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Metabolômica de *Aphis citricidus* (Kirkaldy) (Hemiptera: Aphididae) infectado por *Spiroplasma*

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Dissertação apresentada para obtenção do título de Mestre em Ciências. Área de concentração: Entomologia

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RESUMO

Metabolômica de *Aphis citricidus* (Kirkaldy) (Hemiptera: Aphididae) infectado por *Spiroplasma*

A associação de diferentes seres vivos favoreceu a exploração de novos hábitats e outras fontes de alimento, contribuindo nos processos de adaptação e de diversificação biológica. Essa associação, em que duas espécies passam a viver juntas, é denominada simbiose, e as associações de simbiose podem resultar em relações benéficas (mutualismo), neutras (comensalismo) ou prejudiciais (parasitismo) aos organismos envolvidos. Insetos estabelecem associações de simbiose diversas com outros organismos, como leveduras, vírus, protistas e bactérias. Dentre esses organismos, as bactérias são as mais comumente associadas a insetos. Elas podem se localizar no interior do corpo do hospedeiro, tanto no espaço extracelular ou endocelular (endossimbionte), assim como associadas externamente ao corpo do hospedeiro (ectossimbionte). Endossimbiontes são os que mais diretamente influenciam a fisiologia e o metabolismo do hospedeiro, tornando-se, em alguns casos, essenciais à sobrevivência de seu hospedeiro. Exemplo disso são as bactérias endossimbiontes associadas a pulgões, *Buchnera aphidicola*, que são responsáveis pela síntese de aminoácidos essenciais e vitaminas para complementar os recursos nutricionais explorados pelos pulgões, seiva de plantas, que normalmente apresentam baixo valor nutricional. Mas vários outros simbiontes, mesmo que não estabelecendo essa obrigatoriedade na relação com o hospedeiro, acabam influenciando inúmeros aspectos da bioecologia de seus pulgões hospedeiros, como, por exemplo, a capacidade de defesa de seus hospedeiros a inimigos naturais. *Spiroplasma* são bactérias que influenciam o sistema de reprodução de borboletas, moscas-das-frutas, joaninhas e pulgões, além de influenciar a resposta de seus hospedeiros ao ataque de inimigos naturais, como parasitoides e nematoides. Porém, pouco se sabe sobre os efeitos desse simbionte no metabolismo de seus hospedeiros. Assim, este trabalho teve por objetivo avaliar o efeito de infecções por *Spiroplasma* no metaboloma de seu hospedeiro *Aphis citricidus* (Kirkaldy) (Hemiptera: Aphididae). As análises foram conduzidas utilizando-se de isolinhagens irmãs de *A. citricidus*, infectadas ou não por *Spiroplasma.* Fêmeas adultas de *A. citricidus*, infectadas ou não por *Spiroplasma*, foram coletadas (25 mg/amostra), submetidas aos processos de extração com solução de clorofórmio, metanol e água (1:3:1) e derivatização com n-metil-n-(trimetilsilil)trifluoroacetamida e trimetilclorosilano, para posterior análise por cromatografia gasosa acoplada à espectrometria de massas (GCMS-TOF) para a detectação de metabólitos. Após tratamento e análise dos dados obtidos, foi observado que 40 metabólitos foram afetados significativamente em pulgões infectados por *Spiroplasma*. Dentre os metabólitos de *A. citricidus* alterados pela infecção por *Spiroplasma*, merecem destaque os hidrocarbonetos cuticulares, compostos nitrogenados e ácidos graxos. Os efeitos metabólicos da interação *Aphis citricidus – Spiroplasma* revelou informações importantes sobre a influência do endossimbionte na composição química de seus hospedeiros.

Palavras-chave: Composição química; Interação Inseto-Simbionte; Metabolismo; Simbionte secundário; Simbiose

ABSTRACT

Metabolomics of *Aphis citricidus* (Kirkaldy) (Hemiptera: Aphididae) infected with *Spiroplasma*

The association between different living beings favored the exploitation of new habitats and food sources, contributing to adaptation and biological diversity. This association in which two species live together is named symbiosis, and associations of symbiosis can result in beneficial (mutualism), neutral (comensalism) or prejudicial (parasitism) interactions. Insects established a diverse range of associations with other organisms, such as yeasts, virus, protists and bacteria. Bacteria are by far the most commonly associates of insects. Bacteria can be located within the host body, either at the extra or intracellular space (endosymbionts), as well as on the body surface (ectosymbionts). Endosymbionts are the ones that most influence the host physiology and metabolism, and they may even become essential for the host survival. An example of such dependency is the aphid symbiont *Buchnera aphidicola* that synthesis essential amino acids and vitamins to complement the natural diet of host, which are of a low nutritional value. But many other symbionts that are not essential do the host can affect a number of bioecological aspects of their host aphids, as the host immune capacity to natural enemies. *Spiroplasma* are bacteria that can influence the reproduction system of butterflies, fruit flies, ladybugs and aphids, and interfere with the host immune response to parasitoids and nematodes. However, little is known of the effects of secondary symbionts on their host's metabolism. Thus, this work is aimed to detect the effects of *Spiroplasma*infection on the metabolomics of the apid host *Aphis citricidus* (Kirkaldy) (Hemiptera: Aphididae). Analysis were conducted using sister isolines *A. citricidus*, infected or not with *Spiroplasma.* Infected and uninfected adult females were collected from lab colonied (25 mg/samples), subjected to metabolite extraction in chloroform, methanol and water (1:3:1) and derivatization with n-methyl-n-(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane, followed by metabolite analysis in a gas chromatography coupled with a mass spectrometer (GCMS-TOF). After filtering and data analysis, 40 metabolites were observed in different intensities in between infected and uninfected aphids. The aphid's metabolites influenced by infection with *Spiroplasma* indicated the regulation of the metabolism of fatty acids, hydrocarbons and nitrogen compounds. The metabolic effects of the interaction *Aphis citricus – Spiroplasma* revealed interesting information on the host metabolism regulation by a secondary symbiont and on the role these changes may play on the associations of the host with other organisms.

Keywords: Chemical composition; Insect-Simbionte interaction; Metabolism; Secondary symbiont; Symbiosis

INTRODUÇÃO

Insetos são amplamente associados a microrganismos que influenciam o seu modo de vida e suas interações com outros níveis tróficos (Ferrari & Vavre, 2011). As bactérias estão entre os microrganismos mais comumente associados a insetos e várias das associações insetos-bactérias estabelecidas evoluíram para relações de mutualismo obrigatório (Rio et al., 2004; Sanchez-Contreras & Vlisidou, 2008). Devido à interdependência dos organismos envolvidos nessas associações, vários são os estudos que se debruçaram para identificar a contribuição metabólica oferecida pela bactéria e/ou as relações nutricionais/moleculares entre inseto-simbionte para a manutenção da simbiose (Buchner, 1965; Douglas, 1989; Douglas, 1998; Moran et al., 2008). No entanto, existe também um grande número de associações com bactérias que são facultativas, e que mesmo assim influenciam vários aspectos da biologia e ecologia de seu hospedeiros (Montllor et al., 2002; Russel & Moran, 2006; Oliver et al., 2009; Burke et al., 2010; Xie et al., 2010; Kikuchi et al., 2012). Mas os mecanismos e os efeitos das interações insetos – simbiontes secundários são conhecidos para um reduzido número de bactérias, merecendo destaque as bactérias que interferem nos mecanismos de reprodução dos hospedeiros, como *Wolbachia*, por exemplo (Stouthamer et al., 1999).

Pulgões apresentam interações com bactérias obrigatórias, *Buchnera aphidicola*, e um grupo diverso de bactérias simbiontes facultativas, sendo que todas elas desenvolveram estratégias para sua transmissão transovariana, garantindo sua associação com a progênie produzida (Braig, 1994; Moran & Baumann, 1994; Moya, 2009). *Buchnera aphidicola* é essencial ao pulgão por produzir aminoácidos essenciais e vitaminas, enquanto os diferentes simbiontes facultativos podem influenciar os processos de seleção hospedeira, o desenvolvimento de raças adaptadas à planta hospedeira, a resposta imunológica a patógenos e parasitoides, entre outros (Douglas, 1998; Leonardo & Muiru, 2003; Xie et al., 2011; Farahani et al., 2015; Moran et al., 2015).

A interação pulgão – *B. aphidicola* está entre as interações inseto-simbionte mais bem conhecidas, já tendo sido descrito o mecanismo molecular e a comunicação metabólica existentes (Shigenobu et al., 2000; Wilson et al., 2010). No entanto, pouco se sabe sobre as interações e os efeitos das associações com simbiontes facultativos no metabolismo de pulgões hospedeiros, apesar da existência de informações genômicas para alguns desses simbiontes (Carle et al., 1995; Kondo et al., 2002).

Apesar de *Spiroplasma* já ter sido relatado associado a algumas espécies de pulgões (Fukatsu et al., 2001; Guidolin & Cônsoli, 2017), esse simbionte é mais comumente estudado em associações com *Drosophila* (Williamson et al, 1999; Montenegro et al., 2005; Xie et al., 2010). Nas associações em que é estudado, *Spiroplasma* é descrito afetando o modo de reprodução do hospedeiro, principalmente por causar a eliminação de machos (Hursrt et al., 1999; Jiggins etal., 2000). No entanto, na associação *Spiroplasma* - *Drosophila hydei*, esse simbionte ofereceu maior proteção ao hospedeiro ao ataque de inimigos naturais (Xie et al., 2010).

Estudos comparativos do metabolismo de insetos sujeitos a diferentes condições de estresse auxiliam na compreensão dos eventos fisiológicos envolvidos, sendo possível a obtenção de um grande número de informações sobre o funcionamento do organismo (Overgaard et al., 2007). Análises metabolômicas auxiliaram na identificação das alterações metabólicas causadas pelo estresse e envelhecimento de *Ceratitis capitata* (Pujol-Lereis et al., 2016), choque térmico em *Drosophila melanogaster* (Malmendal et al., 2006), pela infecção de *Apis mellifera* por *Nosema ceranae* (Aliferis et al., 2012), além de auxiliar na identificação dos metabólitos secretados por *Metarhizium* e *Beauveria* em processos de infecção (De Bekker et al., 2013).

Dada à lacuna existente nos estudos dos efeitos da interação *Spiroplasma*-pulgões, a alta incidência desse simbionte em *Aphis citricidus* indica que essa associação se mostra como um modelo biológico promissor para se estudar os efeitos desse simbionte no seu hospedeiro através de estudos comparativos de metabolômica. Assim, nessa dissertação utilizamos de ferramentas de análises multivariadas dos metabólitos produzidos por isolinhagens irmãs de *A. citricidus,* infectadas ou não por *Spiroplasma*, para identificar as alterações metabólicas induzidas por *Spiroplasma* no hospedeiro e, dessa forma, buscar decifrar os efeitos dessas alterações na bioecologia do pulgão, contribui para o entendimento da interação pulgão – *Spiroplasma*.

CONCLUSÕES

- *Spiroplasma* altera o metaboloma *Aphis citricidus;*
- *Spiroplasma* interfere negativamente no metabolismo de ácidos graxos de *Aphis citricidus;*
- *Spiroplasma* interfere positivamente no metabolismo de hidrocarbonetos de *Aphis citricidus;*
- *Spiroplasma* interfere no metabolismo de compostos nitrogenados de *Aphis citricidus,* interferindo no metabolismo associado de *Aphis citricidus – Buchnera aphidicola*.

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