposed genetic manipulation of the vector mosquito itself. Moreover, ethical issues about the release of genetically modified (GM) microbes imply minor concerns than GM mosquitoes. In fact, GM bacteria are currently used in many applications and regulations for their management already exist (Morrissey et al. 2002). Paratransgensis has been already proposed as an innovative way to control insect borne diseases. The triatomine *Rhodnius prolixus*, commonly known as kissing bug, is the vector of the parasitic protozoan *Trypanosoma cruzi* causative agent of the Chagas disease. This insect harbours in its intestinal tract bacteria that provide nutrients to its host and that have been cultivate outside the host, genetically modify to express anti-parasite effector molecules and place them back into the vector (Beard et al. 2001). This paratransgenic insect has shown to be incapable of transmitting the disease, posing the basis for the development of *paratransgenic-related* strategies to control insect borne diseases. Concerning malaria, few studies have already shown the feasibility of approaches based on mosquito guts bacteria to deliver anti-*Plasmodium* effector molecules within the insect (Riehle and Jacobs-Lorena 2005, Riehle et al. 2007). Many bacteria constitute the midgut microflora of some malaria vector mosquitoes (Dong et al. 2009; Meister et al. 2009; Kajla et al. 2010) but, to date, the only stable association with a mosquito host is that described for bacteria of the genus *Asaia* (Favia et al. 2007).

Unlike growing literature in symbiotic bacteria, the yeast microflora composition of mosquitoes remains largely unexplored. Ignatova et al. (1996) documented the isolation of certain *Candida* spp. from different mosquito species and recently, several members of *Pichia* and *Candida* genera have been found in the intestinal tract of the species *Aedes aegypti* (Gusmão et al. 2007, 2010), the mosquito vector of several viral infections including dengue and yellow fever. A major knowledge of the mosquito yeast microflora could clarify if specific roles described in other insect endosymbiotic yeast relationships maybe relevant for mosquitoes as well. Like other insects that feed only on phloem, or wood, or blood, mosquitoes can have a deficient diet, and thus need to augment their nutrition. Male mosquitoes feed exclusively on nectar and fruit juices, whereas hematophagy is a behavioural adaptation essential to females to efficiently complete the gonotrophic cycle, and the possibility of having a blood meal, required after the mating, depends on the availability of a prey (Bruce-Chwatt 1985). The ingestion of carbohydrate sources ensures highly

energetic activities such as flight, mating and host-seeking behaviour, yet it is essential for mosquitoes to acquire additional nutrients missing from the diet to satisfy other physiological needs related to certain anabolic pathways (e.g. gametogenesis). Thus, it is possible that yeast endosymbionts in mosquitoes can benefit their host nutritionally as happens in other insects.

In addition to nutritional supplementation insect-fungal endosymbionts may play other roles in the host biology, such as protection against parasitism and pathogens as largely documented for bacterial symbionts of insects (Moran et al. 2008). This already has been described in the crab *Purtunus trituberculatus* (Wang et al. 2007) that has properties indicating the presence of killer yeast strains. It is possible to suppose a similar implication in mosquitoes as well.

Interestingly, yeasts could also take part in driving some behavioural traits of the insects as oviposition sites selection and host-seeking. Phelan and Lin (1991) revealed that certain beetles are attracted from yeast-associated fermentation volatiles serving as oviposition cues. Concerning mosquitoes, it is known that *Aedes aegypti* females are attracted to oviposition sites by biotic factors originated in the mosquito larvae infected with *Candida* spp. (Reeves 2004). These findings support previous evidence indicating that environmental conditions of the larvae play an important role in regulating the larval ingestion rate, for example, some yeasts released phagostimulant factors able to accelerate the ingestion rate of particulate materials by mosquito larvae (Rashed and Mulla 1989). Thus, the ability of a female mosquito to perceive some environmental stimuli for the oviposition site selection seems to represent an adaptive advantage. It is possible these signals could also drive some mechanisms of host-seeking, for examples volatile organic compounds produced by human skin microbiota act as odours attractive to the malaria vector *Anopheles gambiae* when in search for a blood meal (Verhulst et al. 2009, 2010).

Considering both the contribution to understanding mosquito biology and the potential application for control of vector-borne diseases, for some years we have focused our interest on the characterisation of microbial residents, bacteria and yeasts, associated with different vector mosquito species. Analysis of mosquito organs by transmission electron microscopy
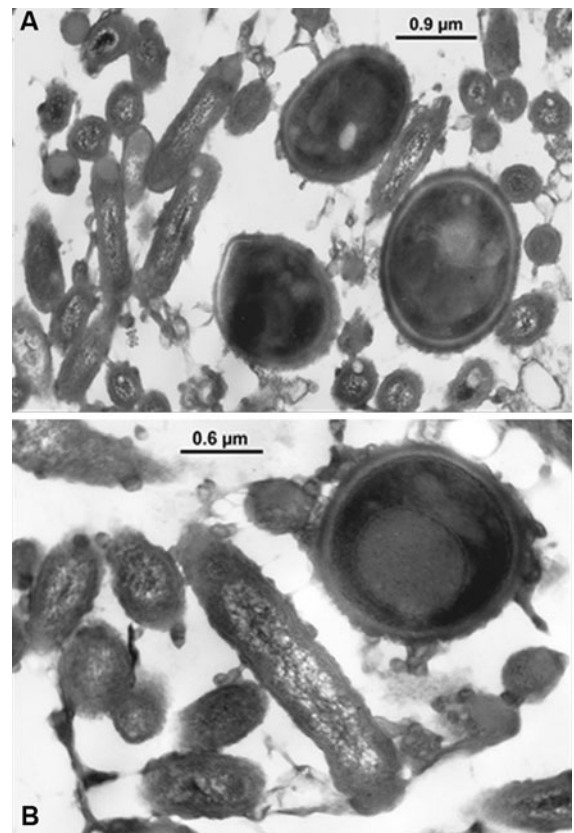


**Fig. 1** Bacteria and yeasts observed by TEM in the midgut lumen of female mosquitoes (**a**, **b**) of the malaria vector species *An. gambiae*

(TEM) detected plenty of bacteria and yeasts in the alimentary canal as well as in the reproductive system (Figs. 1, 2). During these studies, bacteria of the genus *Asaia* have been identified as symbionts inhabiting the mosquito midgut and gonads; both sites are considered very interesting for biological control of pathogens within the vector (Favia et al. 2007, 2008; Damiani et al. 2008, 2010; Crotti et al. 2009). In the same organs we characterised residing yeasts, focusing mainly on *Wickerhamomyces anomalus* (*Pichia anomala*) (Ricci et al. 2010, accepted for publication), that is already known for its biotechnological potential (Walker 2010). We consider the detection of this 'promising' yeast in the body of different mosquitoes species responsible for human pathogen transmission opens interesting possibilities for a potential tool for symbiotic control application of mosquito-borne diseases and offers new insights in the comprehension of mosquito biology.
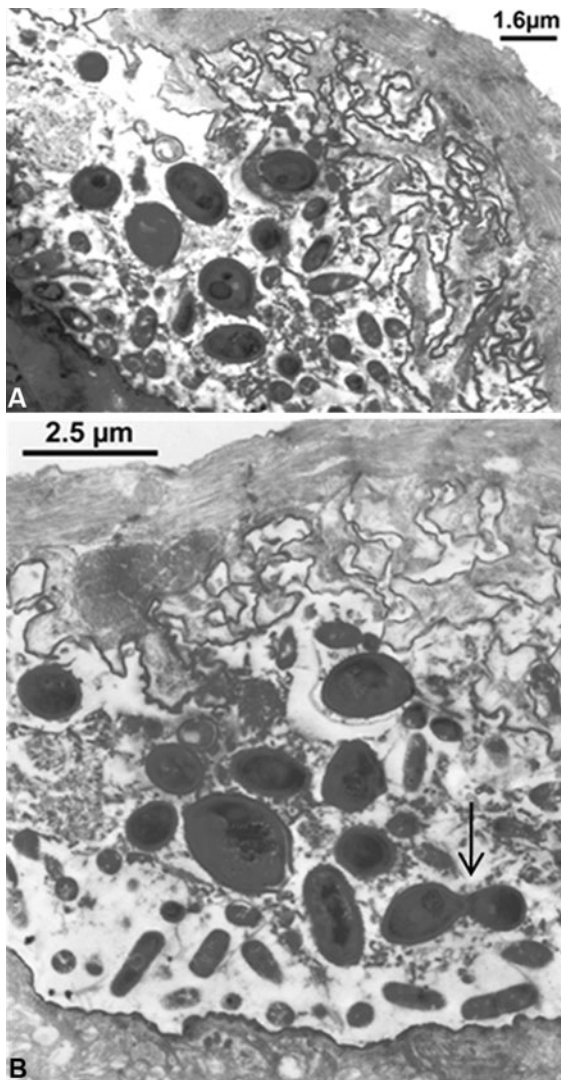
**Fig. 2** TEM images **a**, **b** showing yeasts and bacteria in the gonoducts of male mosquitoes of the malaria vector species *An. stephensi*. *Arrow* in *panel* **b** shows a budding yeast

### *Wickerhamomyces anomalus* in different mosquito species vectoring diseases

Our investigations aim to describe the yeast community associated with four mosquito vectors relevant to human public health, *Anopheles stephensi*, *Anopheles gambiae*, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). These species occur in different geographic localities, vary in the pathogens they are able to transmit and differ in some ecological and behavioural traits, including host specialization and the degree of antropophily (Klowden and Briegel 1994). The two anopheline species analysed, *An. stephensi* and *An. gambiae*, are the primary malaria vectors in Asia and Africa, respectively; *Ae. aegypti* and *Ae. albopictus* (the Asian tiger mosquito) are vectors of several viral diseases (such as yellow fever, dengue, encephalitis and chikungunya) but not human malaria (Paupy et al. 2009). By both cultural-dependent and independent methods we have identified some yeasts, including *Wickerhamomyces anomalus*, associated with the four mosquito species analysed. Further analysis has been carried out to better characterise the relationship between *W. anomalus* and the mosquitoes.

PCR and fluorescence in situ hybridisation specific assays to detect *W. anomalus* revealed the presence of the yeast in different mosquito populations and tissues. Diverse laboratory reared colonies and field collected mosquitoes, belonging to the four studied species, have been used for analysis. The following data emerged from these screenings: (i) the occurrence of *W. anomalus* in different vector mosquito species and in both lab-reared and wild-collected samples from different insectaries (two from Italy, one from Africa) and field collection sites (Italy and Africa) (see Table 1); (ii) the presence of the yeast in the majority of the individuals analysed both males and females (between 65 and 80%, but larger screenings for field populations are needed); (iii) the presence of the yeast in all mosquito developmental stages, from early larvae to pupae and adults; (iv) *W. anomalus* retrieval in both male and female gut and gonads of all the four mosquito species analysed.

The identification of *W. anomalus* in mosquitoes is of interest since this yeast has been found in a variety of habitats (Passoth et al. 2006) but has not yet been explored within any mosquito population. Within other insect populations *W. anomalus* has been isolated from insect frass in Ponderosa pine (Kurtzman 2001), and a screening for yeasts in different insects from agricultural areas revealed the presence of this species only within the inner body of *Drosophila* sp. (Diptera: Drosophilidae), suggesting that *W. anomalus* is not a ubiquitous microorganism in insects with different diet or habitats (Zacchi and Vaughan-Martini 2002). A substantial amount of work on the physiology and genetics of this yeast has been carried out (Passoth et al. 2006) and its use has already been proposed as a

**Table 1** List of different mosquito species and populations investigated for the presence of *W. anomalus*

| Species of mosquitoes analysed | Rearing insectaries and field collection sites | Positive/Total* |
| --- | --- | --- |
| *Anopheles stephensi* | Lab-reared colonies (A) and (B) | 107/150 (A), 33/50 (B) |
| *Anopheles gambiae* | Lab-reared colonies (A) and (C), wild samples (D) | 97/150 (A), 35/50 (C), 18/27 (D) |
| *Aedes albopictus* | Lab-reared colony (A), wild samples (E) | 120/150 (A), 18/25 (E) |
| *Aedes aegypti* | Lab-reared colony (A), wild samples (D) | 118/150 (A), 17/25 (D) |

*Locations* (*A*) insectary of Camerino, (*B*) another Italian insectary, (*C*) African insectary, (*D*) collection site in Africa and (*E*) collection site in Italy. * 50 individuals per three reared generations have been tested from (*A*), tests from other locations referred to single samplings

suitable means of bio-control for applications in the food industry (Palpacelli et al. 1991; Michalcakova et al. 1993; Shepherd et al. 1995; Chen et al. 1998). All these features make *W. anomalus* a very attractive candidate for potential use in the symbiotic control of mosquito borne diseases, in this context it is worth to consider that is generally supposed as non-threatening to humans, although in very rare cases it has been reported as an opportunistic pathogen (Thuler et al. 1997; Kalenic et al. 2001; Pasqualotto et al. 2005).

## *Wickerhamomyces anomalus* colonises in the midgut and gonads of mosquitoes

Occurrence of yeasts or yeast-like particles within the mosquito body is frequently observed during microscopic analysis of dissected organs, especially the gut, but these data have always been underestimated or considered only as an environmental contaminant. Generally the insect gut may be structurally easier to colonize with yeasts. Their presence in the gonads, however, could reflect an adaptive characteristic to supplement nutrients essential for gametogenesis, particularly to augment fecundity. Interestingly, the insect gonads are characterised by a thick system of tracheal trunks that could represent an advantage for meeting oxygen demands (Gibson and Hunter 2005). These findings strongly support the hypothesis that the gonads of the mosquito could be a niche for mutualistic relationships.

The unusual localization of *W. anomalus* in the mosquito reproductive system is an original finding with few reports describing yeasts in the gonads of other insects. One example is the ovary of the leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae), where yeast-like endosymbionts are found in both the follicular cells and the eggs, and are transovarially transmitted to the offspring (Sacchi et al. 2008). This kind of transmission of yeasts in insects has been hypothesised rarely, as in the case of the lacewings *Chrysoperla* (Gibson and Hunter 2005) and some sawflies whose eggs are deposited in willow leaves to induce galls that contain a specific yeast, *Ogataea cecidiorum* (Glushakova et al. 2010).

The presence of *W. anomalus* in the mosquito ovary suggests a possibility of vertical transmission from parents to offspring by eggs infected during the gonotrophic cycle. Larvae possibly could be contaminated by yeasts during hatching by ingestion of the egg chorion as they chew their way out. Furthermore, *W. anomalus* was detected in all the developmental stages of the mosquito, reinforcing the hypothesis of vertical transmission from mother to offspring as already demonstrated for bacteria of the genus *Asaia* in different insect species (Favia et al. 2007, 2008; Damiani et al. 2008, 2010; Crotti et al. 2009). These findings indicate that mosquitoes of the genus *Anopheles* and the leafhopper *S. titanus* may represent very rare cases of insects that have vertical transmission by the eggs with both prokaryotic and eukaryotic symbionts.

Even though possible functional roles of *W. anomalus* in the mosquito body are still to be assessed, potential benefits to mosquitoes harbouring this yeast can be hypothesised. We assume *W. anomalus* has a nutritional function in the mosquito host, particularly for insects consuming a largely carbohydrate diet. Other functions cannot be excluded as, for example, a protective role against pathogenic fungi to mosquitoes. Some *W. anomalus* killer strains have demonstrated antifungal activity against a wide range of fungi and, more generally, can inhibit harmful microorganisms in a great variety of habitats (Polonelli 2000; Passoth et al. 2006). Marine strains of *W. anomalus* have been identified

as killer yeasts, acting against pathogenic fungal strains in the crab *P. trituberculatus* (Wang et al. 2007, 2008). Interestingly, preliminary findings showed that *W. anomalus* isolates from *An. stephensi* possessed possible anti-fungal killer toxin mediated activity in vitro, with possible implications for protection in this mosquito.

Anyway, the presence of some uninfected individuals in each generation of the mosquito populations analysed seems to indicate a not-obligated relationship between *W. anomalus* and mosquitoes, suggesting that this yeast may be a facultative endosymbiont.

## *W. anomalus* as a tool for vector-borne diseases control

The detection of *W. anomalus* in different species of mosquito vectors and its localization in both the insect gut and gonads, open a discussion of an alternative means in which to fight vector-borne infectious diseases by reducing or eliminating vector competence of the insect. Additionally, the ease with which this yeast can be cultured may aid in its potential molecular manipulation, with the advantage to use eukaryotic microorganisms for the delivery of effector molecules directed against pathogens.

On these bases, it is now possible to think of developing methods of mosquito-borne disease control through the manipulation of this yeast to produce in situ anti-pathogen effectors molecules that are able to interfere with pathogens development (Riehle and Jacobs-Lorena 2005). Generally, this kind of approach is termed paratransgenesis and examples of effective paratransgenic approaches for vector-borne disease control have already been achieved (Beard et al. 2001; Durvasula et al. 2008). Several potential molecules have already been identified and tested for their ability to interfere with the transmission of malaria parasite *Plasmodium* sp. (Riehle et al. 2007). The localization of *W. anomalus* in the midgut of the *Anopheles* is intriguing since the midgut phases of the malaria parasite development represents a remarkable bottleneck in the malaria cycle in which the parasite cell numbers are very low with respect to other parasite life-stages in the mosquito (Beier 1998). Interference with the parasite within the midgut would be more effective leading to significant reduction of transmission rates.

In addition, the localization in the female reproductive system suggests a vertical route of transmission for *W. anomalus*, which is a desirable characteristic for microorganisms to use in biocontrol. In fact, even though, vertical transmission of *W. anomalus* to the progeny has not been demonstrated yet, there is the potential to spread recombinant yeasts in field populations. Moreover, since the yeast is present also in the male testes and gonoducts, it may be transmitted to females during mating, thus additional horizontal spreading mechanisms among mosquitoes could occur, as previously demonstrated for the mosquito symbiont *Asaia* (Damiani et al. 2008).

Importantly, *W. anomalus* has already been proposed as an agent for the bio-control of several microorganisms (Fredlund et al. 2002) and killer toxins from *W. anomalus* producer strains have shown an antimicrobial effect on a variety of human pathogens (Séguy et al. 1998; Magliani et al. 2001), including arthropod-transmitted protozoan parasites such as *Leishmania* spp. (Savoia et al. 2002).

Although further investigations will address the question, can *W. anomalus* act directly against pathogens harboured by the mosquitoes, the potential of using *W. anomalus* as an agent for the control of mosquito-borne diseases warrants targeted studies to that effect.

## References

Beard CB, Dotson EM, Pennington PM, Eichler S, Cordon-Rosales C, Durvasula RV (2001) Bacterial symbiosis and paratransgenic control of vector-borne Chagas disease. Int J Parasitol 31:621–627

Beier JC (1998) Malaria parasite development in mosquitoes. Annu Rev Entomol 43:519–543

Benda N, Boucias D, Torto B, Teal P (2008) Detection and characterisation of *Kodamaea ohmeri* associated with small hive beetle *Aethina tumida* infesting honey bee hives. J Apic Res 47:193–200

Bruce-Chwatt LJ (1985) Essential Malariology, 2nd edn. William Heinemann Medical Books, London

Chen HL, Su HP, Lin CW (1998) Characterization of yeast cultures for a flavouring agent in a yoghurt-type product. J Food Sci 63:897–900

Crotti E, Damiani C, Pajoro M, Gonella E, Rizzi A, Ricci I, Negri I, Scuppa P, Rossi P, Ballarini P, Raddadi N, Marzorati M, Sacchi L, Clementi E, Genchi M, Mandrioli M, Bandi C, Favia G, Alma A, Daffonchio D (2009) Asaia, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders. Environ Microbiol 11:3252–3264

Damiani C, Ricci I, Crotti E, Rossi P, Rizzi A, Scuppa P, Esposito F, Bandi C, Daffonchio D, Favia G (2008) Paternal transmission of symbiotic bacteria in malaria vectors. Curr Biol 18:1087–1088

Damiani C, Ricci I, Crotti E, Rossi P, Rizzi A, Scuppa P, Capone A, Ulissi U, Epis S, Genchi M, Sagnon N, Faye I, Kang A, Chouaia B, Whitehorn C, Moussa GW, Mandrioli M, Esposito F, Sacchi L, Bandi C, Daffonchio D, Favia G (2010) Mosquito-Bacteria Symbiosis: The Case of Anopheles gambiae and Asaia. Microbiol Ecol. doi: 10.1007/s00248-010-9704-8

de Bary A (1879) The Phenomenon of Symbiosis. Karl J Trubner, Strasbourgh

Dong Y, Manfredini F, Dimopoulos G (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLoS Pathog 5:e1000423

Dowd PF (1989) In situ production of hydrolytic detoxifying enzymes by symbiotic yeasts of cigarette beetle (Coleoptera: Anobiidae). J Econ Entomol 82:396–400

Dowd PF (1991) Symbiont-mediated detoxification in insect herbivores. In: Barbosa P, Krischik VA, Jones CG (eds) Microbial Mediation of Plant–Herbivore Interactions. John Wiley & Sons, New York, pp 411–440

Durvasula RV, Sundaram RK, Kirsch P, Hurwitz I, Crawford CV, Dotson E, Beard CB (2008) Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector Triatoma infestans. Exp Parasitol 119:94–98

Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M et al (2007) Bacteria of the genus Asaia stably associate with Anopheles stephensi, an Asian malarial mosquito vector. Proc Natl AcadSci U S A 104:9047–9051

Favia G, Ricci I, Marzorati M, Negri I, Alma A, Sacchi L, Bandi C, Daffonchio D (2008) Bacteria of the genus Asaia: a potential paratransgenic weapon against malaria. Adv Exp Med Biol 627:49–59

Fredlund E, Druvefors U, Boysen ME, Lingsten KJ, Schnürer J (2002) Physiological characteristics of the biocontrol yeast Pichia anomala J121. FEMS Yeast Res 2:395–402

Gibson CM, Hunter MS (2005) A reconsideration of the role of yeast associated with Chrysoperla lacewings. Biol Contr 32:57–64

Gibson CM, Hunter MS (2009) Negative fitness consequences and transmission dynamics of a heritable fungal symbiont in a parasitic wasp. Appl Environ Microbiol 75: 3115–3119

Glushakova AM, Maximova IA, Kachalkin AV, Yurkov AM (2010) Ogataea cecidiorum sp. nov., a methanol-assimilating yeast isolated from galls on willow leaves. Antonie Van Leeuwenhoek 98:93–101

Gusmão DS, Santos AV, Marini DC, Russo Ede S, Peixoto AM, Bacci Júnior M et al (2007) First isolation of microorganisms from the gut diverticulum of Aedes aegypti (Diptera: Culicidae): new perspectives for an insect-bacteria association. Mem Inst Oswaldo Cruz 102: 919–924

Gusmão DS, Santos AV, Marini DC, Bacci M Jr, Berbert-Molina MA, Lemos FJ (2010) Culture-dependent and culture-independent characterization of microorganisms associated with Aedes aegypti (Diptera: Culicidae) (L.) and dynamics of bacterial colonization in the midgut. Acta Trop 115:275–281

Ignatova EA, Nagornaia SS, Povazhnaia TN, Ianishevskaia GS (1996) The yeast flora of blood-sucking mosquitoes. Mikrobiol Z 58:12–15

Kajla MK, Andreeva O, Gilbreath TM III, Paskewitz SM (2010) Characterization of expression, activity and role in antibacterial immunity of Anopheles gambiae lysozyme c-1. Comp Biochem Physiol B Biochem Mol Biol 155: 201–209

Kalenic S, Jandrlic M, Vegar V, Zuech N, Sekulic A, Mlinaric-Missoni E (2001) Hansenula anomala outbreak at a surgical intensive care unit: a search for risk factors. Eur J Epidemiol 17:491–496

Klowden MJ, Briegel H (1994) Mosquito gonotrophic cycle and multiple feeding potential: contrasts between Anopheles and Aedes (Diptera: Culicidae). J Med Entomol 31:618–622

Kudo T (2009) Termite-microbe symbiotic system and its efficient degradation of lignocellulose. Biosci Biotechnol Biochem 73:2561–2567

Kurtzman CP (2001) Four new Candida species from geographically diverse locations. Antonie Van Leeuwenhoek 79:353–361

Magliani W, Conti S, Arseni S, Frazzi R, Salati A, Polonelli L (2001) Killer anti-idiotypes in the control of fungal infections. Curr Opin Investig Drugs 2:477–479

Meister S, Agianian B, Turlure F, Relógio A, Morlais I, Kafatos FC, Christophides GK (2009) Anopheles gambiae PGRPLC-mediated defense against bacteria modulates infections with malaria parasites. PLoS Pathog 5: e1000542

Michalcakova S, Sulo P, Slavikova E (1993) Killer yeasts of Kluyveromyces and Hansenula genera with potential application in fermentation and therapy. Acta Biotechnol 4:341–350

Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet 42:165–190

Morrissey JP, Walsh UF, O'Donnell A, Moënne-Loccoz Y, O'Gara F (2002) Exploitation of genetically modified inoculants for industrial ecology applications. Antonie Van Leeuwenhoek. 81:599–606

Palpacelli V, Ciani V, Rossini G (1991) Activity of different killer yeasts on strains of yeast species undesirable in the food industry. FEMS Microbiol Lett 68:75–78

Pasqualotto AC, Sukiennik TC, Severo LC, de Amorim CS, Colombo AL (2005) An outbreak of Pichia anomala fungemia in a Brazilian pediatric intensive care unit. Infect Control Hosp Epidemiol 26:553–558

Passoth V, Fredlund E, Druvefors UA, Schnürer J (2006) Biotechnology, physiology and genetics of the yeast Pichia anomala. FEMS Yeast Res 6:3–13

Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009) *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes Infect 11:1177–1185

Phelan PL, Lin HC (1991) Chemical characterization of fruit and fungal volatiles attractive to dried-fruit beetle, *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae). J Chem Ecol 17:1253–1272

Polonelli L (2000) Human, monoclonal and recombinant candidacidal, pneumocysticidal and mycobactericidal antibodies. Indian J Chest Dis Allied Sci 42:259–263

Rashed SS, Mulla MS (1989) Factors influencing ingestion of particulate materials by mosquito larvae (Diptera: Culicidae). J Med Entomol 26:210–216

Reeves WK (2004) Oviposition by *Aedes aegypti* (Diptera: Culicidae) in relation to conspecific larvae infected with internal symbiotes. J Vector Ecol 29:159–163

Ricci I, Damiani C, Scuppa P, Mosca M, Crotti E, Rossi P, Rizzi A, Capone A, Gonella E, Ballarini P, Chouaia B, Sagnon N, Esposito F, Alma A, Mandrioli M, Sacchi L, Bandi C, Daffonchio D, Favia G. (2010) The yeast *Wickerhamomyces anomalus* (*Pichia anomala*) inhabits the midgut and reproductive system of the Asian malaria vector *Anopheles stephensi*. Environ Microbiol (in press)

Riehle MA, Jacobs-Lorena M (2005) Using bacteria to express and display anti-parasite molecules in mosquitoes: current and future strategies. Insect Biochem Mol Biol 35:699–707

Riehle MA, Moreira CK, Lampe D, Lauzon C, Jacobs-Lorena M (2007) Using bacteria to express and display anti-*Plasmodium* molecules in the mosquito midgut. Int J Parasitol 37:595–603

Rosa CA, Lachance MA, Silva JO, Teixeira AC, Marini MM, Antonini Y, Martins RP (2003) Yeast communities associated with stingless bees. FEMS Yeast Res 4:271–275

Sacchi L, Genchi M, Clementi E, Bigliardi E, Avanzati AM, Pajoro M, Negri I, Marzorati M, Gonella E, Alma A, Daffonchio D, Bandi C (2008) Multiple symbiosis in the leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae): details of transovarial transmission of *Cardinium* sp. and yeast-like endosymbionts. Tissue Cell 40:231–242

Saridaki A, Bourtzis K (2010) *Wolbachia*: more than just a bug in insects genitals. Curr Opin Microbiol 13:67–72

Savoia D, Avanzini C, Conti S, Magliani V, Frazzi R, Polonelli L (2002) In vitro leishmanicidal activity of a monoclonal antibody mimicking a yeast killer toxin. J Eukaryot Microbiol 49:319–323

Séguy N, Polonelli L, Dei-Cas E, Cailliez JC (1998) Effect of a killer toxin of *Pichia anomala* to *Pneumocystis*. Perspectives in the control of pneumocystosis. FEMS Immunol Med Microbiol 22:145–149

Shepherd R, Rockey J, Sutherland IW, Roller S (1995) Novel bioemulsifiers from microorganisms for use in foods. J Biotechnol 40:207–217

Suh SO, Marshall CJ, McHugh JV, Blackwell M (2003) Wood ingestion by passalid beetles in the presence of xylose-fermenting gut yeasts. Mol Ecol 12:3137–3145

Thuler LC, Faivichenco S, Velasco E, Martins CA, Nascimento CR, Castilho IA (1997) Fungaemia caused by *Hansenula anomala*-an outbreak in a cancer hospital. Mycoses 40:193–196

Torto B, Boucias DG, Arbogast RT, Tumlinson JH, Teal PE (2007) Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. Proceedings of the National Academy of Sciences of the USA 104:8374–8378

Vega FE, Dowd PF (2005) The role of yeasts as insects endosymbionts. In: Vega FE, Blackwell M (eds) Insect-fungal associations: ecology and evolution. Oxford University Press, Oxford, United Kingdom, pp 211–243

Verhulst NO, Beijleveld H, Knols BG, Takken W, Schraa G, Bouwmeester HJ, Smallegange RC (2009) Cultured skin microbiota attracts malaria mosquitoes. Malar J 8:302

Verhulst NO, Takken W, Dicke M, Schraa G, Smallegange RC (2010) Chemical ecology of interactions between human skin microbiota and mosquitoes. FEMS Microbiol Ecol. doi:10.1111/j.1574-6941.2010.00908.x

Walker JM (2010) *Pichia anomala*: cell physiology and biotechnology relative to other yeasts. Antonie van Leeuwenhoek (this volume) (in press)

Wang X, Chi Z, Yue L, Li J, Li M, Wu L (2007) A marine killer yeast against the pathogenic yeast strain in crab (*Portunus trituberculatus*) and an optimization of the toxin production. Microbiol Res 62:77–85

Wang L, Yue L, Chi Z, Wang X (2008) Marine killer yeasts active against a yeast strain pathogenic to crab *Portunus trituberculatus*. Dis Aquat Organ 80:211–218

Zacchi L, Vaughan-Martini A (2002) Yeast associated with insects in agricultural areas of Perugia. Ann Microbiol 52:237–244