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ORIGINAL ARTICLE/ARTICLE ORIGINAL

# Lipopeptide biosurfactant from *Bacillus thuringiensis* pak2310: A potential antagonist against *Fusarium oxysporum*

## *Biosurfactant lipopeptidique de* Bacillus thuringiensis *pak2310 : un antagoniste potentiel contre* Fusarium oxysporum

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**KEYWORDS**
Fusariosis;
*Bacillus thuringiensis* pak2310;
Lipopeptide;
Antibiotic;
Antifungal;
Anti-biofilm

**Summary**
*Objectives.* — The aims of the study were to evaluate the effects of a biosurfactant obtained from a novel *Bacillus thuringiensis* on *Fusarium oxysporum* to determine the morphological changes in the structure of the fungi and its biofilm in the presence of the biosurfactant and to evaluate the toxicity of the biosurfactant on HEp-2 human epithelial cell lines.
*Materials and methods.* — The strain was screened and isolated from petroleum contaminated soil based on the $E_{24}$ emulsification index. The biosurfactant was produced on glycerol, extracted using chloroform:methanol system and purified using HPLC. The purified fraction showing both surface activity (emulsification and oil-spread activity) and anti-fusarial activity (agar well diffusion method) was studied using FT—IR and MALDI-TOF MS, respectively. The minimum inhibitory concentration (MIC) and the biofilm inhibitory concentration (BIC) were determined using dilution method. The effect of biosurfactant on the morphology of *Fusarium oxysporum* was monitored using light microscopy and confocal laser scanning microscopy (for biofilm).
*Results.* — The purified surfactant showed the presence of functional groups like that of surfactin in the FT—IR spectra and MALDI-TOF MS estimated the molecular weight as 700 Da. The MIC and BIC were estimated to be 0.05 and 0.5 mg/mL, respectively. The molecule was also non-toxic to HEp-2 cell lines at 10× MIC.
*Conclusion.* — A non-toxic and effective anti-*Fusarium* biosurfactant, that is both safe for human use and to the environment, has been characterized. The growth and metabolite

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production using glycerol (major byproduct of biodiesel and soap industries) also adds up to the efficiency and ecofriendly nature of this biosurfactant.


**MOTS CLÉS**
Fusariose ;
*Bacillus thuringiensis* pak2310 ;
Lipopeptides ;
Antibiotique ;
Antifongique ;
Anti-biofilm

**Résumé**
*Objectifs.* — Les objectifs de cette étude étaient d'évaluer les effets d'un bio-tensioactif obtenu à partir d'un *Bacillus thuringiensis* nouveau, sur *Fusarium oxysporum*, afin de déterminer les changements morphologiques dans la structure du champignon et de son biofilm en présence de l'agent bio-tensioactif et d'évaluer la toxicité du biosurfactant sur des lignées de cellules épithéliales humaines HEp-2.
*Matériels et méthodes.* — La souche a été criblée et isolée à partir de sols contaminés par du pétrole sur la base de l'indice de mise en émulsion E24. Le bio-tensioactif a été produit sur le glycérol, extrait à l'aide de chloroforme:methanol système et purifié en utilisant une HPLC. La fraction purifiée montrant à la fois une activité de surface (émulsification et l'activité de propagation de l'huile) et de l'activité anti-*Fusarium* (méthode de diffusion en gélose par puits) a été étudiée à l'aide de FT—IR et MALDI-TOF MS, respectivement. La concentration minimale inhibitrice (CMI) et la concentration inhibitrice biofilm (BIC) ont été déterminées en utilisant la méthode de dilution. L'effet du biosurfactant sur la morphologie de *Fusarium oxysporum* a été objectivée en utilisant la microscopie optique confocale à balayage laser et la microscopie du biofilm.
*Résultats.* — L'agent tensioactif purifié a montré la présence de groupes fonctionnels, tels que des surfactines dans les spectres FT—IR et MALDI-TOF MS avec un poids moléculaire estimé à 700 Da. La CIM et BIC ont été estimées à 0,05 et 0,5 mg/mL, respectivement. La molécule était également non toxique pour les cellules HEp-2 dans des lignées de cellules 10× MIC.
*Conclusion.* — Un biosurfactant anti-*Fusarium* non toxique et efficace, à la fois sans danger pour la consommation humaine et l'environnement a été caractérisé. La croissance et la production de métabolites en utilisant du glycérol (sous-produit majeur de biodiesel et de savon d'industrie) ajoute également à l'efficacité écologique et à la protection de la nature de ce biosurfactant.


## Introduction

*Fusarium oxysporum*, a plant pathogen, was the etiological agent of the epidemic wilting of bananas in Panama and hence, the name of the disease — Panama Wilt [29] was coined. Several other diseases, such as scabs, crown rot and red blight are caused by this fungus [2]. Although primarily a plant pathogen, recent reports indicate that members of *Fusarium*, especially *F. oxysporum* and *F. solani* are emerging human pathogens as well. Keratitis and onychomycosis are the main superficial infections of *F. oxysporum* in mankind [5,17,33]. Keratitis has been reported mostly in patients who use soft contact lenses [8,14]. Fusariosis or disseminated *Fusarium* infection has been reported worldwide in immune compromised patients, especially the ones with prolonged neutropenia [4,34]. The symptoms of the disease are very similar to that of aspergillosis [4]. Intra-venous amphotericin B is used against aspergillosis, while that has been reported to be ineffective against *Fusarium* infections [4]. Although the infection recedes initially with the administration of azole ring antibiotics, death due to fusariosis seems to be inevitable in immune compromised patients [4,8]. The main route of invasion by the pathogen is through the skin, through cuts, rashes or wounds, like the scratch wounds in cellulitis patients [33]. Once entered, the host body is invaded immensely, releasing toxins that can lead to tissue breakdown and immune suppression [17]. However, the toxins produced by *Fusaria* can contaminate food and can be easily ingested. These toxins have been shown to suppress the immune system of the host and in turn make the host more susceptible to the pathogen attack [11,15,17]. *Fusarium* infections are also accompanied by ecthymagangerosum, a skin infection caused by *Pseudomonas* spp; osteomyelitis; sinusitis and general fungemia [4,33]. Thus, it is necessary to develop new drugs and therapies that can help to manage the disease effectively.

*F. oxysporum* is capable of producing adherent substances form biofilms and produce tissue damaging enzymes [27,40]. Formation of a biofilm has been reported to be the main reason for keratitis and *Fusarium* keratitis has gained more prominence in the recent past with 41% of total cases of keratitis [21] as a biofilm is more resistant to drugs in use. Another study reports that people using soft contact lens are 40% susceptible to *Fusarium* keratitis [1]. Several factors for biofilm formation by the pathogen on contact lens including the material used in fabrication and cleansing solution have also been studied [20]. The same research team has also investigated the formation of biofilms in murine models and its relationship with keratitis [43]. Thus, preventing *Fusarium* biofilms gains importance in the present scenario.

Surfactants are those chemical molecules that have both hydrophobic and hydrophilic groups in them. They are soluble in both polar and non-polar solvents [29]. Surfactants produced by microbes are called biosurfactants. Biosurfactants have many advantages, like biodegradability, lower toxicity, mild production conditions, selectivity and higher