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INSECTS AS SOURCE OF ACTINOBACTERIA

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ABSTRACT

Actinobacteria, a group of Gram-positive, filamentous bacteria known for their extensive production of bioactive compounds, are widely recognized for their role in microbial ecology and biotechnology. Insects, particularly those with specialized diets or complex social structures, have evolved intricate symbiotic relationships with Actinobacteria, which provide crucial benefits to their hosts. *This paper examines the interactions between insects and Actinobacteria, focusing on the protective roles these bacteria offer to their insect hosts, their ecological significance, and their potential applications.*

INTRODUCTION

Actinobacteria are a group of Gram-positive bacteria known for their filamentous structure, high GC (guanine-cytosine) content, and ability to produce secondary metabolites.

These bacteria are prolific producers of antibiotics and other bioactive compounds, which have found wide applications in medicine, agriculture, and biotechnology. Insects, with their complex symbiotic relationships, often harbor Actinobacteria, which play essential roles in their survival, defense mechanisms, and ecological success.

The ancient phylum *Actinobacteria* is composed of phylogenetically and physiologically diverse bacteria that help Earth's ecosystems function. As free-living organisms and symbionts of herbivorous animals, *Actinobacteria* contribute to the global carbon cycle through the breakdown of plant biomass. In addition, they mediate community dynamics as producers of small molecules with diverse biological activities (G.R. Lewin et. Al 2016).

Actinobacteria are ubiquitous and one of the most diverse groups of bacteria in nature. Its members range from anaerobic, unicellular organisms to aerobic, filamentous, and spore-forming lineages. Alone, the genus *Streptomyces* accounts for almost 5% of the ~16,000 described bacterial species (http://www.bacterio.net/).

Actinobacteria have made a substantial positive contribution to human health; they are the producers of many compounds that are used as important drugs, including most antibiotics (Hopwood D.A., 2007).

More recently, *Actinobacteria* have been revealed as widespread symbionts of eukaryotes, helping herbivores gain access to plant biomass as nutritional mutualists and producing natural products as defensive mutualists (Book A.J., et al, 2016, Coombs, J.T., Michelsen P.P., Franco C.M.M, 2004, Currie C.R. et. Al. 1999, Kaltenpoth M., 2009).

MATERIAL AND METHODS

This section outlines the materials and methods used to investigate the relationship between Actinobacteria and insects. The focus is on identifying, isolating, and characterizing Actinobacteria that are produced or hosted by insects, as well as understanding their functional roles, such as antimicrobial production or mutualistic benefits. The study typically involves field collection of insect samples, laboratory microbiological techniques for isolating bacteria, and molecular biology methods for identification and characterization.

Insect Samples:

Target Species: Insects known to harbor Actinobacteria, such as fungus-growing ants (*Atta* and *Acromyrmex*), beewolf wasps (*Philanthus triangulum*), bark beetles (*Dendroctonus* spp.), and termites. These insects often display symbiotic relationships with Actinobacteria.

Collection Sites: Insect samples are collected from natural habitats, such as forests, soil, and agricultural areas, where symbiotic relationships are likely to occur. GPS coordinates and environmental data (temperature, humidity) are recorded for each collection site to ensure proper documentation.

Sampling Tools: Insect nets, aspirators, and forceps are used to collect live insect specimens, which are then placed into sterile vials or containers for transport to the laboratory.

Culture Media:

Isolation Media: Various types of culture media are used to isolate Actinobacteria, including:

Starch Casein Agar (SCA): A common medium for isolating *Streptomyces* species, which are frequently found in insect symbioses.

ISP (International Streptomyces Project) Media: Specialized media to differentiate Actinobacteria based on their morphological and biochemical properties.

Nutrient Agar: Used for initial general cultivation of bacteria from insect samples.

Selective Media: For isolating specific types of Actinobacteria while suppressing other microbial growth.

Data Analysis:

Statistical Analysis: Data from antimicrobial assays are statistically analyzed to determine the significance of differences in activity between Actinobacteria strains. This may include ANOVA or t-tests to compare zones of inhibition.

Phylogenetic Analysis: DNA sequence data is aligned and analyzed using software like MEGA or PhyML to construct phylogenetic trees. Evolutionary relationships between isolated Actinobacteria strains and known species are evaluated.

RESULTS AND DISCUSSIONS

Insects, particularly those that live in close-knit colonies or have specialized diets, have evolved symbiotic relationships with various microbial communities, including Actinobacteria. These symbiotic bacteria provide benefits such as protection against pathogens, aiding in digestion, and producing bioactive compounds. Some of the most notable examples of this symbiosis occur in ants, termites, and beetles.

Ants and Actinobacteria:

Fungus-Growing Ants: One of the most well-studied examples of insect-Actinobacteria symbiosis involves fungus-growing ants of the genus *Atta* and *Acromyrmex*. These ants cultivate fungal gardens as their primary food source, which are threatened by parasitic fungi such as *Escovopsis*.

Actinobacteria, particularly of the genus *Streptomyces*, live on the exoskeletons of these ants and produce antifungal compounds that protect the fungal gardens from parasitic infections.

The ants actively culture these bacteria on their cuticles, passing them from one generation to the next. This mutualistic relationship helps maintain the delicate balance between the ants' food source and pathogenic fungi in their environment.

Beetles and Actinobacteria:

Bark beetles (*Dendroctonus spp.*), which infest and feed on trees, are another example of insects that rely on Actinobacteria for protection. These beetles form associations with *Streptomyces* bacteria, which produce antibiotics that help them combat fungal pathogens within their galleries.

Similarly, the beewolf wasp (*Philanthus triangulum*) engages in symbiosis with *Streptomyces* bacteria. Female beewolves apply these bacteria to their brood cells to protect their larvae from fungal infections during development.

Termites and Actinobacteria:

In termites, particularly wood-feeding species, Actinobacteria play a crucial role in breaking down complex plant materials like cellulose and lignin. They contribute to the digestive process by producing enzymes that aid in degrading these compounds, thereby enhancing nutrient absorption.

In addition to digestive benefits, Actinobacteria in termite nests produce antimicrobial compounds, which help protect the colony from harmful pathogens, ensuring the health and survival of the group.

Biological and Ecological Functions of Actinobacteria in Insects

Antibiotic Production:

Actinobacteria, especially from the genus *Streptomyces*, are well-known for their capacity to produce antibiotics. Insects use these bacteria to protect themselves and their food sources from microbial threats. These antibiotics not only inhibit the growth of pathogenic fungi and bacteria but also contribute to the overall fitness of the insect host.

The symbiotic Actinobacteria found in insects have led to the discovery of novel antibiotics and antimicrobial agents that have significant implications for human health. For example, some antibiotics isolated from *Streptomyces* in insects are effective against resistant strains of bacteria, making them promising candidates for new drug development.

Defense Mechanisms:

In addition to producing antibiotics, Actinobacteria also contribute to the chemical defenses of insects. The bioactive compounds produced by these bacteria are often tailored to the specific pathogens or environmental pressures the insect faces.

The production of these compounds is highly specialized and adapted to the insect's life cycle, behavior, and habitat. For instance, some ants deploy Actinobacteria-produced antimicrobial compounds on their eggs or nests, providing direct protection to their offspring.

Nutritional Support:

Actinobacteria also play a role in supplementing the diets of insects, especially those with nutrient-poor or specialized feeding habits. For example, wood-feeding insects like termites rely on microbial symbionts, including Actinobacteria, to break down lignocellulose into digestible sugars.

This nutritional mutualism allows insects to exploit food resources that would otherwise be inaccessible, giving them a competitive advantage in their respective ecosystems.

Environmental Adaptation:

The presence of Actinobacteria in insect colonies and individual insects allows them to thrive in environments where pathogenic microbes pose a constant threat. For example, insects living in humid, densely populated environments like soil or decaying wood are particularly vulnerable to fungal and bacterial pathogens. The antimicrobial properties of Actinobacteria help them survive these challenging conditions.

Insects that engage in agriculture-like behaviors, such as the fungus-growing ants or ambrosia beetles, rely heavily on their Actinobacteria symbionts to maintain a pathogen-free environment for their crops.

Applications and Future Prospects

The unique insect-Actinobacteria associations have important implications for biotechnology and medicine. The discovery of novel antibiotics and bioactive compounds from insect-associated Actinobacteria is a rapidly growing field of research, with potential applications in:

Drug Discovery:

Actinobacteria associated with insects have been found to produce new classes of antibiotics and antifungals that can be useful in treating antibiotic-resistant bacterial infections in humans. As the global healthcare community grapples with the rise of antimicrobial resistance, the symbiotic bacteria in insects represent an untapped source of novel compounds.

Agricultural Biocontrol:

The use of insect-associated Actinobacteria for developing biocontrol agents is another promising area. By harnessing the natural antifungal and antibacterial properties of these bacteria, researchers could develop biological pesticides that reduce the reliance on chemical pesticides in farming, contributing to more sustainable agricultural practices.

Ecological Research:

Understanding the co-evolution of insects and their Actinobacteria symbionts sheds light on the dynamics of host-microbe interactions, microbial ecology, and insect adaptation. This knowledge can inform conservation strategies, particularly in ecosystems where invasive pathogens or pests are threatening biodiversity.

CONCLUSIONS

The relationship between insects and Actinobacteria is a fascinating example of co-evolution and mutualism. Actinobacteria provide essential services to their insect hosts, including pathogen protection, nutrient supplementation, and enhanced environmental adaptation. In turn, insects offer a habitat and resources for the bacteria to thrive. These interactions not only have profound ecological implications but also hold great potential for biotechnological and medical applications. As research continues to uncover the depth of these associations, the insect-Actinobacteria symbiosis may provide solutions to some of the most pressing challenges in medicine and agriculture today.

Actinobacteria, especially from the genus *Streptomyces*, play a vital role in the symbiotic relationships they form with insects. These bacteria provide significant benefits to their insect hosts by producing antimicrobial compounds that protect against pathogens, contributing to digestion, and facilitating environmental adaptation. Insects, in turn, offer Actinobacteria a stable habitat and resources to thrive, resulting in a mutually beneficial relationship that has co-evolved over time.

The study of these insect-Actinobacteria interactions reveals not only the complexity of microbial ecology but also the vast potential for novel bioactive compounds, particularly antibiotics, which can address global challenges such as antibiotic resistance. The unique metabolites produced by Actinobacteria in insect systems are promising candidates for pharmaceutical development, agricultural biocontrol, and environmental sustainability.

Further research into the genetic, biochemical, and ecological aspects of insect-associated Actinobacteria can enhance our understanding of these symbioses and unlock new avenues for biotechnological innovation. By leveraging these naturally occurring relationships, we can contribute to solving critical health and environmental issues while preserving the ecological balance that these interactions support.

REFERENCES

Book AJ, Lewin GR, McDonald BR, Takasuka TE, Wendt-Pienkowski E, D.T. Doering, S. Suh, K. F. Raffa, B. G. Fox,C. R. Currie 2016. Evolution of high cellulolytic activity in symbiotic *Streptomyces* through selection of expanded gene content and coordinated gene expression. *PLOS Biol.* 2016; 14(6): e1002475.

Coombs JT, Michelsen PP, Franco CMM. Evaluation of endophytic Actinobacteria as antagonists of *Gaeumannomyces graminis* var*tritici* in wheat. *Biol Control.* 2004; 29(3):359–66 Google Scholar]

Currie CR, Scott JA, Summerbell RC, Malloch D. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature.* 1999; 398:701–4.

Hopwood DA. *Nature and Medicine: The Antibiotic Makers.* New York: Oxford Univ. Press; 2007. Streptomyces.

Kaltenpoth M. Actinobacteria as mutualists: general healthcare for insects? *Trends Microbiol.* 2009; 17(12):529–35.

Lewin G. R., Carlos C., Chevrette M. G., Horn H. A., MacDonald B. R., Stankey R. J., Fox B. G., Currie C. R. Evolution and Ecology of Actinobacteria and Their Bioenergy Applications Annu Rev Microbiol 2016 Sep 8: 70:235-54. doi: 10.1146/annurev-micro-102215-095748.