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Selection of antagonistic actinobacteria for Fusarium graminearum control

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Short Report

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Abstract

Fungi of the genus *Fusarium* cause damage in different types of crops such as tomatoes, onions, bananas, among others, causing different diseases such as wilt disease or *Fusarium* Head Blight (FHB). Recent studies show that *Fusarium graminearum* has a high resistance to fungicide and alternative treatment needs to be searched. There are different ways to treat diseases caused by *Fusarium*, between them, the most relevant is the use of biological control agents. The use of actinobacteria as a biological control agent is considered promising, due to the fact that the most important and known characteristic of this bacteria is the production of secondary metabolites and bioactive compounds. In the present study, eleven actinobacteria isolates were identified by morphological characteristics and submitted to antifungal assay against two isolates of *Fusarium graminearum*. As a result of the preliminary identification one isolate was identified as *Nocardia spp.*, seven isolates as *Streptomyces spp.* and three remained unidentified morphologically. The antagonistic assay was performed using the plate confrontation method, against the two strains of *Fusarium graminearum*. The isolate LMAF, previously identified as *Streptomyces avidinii*, showed activity against both *Fusarium graminearum* isolates. The relation between the pathogen and the *Streptomyces avidinii* LMAF remain unsure, due to the fact that it can be direct, where the isolate is producing substances that control the growth, or indirect, where both are competing for nutrients and space. Both cases lead to the potential use of isolate LMAF as a biological control agent.

Introduction

The soil is a diverse environment and shelters an ecosystem, mainly composed of microorganisms. Bacteria, Archaea and Fungi play a fundamental role in soil maintenance, because it recycles organic matter through metabolic processes. Actinomycetota stand out as one of the most prevalent phyla within the soil microbiota, frequently exhibiting high relative abundance in subterranean environments. They are responsible for the maintenance of soil's carbon reserve and macronutrients through extracellular enzymes that play a fundamental role in organic matter degradation[1, 2, 3].

Actinobacteria belong to the Actinomycetota phylum, considered one of the biggest taxonomic units among bacteria. They are Gram-positive bacteria that contain high levels of guanine and cytosine (GC) in its genome[4]. They grow in a branched manner through the extension of their hyphae, forming mycelial structures and reproduce asexually through spores[5]. Its spore chains can present different conformations (short, long, spiral, etc.) and, through optical microscopy, these chains can be used for morphological identification.

Actinobacteria are renowned for their prolific capacity to synthesize a diverse array of secondary metabolites and bioactive compounds of significant biotechnological value [6], establishing them as a cornerstone of research across multiple applications. In the pharmaceutical industry, these secondary metabolites are indispensable, as the structural diversity of the substances produced encompasses a vast majority of clinically relevant antibiotics[7]. Furthermore, within the agricultural sector, their

metabolic repertoire plays a critical role in modulating microbial ecosystems; specifically, Actinobacteria serve as potent biological control agents by suppressing the proliferation of various phytopathogens, thereby safeguarding plant health and enhancing crop productivity[8].

Fungi of the genus *Fusarium* are members of the phylum Ascomycota, which has more than 1500 species. They can cause diseases in different types of crops such as onions, tomatoes, bananas, among others[9]. A known way to deal with *Fusarium* contamination, as it is less harmful to the environment and human consumption, is the use of biological control agents[10].

The aim of the present work is to select actinobacteria with the potential to be used as biological control agents against *Fusarium graminearum*.

Material and Methods

Isolates

In this work, 11 isolates of actinobacteria named as: Actinobacteria LMAF, LMA 1, LMA 2,LMA 3, LMA4, LMA 5, LMA 6,LMA 7,LMA 8,LMA, 9, LMA 10 (Table I) were tested against *Fusarium graminearum* isolates. The actinobacteria were isolated from soil of the Farroupilha park (located in the city of Porto Alegre, Rio Grande do Sul, Brazil) and belong to bacteria library of the Laboratório de Microbiologia Aplicada at the Universidade Federal do Rio Grande do Sul (UFRGS).

Morphological characterization of Actinobacteria isolates

The morphological characterization of the actinobacteria colonies was carried out based on the International *Streptomyces* Project[11] using the International *Streptomyces* Project medium 2 (ISP2) (4 g yeast extract, 10 g malt extract, 4 g dextrose, 15 g agar, 1000 ml distilled water). Actinobacteria were streaked in Petri dishes with ISP2 medium and then incubated at 28ºC for 7 days. The following parameters were analyzed: the color of the colonies on the substrate, color of the spores in the aerial mycelium and edges of the isolated colonies. Images of the colonies were captured with a 50 megapixel camera.

Microstructure morphological characterization

To observe the microscopic structures of actinobacteria spore chains, microcultures were carried out in ISP2 and Starch Casein Agar (SCA) culture media (2 g KNO3, 2 g NaCl, 2 g K2HPO4, 0.05 mg MgSO4, 0.01 g FeSO4, 0.02 g CaCO3, 10 g starch, 0.3 g casein, 15 g agar, 1000 ml distilled water). The microculture plates were incubated at 28ºC for 7 days[11']. The morphology of the spore chains were evaluated for identification at genus level, according to *Bergey's Manual of Determinative Bacteriology*[12]. The images were captured using a camera (MD90 - MShot) attached to the microscope.

Antifungal assay

Actinobacteria samples were previously prepared in SCA culture medium. The fungi were previously incubated in Potato Dextrose Agar (PDA) culture medium (1000 ml distilled water; 140g potato broth; 15g Agar; 10g Dextrose). Two strains of *Fusarium graminearum* were used, named FUG4 and FUG09M134. To observe the antifungal activity of actinobacteria against de *Fusarium* strains, the plate confrontation method was used. For this purposes plates with PDA medium were inoculated at the center with a fungal agar plug, and at equidistant points (2.5cm) from the center four actinobacterial agar plugs were inoculated. The triplicate samples were incubated at 28ºC for 7 days, so that the fungal growth radius could be analyzed, according to Zou et al., (2021). The percentage of inhibition was calculated with the formula: Inhibition = [(C - T) / C] x 100, where C is the growth of the fungus in control, and T is the growth of the fungus in treatment.

Results and Discussion

The morphological characterization of the eleven actinobacteria isolates was conducted by growing them in ISP2 medium. During this process, four different colors were observed in the aerial mycelia, three different colors for the colonies, and two types of edges were observed in all colonies (Table I). Actinobacteria belong to a very diverse group of bacteria is comprised most of soil microbiomes. It has been estimated that only 10% of the known actinobacteria have been isolated from soil. Many of these actinobacteria are found in rhizospheric soil, where they can be associated with plant roots and can provide protection against fungi and other microorganisms[14]. Actinobacteria have the ability to produce secondary metabolites and can be used as biological control agents. Therefore, they are a group of great importance for the agricultural field, as they can help to combat plant diseases[13]. Due to the high diversity of these microorganisms, it is necessary to identify this group in order to achieve more accurate results.

Regarding the microstructure morphological characterization, according to the results obtained the actinobacteria isolate LMA 7 did not form spore chains; instead, fragmentation of the aerial mycelium was observed (Fig. 1c). Isolates LMA 2, LMA 3, and LMA 10 did not form enough spore chains for characterization through morphology of the spore chain. All other isolates were similar in terms of their shape and number of spores (Fig. 1d), with small variations in the angle of the spiral. In this study, SCA was used as it is a less nutritious culture medium than ISP2, forming colonies more likely to produce spores and secondary metabolites. Microcultures carried out in SCA medium formed less fragmented and more abundant spore chains. Studies show that the morphology of the spore chain is a very important tool to identify actinobacteria[15]. In addition, pigment production is an important characteristic of this group, and can be used to help identification[14], which is why the color of the colonies and the aerial mycelium were recorded.

According to *Bergey's Manual of Determinative Bacteriology*, seven of eleven isolates are likely to belong to genus *Streptomyces*, due to their morphological characteristics in microculture such as the formation

of spirals in the spore chains. Given the results, a dominance in the occurrence of the genus *Streptomyces* can be clearly observed. Among different types of habitat, soil is one of the best known for this genus, which has more than 850 species already described[7]. Due to its high diversity and abundance in the soil, the number of isolates belonging to this genus is expected to be high. Actinobacteria isolate LMA 7 was identified as *Nocardia* spp. (Table II) because in addition to not showing spore chains it presented fragmentation of the aerial mycelium. Recent studies showed that this genus is capable of producing antibiotics and can even be effective in controlling *Candida albicans*[16, 17].

From the eleven isolates tested against both *F. graminearum* strains in the antifungal assay, one actinobacteria sample, LMAF, showed effectiveness in controlling the growth of both *F. graminearum*. LMAF showed 33% with FUG4 **(**Fig. 2a**)** and 40% of inhibition in the assay with FUG09M134 (Fig. 2d). Fungi of the genus *Fusarium* are known to cause *Fusarium* wilt, among other diseases, which leads to loss of productivity[18]. There are different ways to treat diseases caused by *Fusarium*, such as the use of post-grow copper to treat the disease[19], or pre-seeding treatments with essential oils to reduce the effects of the disease in the fully grown plant[20]. A different way to control diseases caused by *Fusarium* is the use of biological control agents. In the study by Jing et al., (2020) the use of *Streptomyces* isolates as biological control agents against banana wilt disease, caused by the fungus *Fusarium oxysporum*, was demonstrated.

Phenotypic and molecular identification of strain LMAF were previously established via MALDI-TOF MS and 16S rRNA gene analysis (using primers pA/pH and the Neighbor-Joining method in MEGA X), showing high similarity with *Streptomyces avidinii* (accession number: PP410358) (Data not yet published). Biological control agents protect plants from diseases in different ways. Indirectly, they can induce resistance in plants or compete for space and nutrients with the pathogen. Directly, they can parasitize pathogens or produce antibiotics[22]. According to the results, the interaction between the potential biological control agent and the pathogen is difficult to determine, since there may be the production of antibiotics or it may just be a competition for space and nutrients. In both possibilities there is interaction; therefore, biological control is plausible.

The fungus *F. graminearum* is part of a complex of species known to cause different types of diseases, such as fusariosis or blight, and affect different types of crops, such as corn, rice and wheat[23]. The most common way to combat this fungus is the use of fungicides, but recent studies showed that there is a high occurrence of fungicide-resistant *F. graminearum* [24]. Taking into account the damage caused by *F. graminearum*, and the great potential of actinobacteria to produce various compounds that can be useful in controlling fungal growth, it is necessary to identify new and different biological control agents, as well as the identification of actinobacteria that can perform this function. According to our results, isolate LMAF, previously identified as *Streptomyces avidinii*, showed activity against the two *F. graminearum* tested, which suggests a potential for use as a biological control agent. The use of species

of this genus without a fully identification (species level) was shown in the work of Mattei et al. (2022), where spores of *Streptomyces* sp. were used as biological control against *F. graminearum*.

Conclusion

Isolate LMAF has the potential to be used as a biological control agent. The relationship between actinobacteria and fungi remains undetermined, and may be an indirect interaction, where the bacteria are simply competing for space and nutrients. It also can be direct, where there is production of substances that prevent the growth of fungi. More biochemical, genetic and chemical assays are necessary for more accurate identification of natural products and its production inducers. Thus, the isolate can go through new stages of testing to better evaluate its potential.

Declarations

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Conflicts of interest/Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

Not applicable. This study did not involve human participants or animals.

Consent to participate

Not applicable.

Consent for publication

The authors affirm that all participants provided informed consent for the publication of the data presented in this manuscript.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Declaration of deposition in repositories

Not applicable.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by Yuri Borges Véras and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table I Colony Morphology

Isolate	Aerial mycelium color	Colony color	Edge
LMAF	Yellow	Yellow	Irregular
LMA 1	Yellow	Yellow	Irregular
LMA 2	White	Yellow	Round
LMA 3	Yellow	Yellow	Irregular
LMA 4	White	Yellow	Round
LMA 5	White	Brown	Round
LMA 6	Light Brown	Dark Brown	Irregular
LMA 7	White	Yellow	Round
LMA 8	White	Yellow	Irregular
LMA 9	Grey	Yellow	Round
LMA 10	Yellow	Yellow	Irregular

Table II Spore chain morphological identification		
Isolate	Spore chain	Genus
LMAF	Spira	Streptomyces
LMA 1	Spira	Streptomyces
LMA 2	-	W/I
LMA 3	-	W/I
LMA 4	Spira	Streptomyces
LMA 5	Spira	Streptomyces
LMA 6	Spira	Streptomyces
LMA 7	Fragmented mycelium	Nocardia
LMA 8	Spira	Streptomyces
LMA 9	Spira	Streptomyces
LMA 10	-	W/I
W/I: Without identification		

Figures

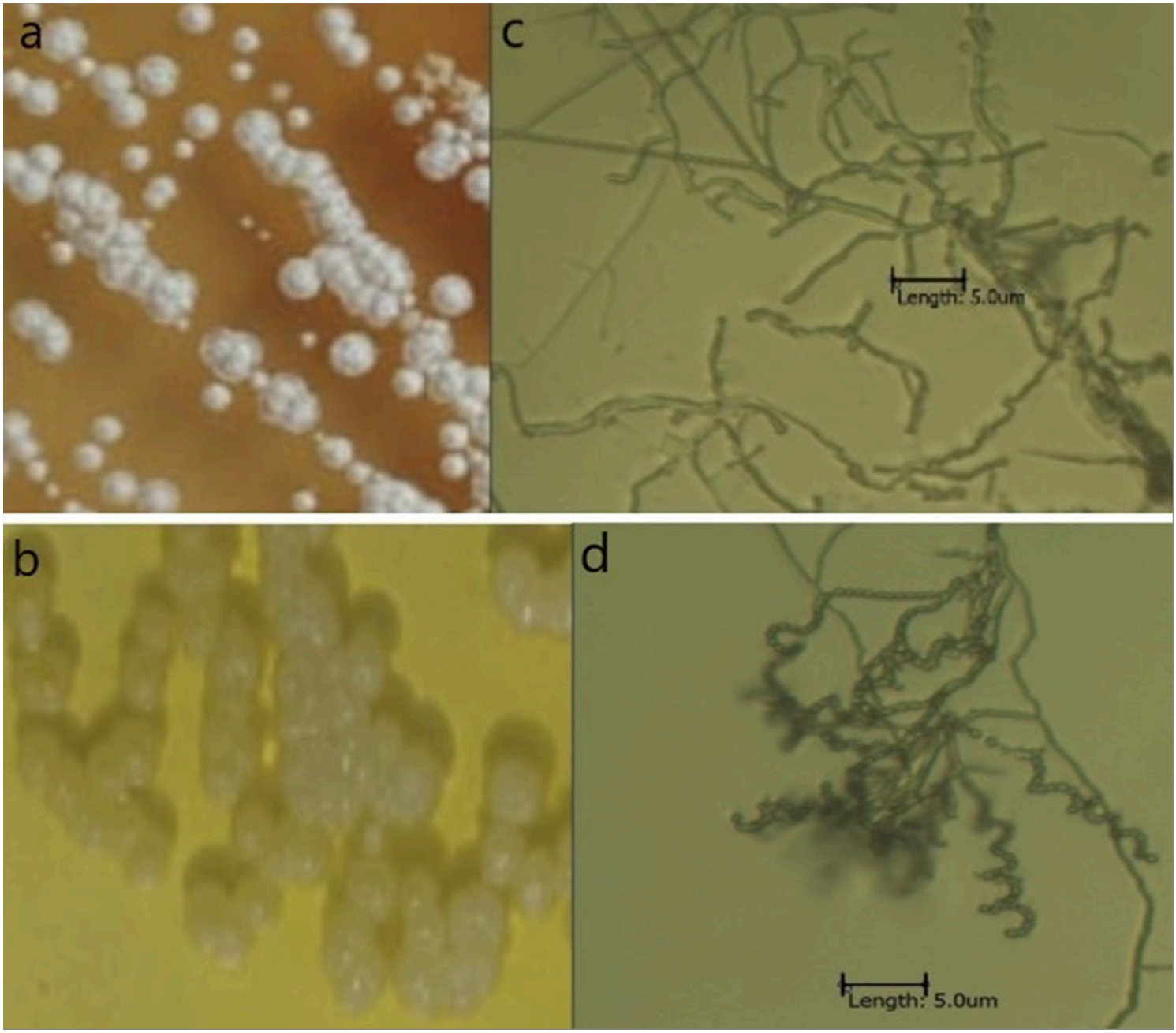


Figure 1

a: Isolate of actinobacteria LMA 7; *b*: Isolate of actinobacteria LMA 4; *c*: Microscopic structure of the aerial mycelium of isolate of actinobacteria LMA 7; *d*: Microscopic structure of the spore chain of isolate LMA 4.

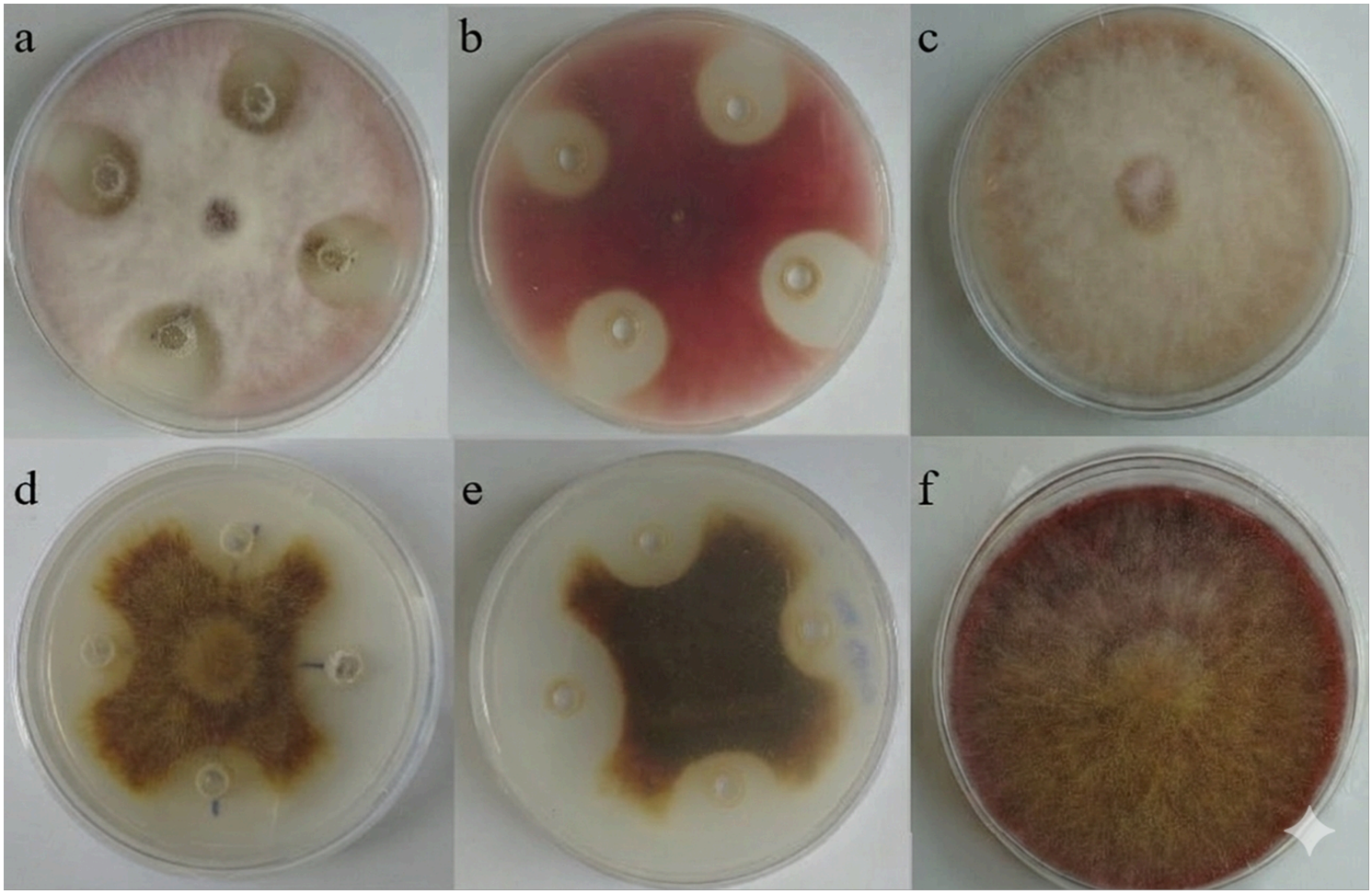


Figure 2

a: Aerial mycelium from antibiotic assay of LMAF against fungus FUG4; *b*: Vegetative mycelium from antibiotic assay of LMAF against fungus FUG4; *c*: FUG4 control; *d*: Aerial mycelium from antibiotic assay of LMAF against fungus FUG09M134; *e*: Vegetative mycelium from antibiotic assay of LMAF against fungus FUG09M134; *f*: FUG09M134 control