

Intestinal bacteria/mutualistic symbionts of triatomines – a review

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Zusammenfassung: Bei den mehr als 150 Arten der Unterfamilie Triatominae (Reduviidae, Hemiptera) saugen alle postembryonalen Stadien Blut. In Lateinamerika übertragen sie *Trypanosoma cruzi* (Trypanosomatidae, Kinetoplastida), den Erreger der Chagas Krankheit, der sich im Darmlumen der Vektoren entwickelt. Dort finden sich ebenfalls Bakterien, die z. T. eine symbiontische Funktion haben. Bei kultivierungsabhängigen Untersuchungen über Ausstriche auf Nährager und der Isolierung werden makro- und mikroskopische Charakteristika, die Gram-Färbung, physiologische Tests und biochemische Api-Systeme zur Identifizierung eingesetzt. Bei Triatominen aus dem Freiland finden sich starke individuelle Unterschiede und bei *Panstrongylus megistus* und *Triatoma infestans* bis zu 16 verschiedene Isolate, bei *Triatoma vitticeps* und *T. klugii* nach kurzer Haltung der Triatominen im Insektarium bis zu 24 Isolate. Da bei *Rhodnius prolixus* das einzige Bakterium mit symbiontischer Funktion ein Aktinomyzeten ist, werden diese Bakterien zusätzlich über die Mykolsäuren und die Sequenzen der 16S rDNA identifiziert. Bei den vier genannten Triatominen treten bis zu fünf Aktinomyzeten auf sowie bei *Triatoma sordida* aus dem Insektarium sieben. Bei kultivierungsunabhängigen Untersuchungen von verschiedenen Triatominen-Arten aus dem Freiland zeigen sich bei den Metagenom-Analysen der Sequenzen der 16S rDNA ebenfalls starke individuelle Variationen, bis zu 1142 Bakterien-Arten und fast immer Aktinomyzeten. Volle symbiontische Funktionen haben bei *P. megistus* ein *Rhodococcus equi*-like Isolat, bei *T. sordida* eine *Gordonia* sp. und bei *T. infestans* *Rhodococcus triatomae*.

Key words: Triatominae, bacteria, mutualistic symbionts, actinomycetes

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Introduction

Trypanosoma cruzi, the causative agent of Chagas disease, is transmitted in Latin America by bloodsucking triatomines. In addition to this flagellate, bacteria were found very early in the intestinal tract (reviewed by VALLEJO & al. 2009). These are transmitted via coprophagy. After identification of the mutualistic symbiont of the triatomine *Rhodnius prolixus*, *Rhodococcus rhodnii*, a function as vitamin B supplier has been postulated, because aposymbiosis effects, such as retarded development of late nymphal instars, increased nymphal mortality rates, disturbed digestion and diuresis, has been reduced in aposymbiotic nymphs after feeding blood supplemented with vitamins B or mutualistic symbionts. However, in investigations of the eight B-group vitamins, different vitamins have been suggested to be produced (reviewed by VALLEJO & al. 2009). In addition, feeding different auxotrophic mutants of *R. rhodnii*, which are unable to synthesize different B-group vitamins, all nymphs develop normal (HILL & al. 1976), an argument against the vitamin B hypothesis. Also an intra- and/or extracellular development of mutualistic symbionts remains questionable (reviewed by VALLEJO & al. 2009). Therefore, we investigate the localisation and development of the intestinal bacteria, identify the intestinal bacteria via cultivation-dependent methods and identify the mutualistic symbiont of the respective species of triatomine. Since the mutualistic symbionts of other triatomines can fulfil a symbiotic function in the breeding of triatomines, the intestinal bacteria have to be isolated from specimens originating from the field (GUMPERT 1962).

Localisation and development of intestinal mutualistic symbionts

In light and electron microscopy, symbionts are present in all three midgut regions of the intestinal tract: a short midgut region, the cardia, in front of the distensible anterior midgut, the stomach, and the narrow midgut, the small intestine (DREWS 1988). The wall of the midgut is composed as a single layer of cells. However, the wall of the cardia is modified possessing higher cells than the cells in the other regions. In many cells, a narrow channel stretches from the lumen to the basal part of the cell enlarging to a vacuole at the end, containing symbionts. According to serial sections, the vacuoles are always connected to the lumen of the cardia (DREWS 1988). Presumably, this modification protects the symbionts against the complement factors present in the ingested blood.

The two regions of the anterior midgut, the cardia and the stomach, are important for the development of the symbionts. Within the first 12 hours after feeding, the number of colony forming units of the mutualistic symbionts of *R. prolixus* and *Triatoma infestans* is reduced by 50–60%. Within 7 days it increases 30- or 80-fold in cardia and stomach of *R. prolixus*, respectively, and 26- or 94-fold in *T. infestans*, respectively. However, these concentrations are considerably reduced by the passage from the stomach to the small intestine (EICHLER & SCHAUB 2002). This development and the killing of the mutualistic symbionts is surprising since the antibacterial activity in the stomach is much higher than in the small intestine (BALCZUN & al. 2008). Those surviving and passed to the rectum are relevant for the coprophagic infection.

Identification of intestinal bacteria and mutualistic symbionts

In cultivation-dependent methods, after plating on nutrient agar and the macroscopic and microscopic classification, bacteria possessing different characteristics are isolated, cultivated and classified via Gram-staining, physiological tests (oxidase, catalase, oxidation/fermentation) and sometimes also api-systems. Isolates resembling actinomycetes are further classified by an analysis of mycolic acids and a sequencing of the 16S rDNA. Eight *Panstrongylus megistus* from the field in Brazil contain 13 different isolates, some of them occurring only in single bugs, some of them in more or all (FISCHER 1989): *Achromobacter* gr., *Pseudomonas* sp., *Arthrobacter globiformis*, *Actinomyces* sp., *Enterococcus faecalis*, *Bacillus subtilis*, *Aureobacterium* sp., *Staphylococcus xylosum* (four different), *Rhodococcus equi*-like and *Corynebacterium pseudodiphthericum*, only the two latter belonging to the actinomycetes. 12 adults/fifth instar nymphs of *T. infestans* from the field (Cochabamba; Bolivia) are colonized by 16 isolates, three of them classified as actinomycetes (REINTJES 1993): *Bacillus coagulans*, *B. licheniformis*, *B. sphaericus*, *B. circulans*, *B. macerarium*, *B. megaterium*, *Bacillus* sp. (two different), *Streptomyces* sp., *Moraxella nonliquefaciens*, *Cellulomonas* sp., *Micrococcus varians*, *Candida* sp., *Dietzia maris* and *Nocardia* sp. (two different). The sequences of the two *Nocardia*-strains isolated by REINTJES (1993) are identical with those denominated as *Rhodococcus triatomae* by YASSIN (2005). Using species investigated after a short period of maintenance in the laboratory, *Triatoma sordida* contains seven actinomycetes (other bacteria not considered) (EICHLER & al. 1996; EICHLER 1998), *Triatoma vitticeps* 24 isolates inclusive 5 actinomycetes (ROGL 2001), *Triatoma klugi* 21 isolates inclusive one actinomycete (HOFFMANN 2002) and *Triatoma brasiliensis* 18 isolates inclusive four actinomycetes (HOFFMANN 2002). The actinomycetes belong to the genera *Rhodococcus*, *Dietzia*, *Gordonia* and *Nocardia*, with *Gordonia terrae* occurring regularly. Using cultivation-independent methods, DNA of intestines is isolated to sequence 16S rDNA for metagenomic approaches. Using *Triatoma brasiliensis*, *T. pseudomaculata*, *T. maculata*, *T. dimidiata*, *T. sanguisuga*, *T. protracta* and *Rhodnius pallescens* from the field, again the diversity between individual specimens of the same species is evident (GUMIEL & al. 2015, MONTROYA-PORRAS & al. 2018, ORANTES & al. 2018, RODRIGUEZ-RUANO & al. 2018, OLIVEIRA & al. 2018). 1,142 bacterial species but no actinomycetes are present in *T. dimidiata* (ORANTES & al. 2018), but the other identifications indicate many actinomycetes, belonging to different genera.

To classify bacteria as mutualistic symbionts, eggs of the triatomines are disinfected, mainly using 1.5% calcium hypochlorite and 1.5% Chloramin T in ethanol. The aposymbiotic nymphs are kept axenic in the first and second instar and fed with pig blood. In the third instar, they are infected via sheep blood, each group receiving a mixture with a different isolate, mostly an actinomycete. This is only possible

after cultivation-dependent identifications. Bacteria with symbiotic function should establish in the intestinal tract and after feeding sheep blood in the fourth and fifth nymphal instar allow moulting to the imago. After feeding sheep blood to the adults, reproduction rates in groups possessing mutualistic symbionts should be the same as in the original colonies. In such approaches, monoxenous systems of mutualistic symbionts and triatomine species are identified. With the exception of *Rhodococcus rhodnii* in *R. prolixus*, all were unknown: *Rhodococcus triatoma* in *T. infestans*, a *Rhodococcus equi*-like isolate in *P. megistus* and *Gordonia* sp. in *T. sordida* (FISCHER 1989, REINTJES 1993, EICHLER & al. 1996, EICHLER 1998, YASSIN 2005). Some of the other bacteria enable the nymphal development, but do not allow a good reproduction. In triatomines from the field a high number of different bacteria develop, but mutualistic symbionts do not necessarily have to be present, if the nymphs feed on mice or chicken. So far, all mutualistic symbionts are actinomycetes.

Conclusions

This review clearly shows the extracellular development of mutualistic symbionts in *T. infestans* and emphasizes the necessity to identify the symbiotic compounds delivered to the triatomine. Since so far all mutualistic symbionts are actinomycetes, their unique compound, mycolic acids, should be considered. The comparison of the intestinal bacteria demonstrates the strong differences between individuals from the same location and between different species. During coprophagy or during probing before blood sucking different bacteria get access to the intestine. Some are killed by antibacterial compounds, but some can establish in the intestinal tract. The characteristics of mutualistic symbionts present in the rectum and those digested in the small intestine should be evaluated. Mutualistic symbionts should be identified for more species of triatomines, and their interactions with other intestinal bacteria and *T. cruzi* should be investigated.

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