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Antagonistic Effect of Hard Palate Isolates on Selected Fungal Plant Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The search for antifungal agents with possible novel application in battling plant diseases cannot be overemphasized. Hence, a study was carried out to investigate the antagonistic ability of some hard palate isolates; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Micrococcus* sp. and *Aspergillus flavus* against four test phytopathogenic fungi - *Colletotrichum lindemuthianum*, *Colletotrichum capsici, Sclerotium rolfsii* and *Fusarium oxysporum*, monitored over a period of five days incubation. All isolates were subjected to screening against the plant pathogens and most of the isolate showed antagonistic activity. Bacillus sp. and *Micrococcus sp*. (57.0±0.0 and 51.1±0.1: P=.05) showed the highest antagonistic activity after day 5 and 4 respectively against *Colletotrichum lindemuthianum*. A steady inhibition of *Colletotrichum capsici* by *Aspergillus flavus* (51.2±0.2 to 52.0±0.0) was observed from day 3 to 5. Antibiotic sensitivity was carried out on the isolated bacteria. Most of the bacterial isolates were susceptible to gentamycin (15-19±0.0: P=.05), while none of the organism showed susceptibility to pefloxacin and streptomycin (0.0±0.0). *Bacillus* sp. showed susceptibility to chloramphenicol and gentamycin; and *Micrococcus sp.* to cotrimoxazole and erythromycin. *Pseudomonas aeruginosa* (0.0±0.0) was resistant to all the antibiotics. This research paper further discussed the use of hard palate microflora as possible source(s) of biocontrol agents in the control of plant diseases, especially those caused by the selected test fungal pathogens.

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Keywords: Antagonism; pathogenic fungi; hard palate; biocontrol; antibiotic.

1. INTRODUCTION

The role of oral microorganisms is of particular interest in two major dental diseases; dental caries and periodontal disease [1]. Bacteria reside in great numbers in many places, both inside and on the surface of the human body. In many cases, like in the gut, the presence of bacteria is beneficial. Bacteria in the mouth cause the formation of cavities, and can increase the chance of other disease and illness. Many different types of bacteria live in the mouth, and some types simply pass through depending on the types of food consumed [1]. The mouth is home to millions of bacteria. The dark, wet, and warm environment of the mouth, with the occasional meal running through it, makes it an excellent niche for microbes to live. Over the past 40 years, scientists have been arduously working to discover the over 500 different species of bacteria in and around the mouth known today. Each habitat within the oral cavity offers differing environmental conditions, and as such is colonized by a different microbial flora. The oral environment is constantly in flux [1]. The hard palate is a thin horizontal bony plate of the skull, located in the roof of the mouth. It spans the arch formed by the upper teeth. It is formed by the palatine process of the maxilla and horizontal plate of palatine bone [2].

The mouth harbors a diverse, abundant and complex microbial community. This highly diverse microflora inhabits the various surfaces of the normal mouth. Bacteria accumulate on both the hard and soft oral tissues in biofilms. Bacterial adhesion is particularly important for oral bacteria. Oral bacteria have evolved mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues. A dynamic equilibrium exists between dental plaque bacteria and the innate host defense system [2]. Oral microflora play active role in the normal development of the mouth and in the maintenance of health at a site. The presence of a resident microflora prevents disease by reducing the chance of colonization by exogenous species. This barrier effect is termed 'colonization resistance'. Some strains of *Streptococcus salivarius* strains produce a bacteriocin (enocin or salivaricin) with activity against Lancefield Group A streptococci [3]. *Streptococcus mutans* can produce antibodies that inhibit cariogenic (dental caries) process. Bacteriocins are also produced by some strains of Lactic acid bacteria (LAB). They are antimicrobial peptides with activity against strains closely related to the producer microorganism. Some bacteriocins are also active against Gram positive food borne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis* and spores of *Clostridium perfringens* [3].

Bacteria-bacteria interactions increase their affinity for a communal lifestyle. In the absence of coaggregation and co-adhesion, the multi-specie biofilms would not be able to form. Coaggregation is the recognition and communication between bacteria in suspension that will clump together to form an aggregate that can bind to the biofilm; while coadhesion is the adhesion of individual bacteria cell in suspension with a cell that is already a member of the biofilm. These interactions are mediated by complementary protein-adhesin and saccharide-receptors. Coaggregation between streptococci and actinomyces, and initial colonizers, help them bind to the acquired pellicle as well as manipulate spatiotemporal development of plaque. Cell-cell interactions are formed through lectin-like receptors; which involves a protein adhesion recognizing the streptococcal receptor polysaccaharide (RPS). Streptococci can interact between each other [4]. Coaggregation and further addition of later colonizers to the biofilm are dictated by the bacteria's nutritional and environmental needs. The earlier colonizers are essential to the formation of the biofilm because they change the environmental conditions for the next layer of bacteria to adhere [4]. The overall structure throughout the biofilm is dictated by antagonistic and synergistic interactions between the bacteria. The composition of the biofilm has a basic organizational method, but the actual percentage of each bacterium present differs between individuals most likely because of interactions that occur [5].

Antagonism is a phenomenon reflected primarily in the struggle for existence. Antagonistic relations can be traced most clearly between a predator and its prey (predation) and between a parasite and its host (parasitism). Antagonism also applies to competitive relations (competition)

- for example, competition for light or mineral nutrition among plants and for the same food among animals [6]. Antagonism of microorganisms which is also known as antibiosis is the suppression of some species of microorganisms by others. The mechanism of antagonism is varied and often obscure. Antagonists more often than not act on their competitors with metabolic products (allelopathy), including antibiotics, or displace the competitors by means of more intensive reproduction or primary utilization of food. Repeated attempts were made as early as the 19th century to treat diseases caused by bacteria. However, these attempts were unsuccessful because of the use of unpurified preparations [7]. Microbial antagonists are extensively used in the production of antibiotics. Useful microbial antagonists inhibit the growth of many phytopathogenic bacteria and fungi. Antagonists can also be used in many branches of the food industry [6]. Antibiotic sensitivity tests are used in the laboratory to determine antibiotic sensitivity pattern of microorganisms. A microbiologist can find the most suitable antibiotic for use in treatment where a particular bacterium is implicated, thereby reducing the development of antibiotic resistance in a strain of bacteria. This is due to the use of only one type of antibiotic in treatment, rather than several different drugs [8].

This study aimed at the isolation and identification of microorganisms present in human hard palate. Also, to determine the antagonistic effect of the isolates from the hard palate against some selected phytopathogenic fungi, and antibiotic susceptibility pattern of the isolates. Research into the search for antifungal agents with possible novel application in battling plant diseases cannot be overemphasized.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Sterile swab stick was used to collect sample from the mouth of the student (hard palate) by using the swab stick to rub the palate [9].

2.2 Analysis of Specimen

Media were prepared according to manufacturer's specification (2.8 g of Nutrient Agar into 100 ml of water, 4.8 g of MacConkey media into 100 ml of water, 5 g of Malt Extract Agar into 100 ml of water, 3.6 g of Potato Dextrose Agar into 100 ml of water and 3.8 g of Mueller Hinton into 100 ml of water). Streak plate method was used for the isolation of the microorganisms. In the streak method, labeled swab stick samples were properly streaked on respectively prepared solidified agar plates, incubated for 24 hrs at 37ºC (Nutrient agar for bacteria) and 48-72 hrs at 25ºC (Potato Dextrose Agar for fungi) [10].

2.3 Isolation and Identification of Bacterial and Fungal Isolates

Distinct colonies of bacteria were purified by repeated subculture on the respective isolation media, and preserved on slants at 4ºC according to Olutiola et al. [11]. Morphological and biochemical tests to identify isolates were carried out using the methods of Fawole and Oso [10] and Bergey's Manual of Systematic Bacteriology [12]. Biochemical tests carried out in the conventional method include fermentation of carbohydrate, catalase, motility, coagulase. Fungal isolates were subcultured using the same isolation media and their identification made possible using macroscopic and microscopic (stereomicroscope) fungal features. Fungi were identified using the cotton-blue in lactophenol method [13].

The hard palate isolates used in this investigation (*Bacillus* sp., *Micrococcus* sp., *Staphylococcus aureus,* and *Pseudomonas aeruginosa*. and *Aspergillus flavus*) were maintained by routine culture on agar slants, stored at 4ºC between transfers. Additional subcultures (24 hrs, 25ºC) were made in fresh medium before use in the experiment.

2. 4 Collection of Test Pathogenic Fungi

Pure cultures of fungi namely; *Collectotrichum capsici, Collectrotrichum lindemuthianum, Scelerotium rolfsii* and *Fusarium oxysporum* (plant pathogens) were obtained at the Department of Crop Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria.

2.5 Antibiotic Susceptibility Assay

The commercial antibiotic sensitivity discs (OptuDiscs) were employed, and contained ten (10) antibiotic impregnated discs namely

Ofloxacin (OFL-5 µg), Streptomycin (STR-10 µg), Chloramphenicol (CHL-30 µg), Ceftriaxone (CFT-30 µg), Gentamycin (GEN-10 µg), Perfloxacin (PEF-5 µg), Cotrimoxazole (COT-25 µg), Ciprofloxacin (CPX-10 µg), Erythromycin (ERY-5 µg), and Amoxycillin (AMX-25 µg). The discs were picked with sterile forceps and placed on the surface of the solidified Mueller Hinton agar with bacterial streak. The plates were incubated at 37ºC for 24 hrs. Thereafter, plates were examined for clear areas/zones around the disc. The zones of inhibition were measured in 'mm' [14]. According to the guideline of National Committee for Clinical Laboratory Standards [15], multidrug resistance was detected by using disk diffusion test. Any growth with less than 12 mm in diameter around the disk was considered indicative of drug resistance; growth with 12-14 mm zone was considered to be intermediate, while growth greater than 14mm in diameter around the disk as sensitive (susceptibility of the bacteria).

2.6 Detection of Antagonistic Activity

In order to detect antagonistic activity of bacterial isolates towards the growth of the fungal cultures, Fokkema [16] method was employed. Using the conventional streak method, a 40mm streak was made from 24 hr-old culture of the tongue isolates, 23 mm away from the centre of a Petridish. Using a 7 mm diameter sterile cork borer, the growing edge of a 4-day old test fungal culture was aseptically cut and placed at the centre of the plate already inoculated with the antagonist. Plates were incubated at 25℃ and monitored for 5 days. Observations were made every 24 hrs for 5 days on the inhibition of mycelial growth of the fungal pathogen by the antagonist. Percentage inhibition was calculated using the formula:

$$I = (r1 – r2/ r1) *100\%$$

where I = percentage of inhibition, r1 = radius of the pathogen away the antagonist, r2 = radius of the pathogen towards from the antagonist.

2.7 Data Analysis

Statistical analysis was carried out using Analysis of Variance (ANOVA) at 95% confidence interval in the SPSS 16 windows version, and Duncan's New Multiple Range Test (DNMRT) used for separation of means.

3. RESULTS

The morphological and some biochemical characteristics for bacteria and fungus isolated from the hard palate were presented in Table 1. In this study, *Staphylococcus aureus* was unable to inhibit all of the selected pathogenic fungi. Table 2 shows the antagonistic result of *Staphylococcus aureus* isolated from the hard palate of human mouth against some selected pathogenic fungi. There were no significant differences within and across groups for *S. aureus* against all test phytopathogenic fungi. *Pseudomonas aeruginosa* (Plate 1b) was able to inhibit the growth of these selected fungi with the maximum inhibition of 30.2±0.2% (Fig. 1) on the third day between *Pseudomonas aeruginosa* and *Sclerotium rolfsii,* and 19.1±0.1% % between *Pseudomonas aeruginosa* and *Colletotrichum lindemuthianum* on the second day.

Bacillus sp. (Plate 1c) showed the highest inhibition rate of all the isolated microorganisms from the human mouth. Between days four and five, there was significant difference across groups of test fungi (40.0 ± 0.0^{a} to 57.0 ± 0.0^{e}: P = .05) (Fig. 2). The maximum inhibition percentage of 57.0±0.0% (Fig. 2) was observed on the fifth day between *Bacillus* sp. and *Colletotrichum lindemuthianum.* The minimum inhibition was observed on the first day between *Bacillus* specie and *Colletotrichum lindemuthianum. Micrococcus* sp. was able to inhibit the growth of these selected fungi with the maximum inhibition of 51.3±0.1% (Fig. 3) on the fourth day between *Micrococcus* sp. and *Colletotrichum lindemuthianum,* and also between *Micrococcus* sp. and *Fusarium oxysporum. Aspergillus flavus* was able to inhibit the selected pathogenic fungi. The maximum inhibition of 52.0±0.0% (Fig. 4) was observed on the fifth day between *Aspergillus flavus* and *Colletotrichum capsici.*

Table 3 showed the different zones of inhibition of the bacterial isolates to different antibiotics. The highest zone of inhibition was found in cotrimoxazole against *Micrococcus* sp., while the lowest zone of inhibition was found in ciprofloxacin against *Micrococcus* sp. and ceftriaxone against *Pseudomonas aeruginosa*. All the bacterial isolates showed susceptibility to gentamycin, while none of the organisms showed susceptibility to perfloxacin and streptomycin.

Table 1. Morphological and biochemical characteristics of microorganisms isolated from the hard palate

	A	B	C	D			E
Pigmentation	Yellow	White	Cream	Yellow		Cultural	Green mycelia
Elevation	Raised	Raised	Flat	Raised		Characteristic	growth
Surface	Smooth	Smooth	Smooth	Smooth			
Shape	Cocci	Rod	Rod	Cocci			
Edge	Entire	Entire	Rhizoid	Entire			
Catalase	+	+	+	+	Fungal		
Coagulase	+	-	-	-	isolate		Upright
Motility	+	+	+	-		Microscopic	conidiophores.
						Observation	One-celled,
							globuse
							conidia
Gram reaction	+	-	+	+			
Lactose	A	AG	AG	AG			
Glucose	A	AG	AG	AG			
Galactose	AG	AG	AG	AG		Suspected	*Aspergillus* sp.
Maltose	AG	-	AG	-		Fungus	
Sucrose	A	-	AG	AG			

KEY: + = Positive, - = Negative, A = Acid production, AG = Acid and gas production
Isolate A = Staphylococcus aureus; Isolate B = Pseudomonas aeruginosa, Isolate C = Bacillus sp., Isolate D = Micrococcus sp. and Isolate D = Aspergillus sp

Table 2. Percentage inhibition of *Staphylococcus aureus* from the hard palate against selected fungi

Selected fungi	A	B	C	D
Days	**IA (%)**	**IB (%)**	**IC (%)**	**ID (%)**
1	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]
2	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
3	0.1±0.1[a]	0.0±0.0[a]	0.0±0.0[a]	0.1±0.1[a]
4	0.0±0.0[a]	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]
5	0.1±0.1[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]

[a]*Means in the same column and across groups/treatments sharing a common letter are not significantly different (P = .05) by Duncan's multiple range test; Key: A = Colletotrichum lindemuthianum, B = Colletotrichum capsici, C = Fusarium oxysporum, D = Sclerotium rolfsii; IA(%) = Percentage of inhibition of A, IB(%) = Percentage of inhibition of B, IC(%) = Percentage of inhibition of C, ID(%)= Percentage of inhibition of D*

4. DISCUSSION

The production of certain substances that inhibit other microorganisms in the microbial environment of the oral cavity could serve as aggressive by-product that may eliminate competitors and pathogens. Bacterial antagonism may be one of the mechanisms which regulate the bacterial flora of the hard palate. Antagonism was measured by zone of inhibition between the fungal plug and bacterial streak.

There were variations in the inhibitory activities of *Bacillus* sp. The antagonistic activity of this organism in inhibiting the growth of *Fusarium oxysporum, Colletotrichum capsici*, *Sclerotium rolfsii* and *Colletotrichum lindemuthianum* was more observable compared to other hard palate isolates. *Colletotrichum* sp. is one of the most important plant pathogens worldwide causing the economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits. Among these hosts, chilli (*Capsicum* spp.), an important economic crop worldwide, is severely affected by anthracnose which may cause yield losses of up to 50% [17]. *Fusarium oxysporum* is a fungal disease of plants that cause severe losses in many vegetables and flowers, field crops, such as cotton, and plantation crops, such as banana, date palm and oil palm. *F. oxysporum* plays the role of a silent assassin. The pathogenic strains

of this fungus can be dormant for 30 years before resuming virulence and infecting a plant. This phytopathogenic fungus is infamous for causing a condition called *Fusarium* wilt. Southern blight caused by the soil borne fungus *Sclerotium rolfsii* is a serious disease of a wide variety of plants, including field, vegetable, fruit, ornamental crops and also turf. The disease occurs worldwide, but predominantly in warm climates [18].

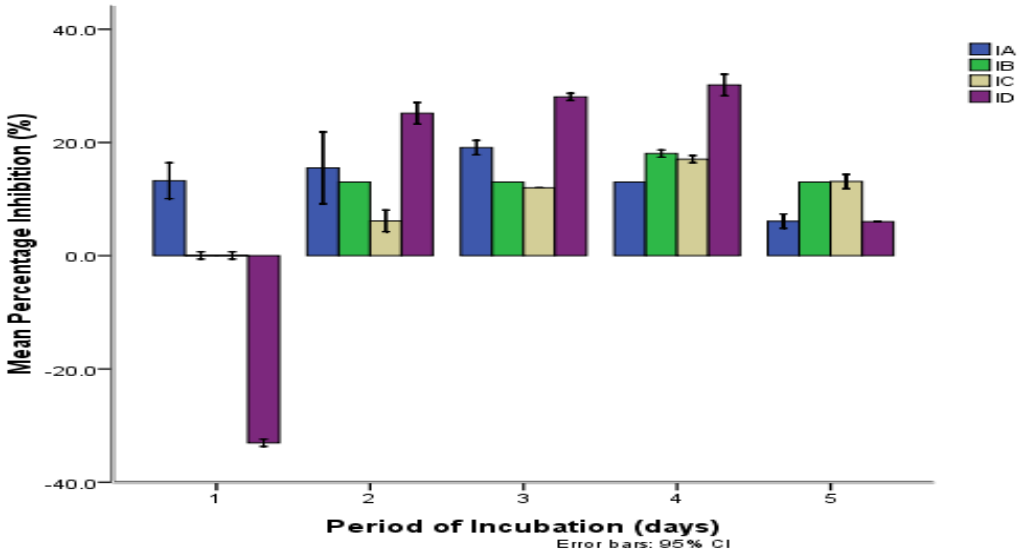


Fig. 1. Percentage inhibition of *Pseudomonas aeruginosa* from the hard palate of the mouth against some selected fungi

Key: A = Colletotrichum lindemuthianum, B = Colletotrichum capsici, C = Fusarium oxysporum, D = Sclerotium rolfsii; IA(%) = Percentage of inhibition of A, IB(%) = Percentage of inhibition of B, IC(%) = Percentage of inhibition of C, ID(%)= Percentage of inhibition of D

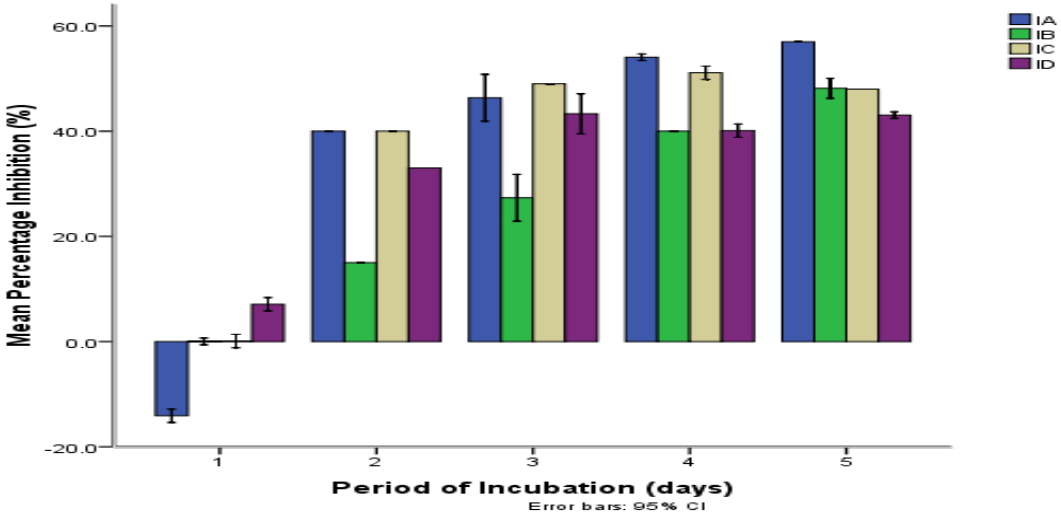


Fig. 2. Percentage inhibition of *Bacillus* sp. from the hard palate of the mouth against some selected fungi

Key: A = Colletotrichum lindemuthianum, B = Colletotrichum capsici, C = Fusarium oxysporum, D = Sclerotium rolfsii; IA(%) = Percentage of inhibition of A, IB(%) = Percentage of inhibition of B, IC(%) = Percentage of inhibition of C, ID(%)= Percentage of inhibition of D

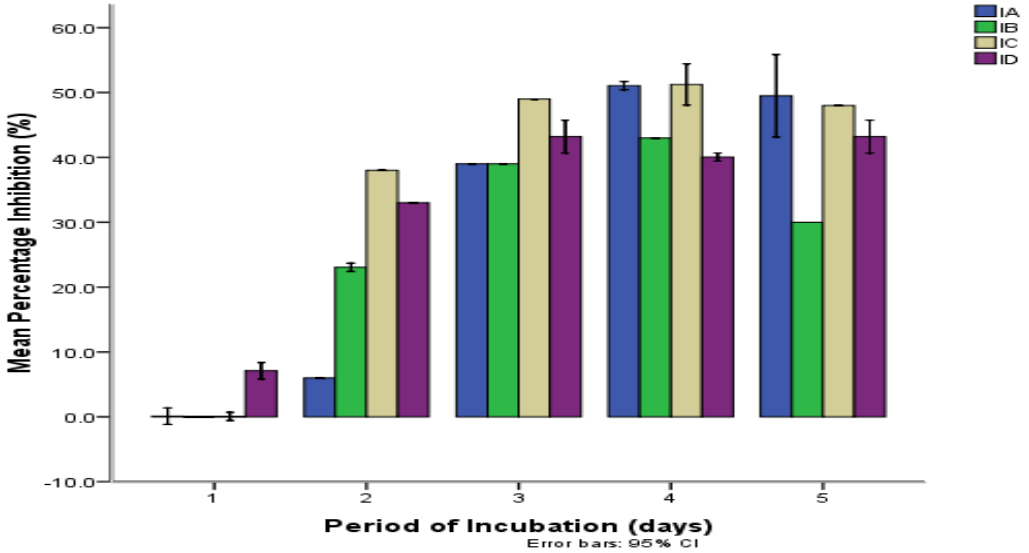


Fig. 3. Percentage inhibition of *Micrococcus* sp. from the hard palate of the mouth against some selected fungi

Key: A = Colletotrichum lindemuthianum, B = Colletotrichum capsici, C = Fusarium oxysporum, D = Sclerotium rolfsii; IA(%) = Percentage of inhibition of A, IB(%) = Percentage of inhibition of B, IC(%) = Percentage of inhibition of C, ID(%) = Percentage of inhibition of D

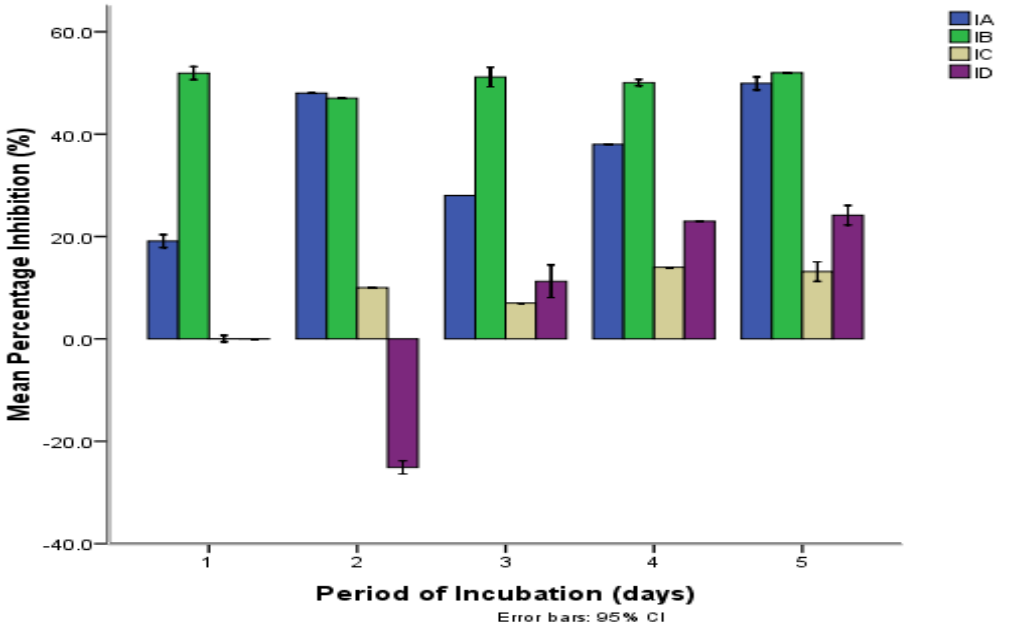


Fig. 4. Percentage inhibition of *Aspergillus flavus* from the hard palate of the mouth against some selected fungi

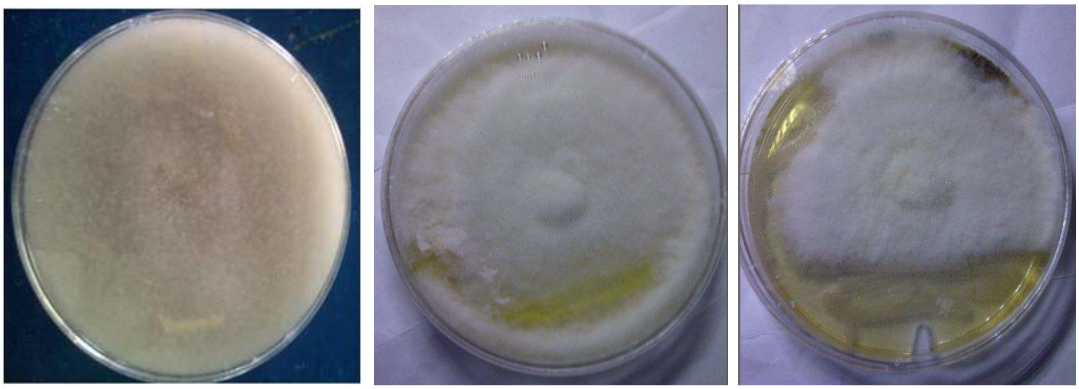
Key: A = Colletotrichum lindemuthianum, B = Colletotrichum capsici, C = Fusarium oxysporum, D = Sclerotium rolfsii; IA(%) = Percentage of inhibition of A, IB(%) = Percentage of inhibition of B, IC(%) = Percentage of inhibition of C, ID(%) = Percentage of inhibition of D

Table 3. Antibiotics sensitivity pattern of the bacterial hard palate isolates

Isolates	Zone of inhibition (mm)									
	STR	**CFT**	**GEN**	**PFX**	**COT**	**ERY**	**AMX**	**OFL**	**CHL**	**CPX**
Bacillus sp.	0.0±0.0[a]	12±0.0[b]	19±0.0[c]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	13±0.0[b]	0.0±0.0[a]	20±0.0[b]	0.0±0.0[a]
Micrococcus sp.	0.0±0.0[a]	0.0±0.0[a]	16±0.0[b]	0.0±0.0[a]	23±0.0[b]	18±0.0[b]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	11±0.0[b]
Staphylococcus aureus	0.0±0.0[a]	0.0±0.0[a]	15±0.0[b]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	20±0.0[b]	0.0±0.0[a]	16±0.0[c]
Pseudomonas aeruginosa	0.0±0.0[a]	11±0.0[b]	12±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	12±0.0[b]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]

[a,b,c]*Means in the same column not sharing a common letter are significantly different (P = .05) by Duncan's multiple range test*

Key: OFL= Ofloxacin, STR= Streptomycin, CHL= Chloramphenicol, CFT= Ceftriaxone, GEN= Gentamycin, PFX= Perfloxacin, COT= Cotrimoxazole, CPX= Ciprofloxacin, ERY= Erythromycin, AMX= Amoxycillin



A **B** **C**

Plate. 1. Antagonistic effect of hard palate isolates against fungal pathogens (Picture after fifth day)

[A = Staphylococcus aureus on Collectrichum lindemuthianum, B = Pseudomonas aeruginosa against Colletotrichum capsici, C = Bacillus sp. on Colletotrichum capsici]

Staphylococcus aureus had no effect on all of the selected fungi. No significant difference was observed within and across groups for *S. aureus* against all test phytopathogenic fungi (0.0-0.1±0.0[a]: *P* = .05). The inability of *Staphylococcus aureus* to inhibit the selected fungal pathogens might be due to the presence and/or secretion of lysostaphin, an antimicrobial substance which has effect on bacteria, but no effect on fungi [19]. Other isolated bacteria (*Pseudomonas aeruginosa* and *Micrococcus* sp.) were also able to inhibit the selected pathogenic fungi in one way or the other. Other studies have reported antagonistic activity among *Pseudomonas* species including *P. fluoresecens* and *P. corrugate*. Control of pathogens using *Pseudomonas* species has been reported to be due to competition for iron, antibiosis [20]. Significant difference (-33.1±0.1[a] to 13.3±0.4[b]: *P* = .05) was observed across groups/treatments at day one (Fig. 1) with *P. aeruginosa* against all test fungi. The initial negative percentage inhibition with *P. aeruginosa* against *S. rolfsii* was indicative of an early synergistic effect, which was no longer observed on subsequent days. Both microorganisms may have produced metabolites that contributed to this effect. Steady antagonism between days two and four may be as a result of the antagonist's ability to quickly adjust to conditions of growth within the medium and inhibit the test fungal pathogen. This may be related to differences in types or amounts of enzymes [21], and other antagonistic products (antibiotics) produced [22,23]. Further studies will be required to investigate factors involved in suppression of the test fungal pathogens used in this study.

There was a marked significant difference and steady increase in antagonistic effect of *Bacillus* sp. against all test fungi observed as positive percentage inhibition and movement of the pathogen away from the antagonist. There was significant difference (-14.1±0.1[a] to 40.0±0.0[b]: *P* = .05) across the groups/treatments for *Bacillus* sp. against all test fungi at day one and two. The initial movement of *Colletotrichum lindemuthianum* towards the antagonist may be as a result of the antagonist adapting to conditions of growth within the medium. Movement away from the antagonist increased from day two to five with all phytopathogens. Significant difference across groups of test fungi (40.0±0.0[a] to 57.0±0.0[e]: *P* = .05) at days four and five (Fig. 2) for this antagonist could be attributed to the difference in nature, and hence, response of each pathogen. *Bacillus* species are known to be strong inhibitors of some fungi. *Bacillus* sp. produce antibiotics and antifungal metabolites such as bacitracin, gramicidin, polymyxin, tyrotricidin, bacilysin, chlotetaine, iturin A, mycobacillin, bacilomycin, mycosubtilin, fungistatin, and subsporin [19]. Antagonism of *Bacillus* species against several pathogens had also been reported [24]. In the case of *Micrococcus* sp. no significant difference (0.1±0.1[a] to 7.1±0.1[a]: *P* = .05) was observed across treatments at day one. The microorganisms could have been adapting to available nutrients and other conditions of growth within the medium. Day two to five showed significant difference (6.0±0.0[b] to 51.3±0.4[e]: *P* = .05) across treatments and steady antagonism of *Micrococcus* sp. against all test phytopathogenic fungi (Fig. 3) observed as

movement of pathogens away from the antagonist. However at day five, antagonism seemed to reduce with *Micrococcus* sp. against all test phytopathogenic fungi. Maximum antagonistic effect for this bacterium would be best observed and exploited after four days. The inhibition of phytopathogenic microorganisms by *Micrococcus* sp. could probably be due to its ability to produce organic acids and bacteriocins. Similar submission was made by Agarry et al. [25].

The isolated fungus (*Aspergillus flavus*) was able to show some inhibitory effect. There were some significant differences across all groups of test fungi on all days of incubation (Fig. 4). The test phytopathogens showed varied response to the antagonist. These fungi exhibit different physiology in their ability to respond to stimuli and metabolic by-products which may have been released by the antagonist into the medium of growth. Antagonism was however more marked and stable with *Colletotrichum capsici* from day two through to five. There was no significant difference in the treatment of *Colletotrichum capsici* with *Aspergillus flavus* at days one and five (51.2 ± 0.1^{d} to 52.0 ± 0.0^{d}: $P = .05$). Significant difference (-25.1 ± 0.1^{a} to 49.9 ± 0.1^{e}: $P = .05$) and fluctuations in antagonism with *A. flavus* and other test fungal plant pathogens could be attributed to the ability of both antagonist and test fungi being able to express metabolic biocompounds that counteract and cancel out the effect of the other [26]. In line with the present study, Aktar et al. [27] also demonstrated the antagonistic effect of *A. flavus* against *Colletotrichum* sp. and *Fusarium oxysporum*, with percentage inhibition range between 11-46%. Results were attributed to the production of volatile and non-volatile compounds which could be produced by the antagonist. This study hypothesizes that biocompounds produced by *Bacillus* sp., *Micrococcus* sp. and *A. flavus* could be useful as potential biocontrol agents.

In the antibiotic susceptibility test there were significant differences across treatments, and no significant difference within streptomycin and perfloxacin treatments. The antibiotics are from different sources and exert their antibiosis using different mechanisms. The highest zone of inhibition was found in cotrimoxazole against *Micrococcus* sp., while the lowest zone of inhibition was found in ciprofloxacin against *Micrococcus* sp. and ceftriaxone against *Pseudomonas aeruginosa*. *Bacillus* sp. was found to be resistance to ceftriaxone, but sensitive to chloraphenicol and gentamycin. *Micrococcus* sp. was sensitive to gentamycin, cotrimoxazole and erythromycin, but resistant to other antibiotics. *Staphylococcus aureus* was sensitive to gentamycin, ofloxacin and ciprofloxacin, but resistant to other antibiotics. *Pseudomonas aeruginosa* showed sensitivity to gentamycin but was resistant to other antibiotics. All the bacterial isolates were susceptible to gentamycin, while none of the microorganisms showed susceptibility to pefloxacin and streptomycin. This might be due to frequent exposure of the isolates to these antibiotics which might have led to development of resistance. Andrew [28] had made similar observations where antibiotic susceptibility of samples taken from the entire dentition of the supragingival region showed the resistance to perfloxacin. *Pseudomonas aeruginosa* was the least inhibited by the selected antibiotics. This was probably due to frequent exposure of this isolate to these antibiotics which lead to development of resistance. Resistance of the isolates may take the form of a spontaneous or induced genetic mutation or the acquisition of resistance genes from other bacterial species by horizontal gene transfer via conjugation, transduction, or transformation [29].

5. CONCLUSION

Bacillus sp. and *Micrococcus* sp. had the highest positive antagonistic effect on all the selected test fungi. *Pseudomonas aeruginosa* also showed antagonistic effect against the selected pathogenic fungi. *Staphylococcus aureus* showed no inhibitory effect on the selected fungi. The isolated fungi, *Aspergillus flavus* had little or no effect on some selected pathogenic fungi. Therefore, *Bacillus* sp. and *Micrococcus* sp. could be useful as biocontrol agents against the phytopathogenic fungi used in this study. Exploiting this area of research would provide more information and pave way for the use of biological methods in disease management. Pure strains of *Bacillus* sp. and *Micrococcus* sp. could be useful as biocontrol agents of *Colletotrichum capsici* and *Sclerotium rolfsii*. These microbes could also serve as good candidates for the biocontrol of *Fusarium oxysporum*. However, further research; especially into the isolation, identification and characterization of the substance(s) which may be responsible for the antagonism observed is still required. Based on the antibiotics sensitivity test carried out on the bacterial isolates, ofloxacin and erythromycin could be used in the treatment of oral infections

caused by corynebacterial bacteria; and ciprofloxacin and streptomycin in treatment of infections caused by *Micrococcus* sp.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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