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Antibiotic Producing Fungi in Sewage: Inhibitory Effect on 4 Bacterial Test Strains, and Different Fungal Types

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Abstract

Fungi were isolated from raw sewage and sewage that had trickled down soil columns from a waste water treatment plant in Karlsruhe, Germany, using the laboratory techniques.*Fusarium sporotrichioides Sherb, Penicillium funiculosum*, and *Trichoderma harzianum Rafai* were named as isolates from raw sewage. *P. notatum Westling, P. meleagrinum Biourge, Aspergillus flavus, Link ex Gray, A. repens, A. fumigatus Fresenius*, and *A. fischeri Wehmer* were among the fungi found in the effluent of the soil columns that were isolated under absolutely anaerobic conditions. *Fusarium poae (Peck) Wollenw.* and *Penicillium chrysogenum Thom.* were isolated when samples were cultured in anaerobic jars with nitrate. The coloration, smell, and other fungal traits, such as conidial and conidiophore size, etc., were used to identify the organism. The fungi's antibiotic properties against bacteria were investigated. A little amount of the fungal mycelium was plate-plated on new Nutrient agar and Sabouraud agar after isolation. After two days of incubation, bacteria were cross-streaked toward the fungal colonies on the plates. On the plates, six strains of *E. coli*, Gram-negative *Pseudomonas* species, and aerobic Gram-positive *Enterococci* species were streaked in the direction of the fungi. After that, the plates were incubated in an aerobic environment. Similar to this, five anaerobic Gram-positive Bifidobacterium species strains that were isolated from

sewage were cross streaked on fungal plates and then further cultured under anaerobic conditions. When compared to *A. flavus* and *A. fumigatus, P. chrysogenum and A. repens* were more effective at inhibiting Enterococci. Only *P. chrysogenum, A. fumigatus*, and *A. repens* inhibited *Pseudomonas sp. A. fumigatus* and *P. chrysogenum var meleagrinum* only little inhibited E. coli, but *P. chrysogenum* and *A. flavus* very successfully did so.*Pre-growing P. chrysogenum, P. notatum*, and *P. meleagrinum* on plates for two days in an aerobic environment was followed by cross-stripping with test strains of Bifidobacterium and incubation under strictly anaerobic conditions. Both *P. chrysogenum and P. notatum* had the greatest inhibitory effects on bifidobacterium.

Keywords: Raw sewage, Aerobic, Anaerobic, Antibiotic, Resistance, Penicillium, Penicillin, *Aspergillus, Pseudomonas, E. coli, Enterococci* and *Bifidobacterium*

1. Introduction

Fungi are essential for nutrient cycling because of their capacity to break down cellulose and lignin (Pointing and Hyde, 2001), while it can also be a major source of fatal diseases in humans, animals, and plants (Hyde et al., 2018). Another imporatant fact is that consideable number of these speceis are found in polluted habitats (bridge cook, 1954). But these fungal species are also understudied and underutilised. Soil fungi of the genera *Aspergillus*, *Penicillium* and *Trichoderma* are very prolific antibiotic producers.

Antibiotics are substances produced by living organisms, typically microorganisms, that are toxic to other microbes. Although some simple byproducts of primary metabolism, including alcohols and aldehydes, may also have antibacterial activity, antibiotics are formed from the complex secondary metabolism (Kalemba et al., 2003). These compounds may be generated by fungi or bacteria as volatile or non-volatile molecules. Many fungi produce non-volatile antibiotics, which are part of a wide variety of chemically varied secondary metabolites (Leylaie & Zafari, 2018). Often several kinds of antibiotics are produced by the same species, resulting in a very broad action spectrum. Citrinin, patulin, gliotoxin and penicillic acid are some of these substances produced by *Penicillium* and *Aspergillus* species (Ciegler et al., 1971). Helvolic acid (fumigacin) is produced by certain strains of *A. fumigatus* isolated from a variety of soils and composts on Czapek Dox medium. Helvolic acid is active chiefly against Gram-positive, pathogenic and phytopathogenic bacteria. Waksman & Harning (1943), who studied and isolated the antibiotic, called it fumigacin. English investigators isolated the antibiotic produced under identical conditions by strains of *A. fumigatus* and called it helvolic acid. It was later shown that fumigacin is a mixture of helvolic acid and the familiar antibiotic gliotoxin (Bilai, 1963; Olga, 1986). However, the focus in the past has been on the same bacterial and fungal genera, such as Streptomyces in the Actinobacteria and common soil moulds like Aspergillus and Penicillium in the filamentous fungi (Karwehl and Stadler, 2017).

Isolated filamentous fungi from sewage include *Penicillium* sp, *Aspergillus* sp, *Trichoderma* sp, *Spicaria* sp and *Hyaloflorae* sp (Fakhrul et al., 2002). The filamentous fungi are naturally present in raw sewage or sewage sludge either as spores or vegetative cells. These can be cultured on synthetic or semi-synthetic substrates making it possible to evaluate their

metabolism. They can metabolize a wide spectrum of organic substances during growth (Hamer and Mason, 1987). It is generally known that filamentous fungi produce compounds with antibacterial properties, some of which have served as the foundation for the creation of fresh, therapeutically significant antimicrobial drugs (Bilai et al., 2012). The production of penicillin is primarily characteristic of representatives of Penicillium, e.g. of *P. chrysogenum.* Russian scientists used penicillin from *Penicillium* sp for inhibition of bacteria (Bilai, 1963). *P. chrysogenum* was found to produce several natural types of penicillins as well as compounds that were isomeric with the natural penicillins. Man can tolerate large doses of purified penicillin (some 1 to 10 million units over a period of 20 to 30 days).

Species of Penicillium and Aspergillus are also sugar fungi but some species add to the environment complex substances such as antibiotics and organic acids, possibly as waste products of metabolism or as aids in maintaining their position in competition with other organisms. Species of *Cladosporium*, *Alternaria*, *Cephalosporium*, *Fusarium*, and *Trichoderma* are able to break down cellulose but appear to prefer simple carbohydrates (Cooke and Pipes, 1969). The unit for measurement of antibacterial properties of penicillin is 0.6 µg of a mixture of a chemically pure preparation of benzyl penicillin and p-hydroxybenzyl penicillin in the proportion of 99.75 : 0.25. There is no antibacterial activity when the pH is below 2.0 or above 8.0 and at an incubation temperature above 60^0C.

Berdy, (1974) lists 768 fungi, which produce antibiotics. Among them about 500 antibiotic producers belong to Deuteromycotina, 140 species to the Basidiomycotina and only 14 to the aseptate groups. This is far from a complete list as there are thousands of species which have yet to be tested or for which there are no published records.

The rapid expansion of fermentation biotechnology over the past 30 years has led to a greater awareness of the usefulness of filamentous fungi for the production of large amounts of organic acids, antibiotics, enzymes, hormones and fuel from inexpensive carbon source or waste ingredients (Smith et al., 1981). *A. flavus*, for instance, produces kojic, aspergillic (granegillin), hydrooxyaspergillic, neooxyaspergillic acids and orizazin from culture fluid depending on the culture conditions. In older cultures of *P. chrysogenum* penicillin was no longer produced, but the amount of certain amino acids in the culture fluid increased again Narasimha and Venkateramen (1952).

Several strains of *P. notatum* and *P. chrysogenum* produce notatin, also called penicillin A, penicillin B, or penicidin. Notatin possesses a broad spectrum of action against Gram positive and Gram-negative bacteria (Bilai, 1963). Notatin ́s mechanism of antibacterial action, is due to the formation of hydrogen peroxide when glucose is oxidized in the presence of oxygen. According to Coulhard (1945), notatin inhibited growth of *E. coli* completely at a dilution of up to 10^{-6}.

The four best-known penicillines of *P. chrysogenum* are F, K, G and X (in the American terminology) or I IV II and III (in the English terminology), respectively, designated 2-pentenyl penicillin, n- heptyl penicillin, benzyl penicillin and n hydroxybenzyl penicillin, with respect to the substitution of 6-amino penicillinoic acid. Benzyl penicillin is the most valuable and is produced commercially for therapeutic use in the form of its sodium salt. In

one study the nature of morphological changes in the mycelia of *P. chrysogenum* during various growth stages is definitely related to the production of penicillin (Bekker, 1957).

In this study fungi were isolated in the lab of the Karlsruhe Institute of Technology from a waste water treatment plant in Karlsruhe, Germany, as well as anaerobic soil columns and anaerobic jars with nitrate. Thus, fungi were isolated from sewage under aerobic, anaerobic and anoxic conditions. Isolation of cultures was on Nutrient, Sabouraund and Czapek agar (selective agar). The antibiotic activity of the isolated fungal species against Gram positive and Gram-negative bacteria was studied.

2. Materials and Methods

2.1 Sources of Organisms

Fungal species were isolated from raw sewage or sewage after filtration through a sandy column under aerobic, anaerobic and anoxic conditions. To test anoxic growth a nitrate containing medium was used

Experimental set up: Raw sewage was sampled at the sewage treatment plant of Karlsruhe, Germany. The sewage was kept under N_2 atmosphere and was trickled through 125 cm of sand in glass columns.

2.2 Media and Isolation Procedures

Sewage was repeatedly diluted 10-fold by mixing of 1 ml of sample with 9 ml of NaCl solution (3 %). The Nutrient agar contained peptone (5 g), meat extract (3 g), glucose (1 g) and agar (15 g) per 1 L of distilled water. The pH was 7.0. Sabouraud agar contained peptone (10 g), glucose (40 g), agar (15 g) and distilled water (1000 ml). The pH of the solution was maintained at 5.6 (Aneja, 2002).

Portions of 0.1 ml of the raw sewage or of column effluent at different dilutions were spread onto Nutrient and Sabouraud agar medium. Fungal colonies developed and these were transferred carefully onto fresh Nutrient and Sabouraud agar plates for isolation of individual colonies.

Raw sewage samples and column effluent in various dilutions in NaCl were also spread on plates for isolation of denitrifiers. These were kept under anaerobic conditions. The medium for denitrifiers (Drews, 1983) contained: meat extract (1 g), peptone (5 g), yeast extract (2 g), NaCl (15 g), KNO_3 (10 g), agar (20 g). The pH of the solution was adjusted to 7.4 by adding NaOH solution. For fungal identification Czapek agar was used that was composed of: $NaNO_3$ (3 g), KH_2PO_4 (1 g), KCl (0.5 g), $MgSO_4.7H_2O$ (0.5 g), $FeSO_4.7H_2O$ (0.01 g), sucrose (30.0 g), agar (15.0 g) and distilled water (1000 ml). The sugar was prepared separately and added to the mixture as the last component, prior to the sterilization.

2.3 Maintenance of Isolates

Isolates were kept on Nutrient agar slants under a N_2 gas atmosphere. Every two months they were transferred to fresh medium.

2.4 Growth Conditions

Three replicates were carried out with fungal cultures on Nutrient and Sabouraud agar. Samples were streaked from the periphery towards the center. Czapek medium was suitable to find out the pigmentation and exudates production of *Penicillium* sp. at incubation temperatures of 25 - 28 ^{0}C. All cultures were incubated at laboratory temperature, only the incubation temperature of fungi with *Bifidobacterium* was at about 37^{0}C. Sterile conditions were maintained.

2.5 Microscopy, Identification of Fungi and Analysis

Fungi were microscopically identified by staining with lactophenol cotton blue and by determining the length and width of conidiophores and conidia, under an oil immersion lens (100X). Species identification was by examining the size and shape of phialospores and conidiophores, the shape and arrangement of phialides, colour and appearance. Odour of the fungi was also recorded, in some of the species as fungi gave a distinct characteristic odour on the applied media. Fungal population density was calculated using MPN technique.

A microscope type Axioskop with an Axiocam equipment, (Carl Zeiss Vision GmbH, Germany) was used for the study of fungal characteristics. Fungi were identified according to Domsch and Gams, (1980), Olga, (1986) and Bilai, (1963).

3. Results

A variety of fungal cultures were isolated from raw sewage and soil column effluents. These were acquired under anaerobic, oxic, and aerobic conditions. One of the fungi isolated from raw sewage, *P. chrysogenum*, was discovered in all three conditions, including aerobic, anaerobic, and anoxic conditions (Table 2). In addition to Nutrient agar, selective agar media like Sabouraud and Czapek agar were employed to screen for distinguishing characteristics, unique colorations, and pigmentations. The isolated fungal strains from raw sewage or sewage that had been filtered through sand belonged to the genera *Penicillium*, *Aspergillus*, *Fusarium*, and *Trichoderma* based on their growth, odour, colour, and morphological characteristics (Table 1). In comparison to the other isolated genera, *Penicillium* sp. and *Aspergillus* sp. were more prevalent. Repeated plate culture revealed reproductive taxonomic structures after a few days of incubation at room temperature (28 ℃).

The fungal density in Nutrient agar in anaerobic columns after 25, 50, 75, 100 and 125 cm trickling through sand was 4.25*10^5, 7.5*10^4, 5*10^3, 1.5*10^5, 7.5*10^5 and 1.0*10^4 / ml respectively, including *A. fumigatus, A. repens, P. notatum, A. flavus* and *P. melegranium*. The fungi obtained under the different conditions are listed in Table 1. After aerobic incubation in Sabouraud medium, *P. funiculosum, F. sporotrichioides, T. harzianum,* and *Verticillium tenerum* were recovered. Under anaerobic growth circumstances, *P. meleagrinum, A. flavus, P. notatum, A. fumigatus*, and *A. repens* were obtained in nutrient media. *P. chrysogenum* and *F. poae* were grown in denitrifying agar with nitrate under anoxic conditions. *P. chrysogenum*, which was isolated artificially, could flourish in both aerobic and anaerobic environments (Table 2 & 3).This fungal strain is more impactful on *A. fumigatus* in nutrient medium as well as in Sabouraund agar. Using Czapek agar, characteristic traits of Penicillium sp. were

investigated (Figure 1).

The population of aerobically developing *P. funiculosum* strains was 1.5*103/ml, while *T. harzianum* covered the entire petriplate. The fungal density in untreated sewage was 6.8*103/ml. Anaerobic strains included *P. meleagrinum, A. flavus, P. notatum, A. fumigatus*, and *A. repens*. In nutrient agar, sabouraud agar, and czapek agar, the CFU of A. repens were each 1.0*104/ml, 7.5*103/ml, and 4.65*105/ml, respectively. *P. meleagrinum* was found to be more abundant in nutritional agar (7.5*105/ml) than in Sabouraud agar (5.25*105/ml). *P. notatum* was found to be 5*103/ml in nutrient agar and 5*103/ml in Sabouraud agar, whereas *A. fumigatus* was found to be 7.5*104/ml in Nutrient agar and 5*104/ml in Sabouraud agar. *A. flavus* was found to be 1.5*105/ml in nutrient agar, 6*105/ml in sabouraud agar. On Czapek agar, there were numerous fungal colonies of *A. repens, A. flavus, A. fumigatus*, and *A. repens*. *F. poae* and *P. chrysogenum* were anoxically growing strains that utilised nitrate as an electron source. Their populations were 500/ml in Sabouraud agar, 1000/ml in Czapek agar and 2.27*103/ml in Denitrifying agar, respectively (Table 3).

Table 1. Fungi isolated from sewage

Isolate identified / Source	**Characteristic features**
T. *harzianum* aer	Isolation on Sabouraud agar green to dark green colonies. Covered the whole petridish after two days, forming light and dark green concentric rings. Conidiophores compactly branched, the apex usually bearing a solitary phialide. Conidia (spores) of 3 µm subglubose to short oval and smooth, nearly spherical.
F. *sporotrichioides* aer	Isolation on Czapek agar. Colonies on Czapek agar white or slightly green with exudates, on Sabouraud agar light pink. Phialides 5-18*3.5-5 µm, which proliferate sympodically. Microconidia often as numerous as macroconidia, singly or in short chains, globose lemon shaped and elongated. Microconidia pyriform, 6-16*3-4 µm 0-2 septa. Macroconidia 3-5, septate 29-46*3.7-5.3µm.
P. *funiculosum* aer	Isolation on Sabouraud agar with clear red patches on the underside of the petriplate. Monoverticillate, metulae growing at several levels. Conidia broadly oval to ellipsoidal, sometimes pyriform. Distinct earthy / aromatic odour. Colonies 5-6 cms in diameter. Yellow exudate in two layers. On Czapek agar white, slight green tinch, conidiophore of 100-300 µm and 3 µm wide, phialides of 6 µm in length and 2 µm wide and conidia of 2.5 µm.
A. repens aer, ana	Isolation on Sabouraud agar, sap green centre. Light to strong aromatic odour. On Czapek flat, yellow green or gray green colonies, reverse yellow green, dark brown at margin.
A. fischeri / *Neosartorya fischeri* aer, ana	Isolation on Sabouraud agar with white, columnar heads. Because of the dense production of conidial heads they are sometimes arbitrarily classified as *Penicillium*. Formation of conidiophore head 60 µm length and 5-10 µm width, philades 7 µm length and 2 µm width. Conidia 2-2.5 µm.
A. fumigatus aer, ana	Colonies with earthy odour, vary in size. Dark sap green, white periphery, exudate in center Single stage sterigmata 6-8*2-3 µm, located parallel to the axis of conidiophore Forming conidiophore of 300 µm in length, 2-8 µm in width. Meticulae 6- 10 µm and conidia 2 µm.
A. flavus aer, ana	Globose to subglobose conidia on Sabouraud agar. Fine roughened yellow green coloured colonies. Yellow-to-yellow green or green. Antibiotic odour. On Czapek agar, center sap green, periphery white upto 2.7 cms in diameter (distinct), spreading of the hyphae in the periphery in second week, covering whole plate. Forming conidiophore of 400-1000 µm in length and 5-15 µm in width. Phialides 6-8 µm and conidia 1-2µm, yellow with fine spines and variable sizes.
P. meleagrinum aer, ana	Isolation on Sabouraud agar. Colonies coiled mycelia folded upon itself many times. White periphery with slightly green or dark coloration. Light to strong aromatic odour. Forming yellow exudate on Czapek agar. Medium changing to yellow (pigmentation), on the 3rd day, on the 7 day pinkish red colouration. Forming conidiophore of 250 µm length and 2.5-3.3 µm wide, phialides of 10 µm, spore of 2.5 - 3 µm in size.

P. notatum aer, ana	Isolation on Sabouraud agar with sap coloured centre and white periphery. Antibiotic odour. Abundant yellow, yellow brown surface. On Czapek agar mycelium white, under side folded, 3-3.5 cm after two weeks. Conidiophore 250-500 µm in length and 3 µm in width, phialides 8 µm and conidia 2 µm with very long conidia arranged in the form of chains when attached to the phialides.
P. chrysogenum aer, anoxic	Typical fruity odour suggesting apples or pinapples. On Czapek agar velvety appearance. Colouration of the media distinct yellow. Margins of colonies white. 1-2 mm wide. Brown yellow exudates fusing to larger drops, reverse yellow, brown. One or more branches lead out from the main axis. Termination verticils of 2 to 5 metulae bearing sterigmata. On the 7th day pinkish orange on Czapek agar. Forming conidiophore of 150-350 µm in length and 3.0-3.5 µm in width, phialides 6 µm in length and 2 µm in width, conidia of 2-4 µm.
F. poae anoxic	Isolation on Sabouraud agar. Pink colouration with slight yellow exudate in the centre and white colouration on Nutrient agar. *Fusarium* has a typical fruity odour. Spherical microconidia, flask shaped phialides on compact, branched stripes distinguished from other species. Macroconidia usually sparsely produced, varying in shape, mostly with 3 septa, flask shaped phialides often appearing like bunches of grapes when examined under low power. Forming a conidiophore head of 26 µm and conidia of 4-6 µm.

* Isolated aerobically from raw sewage (aer), anaerobically from raw sewage after trickling through a 1.25-meter sand column at anoxic growth conditions (ano) and under anoxic conditions with a nitrate containing medium (anoxic).

Table 2. Fungal types identified from raw sewage and from sewage after trickling through soil columns under aerobic, anaerobic and anoxic conditions

Raw Sewage			**Soil columns effluents**		
Aerobic	**Anaerobic**	**Anoxic**	**Aerobic**	**Anaerobic**	**Anoxic**
T. harzianum	-	-	*A. repens*	*A. repens*	
F. sporotrichioides	-	-	*A. fischeri*	*A. fischeri*	
P. funiculosum			*A. fumigatus*	*A. fumigatus*	
Verticillium tenerum	-	-	*A. flavus*	*A. flavus*	
P. chrysogenum	*P. chrysogenum*	*P. chrysogenum*	*P. meleagrinum*	*P. meleagrinum*	
		F. poae	*P. notatum*	*P. notatum*	
-	-	*Monocillium mucidum*			

*Under anoxic conditions no fungal types were observed in soil column.

Table 3. Changes of fungal population densities with increasing trickling depth through a sandy soil

Fungal organism / different dilution factor	**Raw sewage CFU/ ml.**			**After trickling through sand columns in CFU/ ml.**														
				25cm (column 5)			**50cm (column 4)**			**75cm (column 3)**			**100cm (column2)**			**125cm (column 1)**		
	S	**C**	**D**	**N**	**S**	**C**	**N**	**S**	**C**	**N**	**S**	**C**	**N**	**S**	**C**	**N**	**S**	**C**
Aerobic condition																		
Different types of fungi (10^{-1})	$6.8*10^{3}$																	
P. funiculosum (10^{-1})	$1.5*10^{3}$	**5**																
T. harzianum (10^{-1})	**M**	**M**																
Anoxic condition																		
F. poae (10^{-1})	500	500	1000															
P. chrysogenum (10^{-1})	500	500	$2.27*10^{3}$															
Facultatively anaerobic																		
P. chrysogenum var *meleagrinum* (10^{-4})				$1.0*10^{4}$	$7.5*10^{3}$	$4.65*10^{5}$												
A. flavus (10^{-5})				$7.5*10^{5}$	$5.25*10^{5}$	M												
P. notatum (10^{-5})				$1.5*10^{5}$	$6*10^{5}$	$8..25*10^{5}$												
A. fumigatus (10^{4})							$5*10^{3}$	$5*10^{3}$	M							$4.25*10^{5}$	$2.175*10^{5}$	M
A. repens (10^{5})										$7.5*10^{4}$	$5*10^{4}$	M						

N: Nutrient agar, S: Sabouraud agar and C: Czapek agar.*M: Many fungi, D:denitrifying agar The effluent sample had no fungi under aerobic condition. Those obtained anaerobically were able to grown under aerobic conditions, generally more growth was observed in nutrient agar in case of *Aspergillus* and more growth of *Pencillium* in sabouraud agar plates.

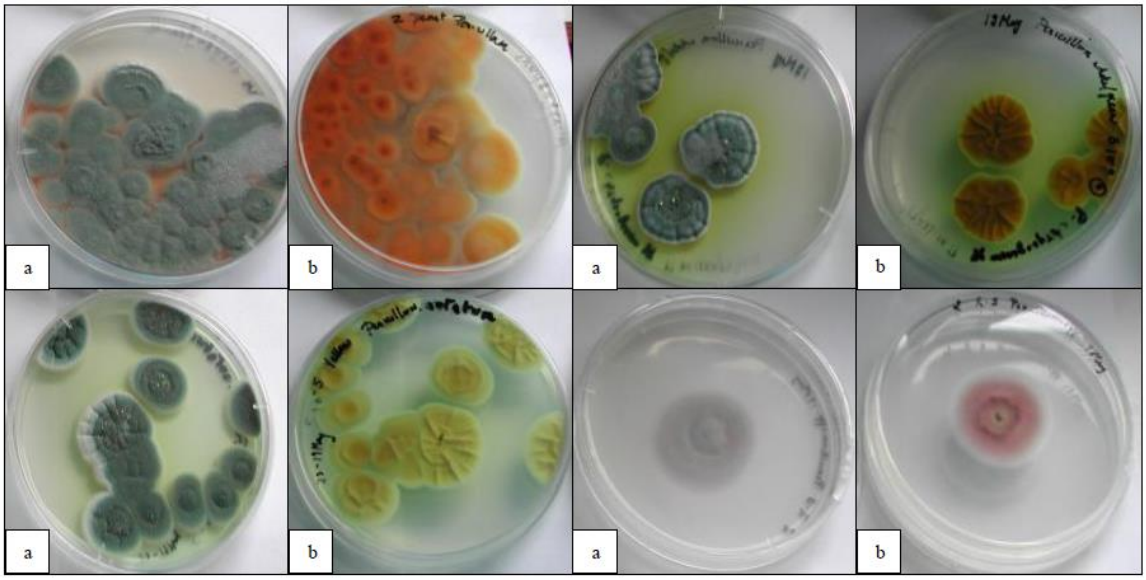

Figure 1. Characteristics of *Penicillium* species for identification on Czapek agar of seven day old culture of *Penicillium* species giving characteristic colouration to the agar medium.From top left *P. chrysogenum*, *P. meleagrinum*, and below *P. notatum* and *P. funiculosum* a, b indicate upper and lower side of the culture plate

P. funiculosum, P. meleagrinum, P. chrysogenum, P. notatum, A. fumigatus, A. flavus, and *A. repens* were tested for their ability to inhibit the growth of bacteria such as Gram-negative *Pseudomonas* and Gram-positive *Enterococcus*, *E. coli*, and anaerobic *Bifidobacterium*. The test bacteria were streaked from the plate's edge toward the fungal colonies after the fungus had been cultured for two days in the centre of the Nutrient and Sabouraud agar plate. Inhibition zones on the plates were measured after three days and after 14 days of further incubation. The test also included the fungus *F. poae*, which does not produce antibiotics.

Table 4. Inhibition of growth of 6 strains of *Enterococci*, *Pseudomonas*, *E. coli* and 5 strains of *Bifidobacterium* species by fungi. All organisms were isolated from sewage and column effluent

Fungal type 6 replicates	*Enterococci*	*Pseudomonas*	*E.coli*	*Bifidobacterium*
cm average inhibited (no of tested strains (6&5)) In brackets indicate no of strains				
Fusarium	ni 1 (1)	ni	Ni	vi 1.5-1.7 (3) 0.2 (1)
P. chrysogenum	i 0.1-0.2 (2) 0.7-2.5 (4)	vi 0.1-0.3 (2) 0.6-1.9 (4)	i 0.7-2.6 (5)	i
P. funiculosum	ni	ni	ni	vi
P. meleagrinum	vi 2 (1)	vi 0.5-1 (1)	vi 0.1-0.6 (2) 1-2.4 (2)	i
P. notatum	ni	vi 0.1-0.7 (1)	vi 1-3 (5)	i
A. fumigatus	i	vi 0.5-1 (2) 1.5-2 (2)	i 0.5 (1) 2-2.4 (4)	vi
A. repens	i 0.5-3 (5)	vi 0.2-0.3 (3) 0.6-0.2 (1)	vi	vi
A. flavus	i 0.5-0.7 (2)	ni 0.2 (1)	i 0.5 (1) 1(1)	vi

i: total inhibition of bacterial growth, ni: no inhibition of bacterial growth and vi: inbibition to variable extent.

P. chrysogenum, A. flavus, A. repens and *A. fumigatus*, were resistant to Enterococci sp., but *P. meleagrinum* was only slightly resistant. *P. meleagrinum*, *P. notatum*, and *A. repens* were somewhat resistant to *E. coli* sp., while *P. chrysogenum*, *A. fumigatus*, and *A. flavus* had the most inhibitory impact. Except for *P. funiculosum*, against which Bifidobacterium was ineffective, the organism was resistant to three different Penicillium species. The species of Pseudomonas was not extremely resistant to fungi (Table 4).Under aerobic conditions, *P. funiculosum* had no effect on bacterial growth, and *P. meleagrinum* and *P. notatum* were only marginally effective. *P. funiculosum* overgrew the bacterial streaks in the later weeks.

Enterococci, *Pseudomonas*, and *E. coli sp.* were inhibited by *P. chrysogenum*, *A. fumigatus*, and *A. flavus* in Nutrient agar (Figure 2) and by *P. chrysogenum* and *P. notatum* in Sabouraud agar (Figure 3). Compared to Nutrient agar, which has a neutral pH, Sabouraud agar's ingredients have higher sugar and a lower pH. When used against *Enterococci* and *E. coli sp.*, *A. repens* was effective (Figure 2).

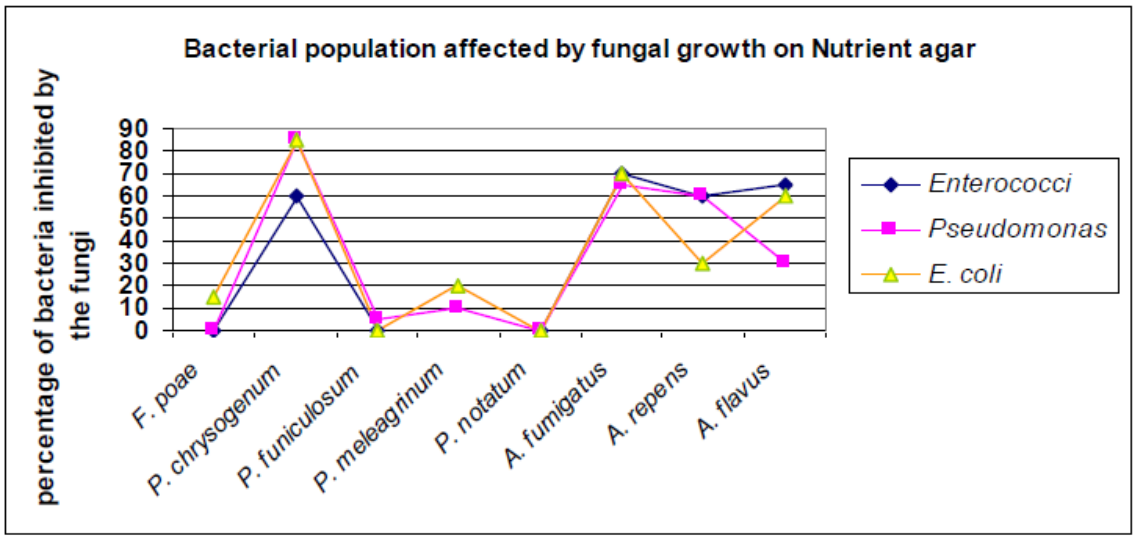

Figure 2. Bacterial population affected by fungal growth

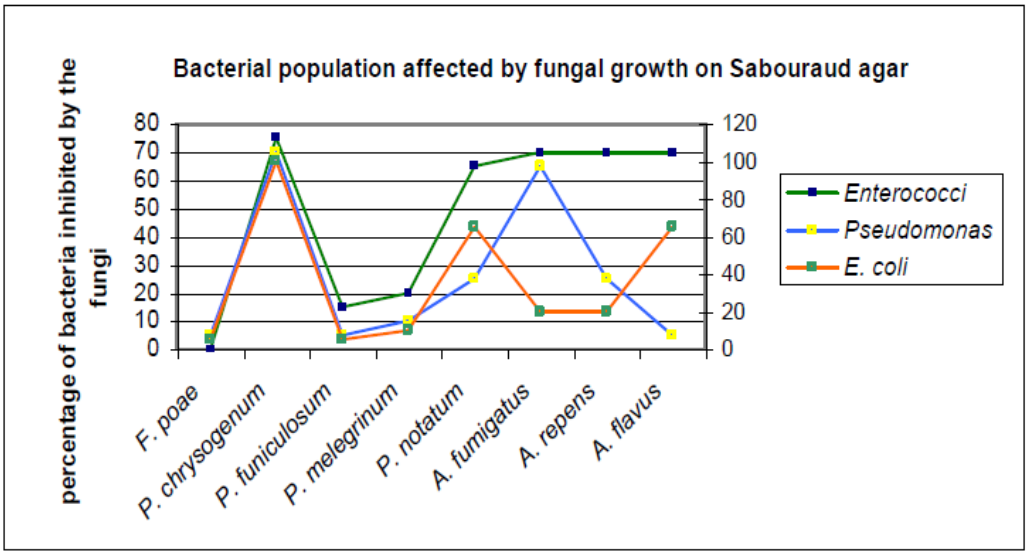

Figure 3. Bacterial population affected by fungal growth

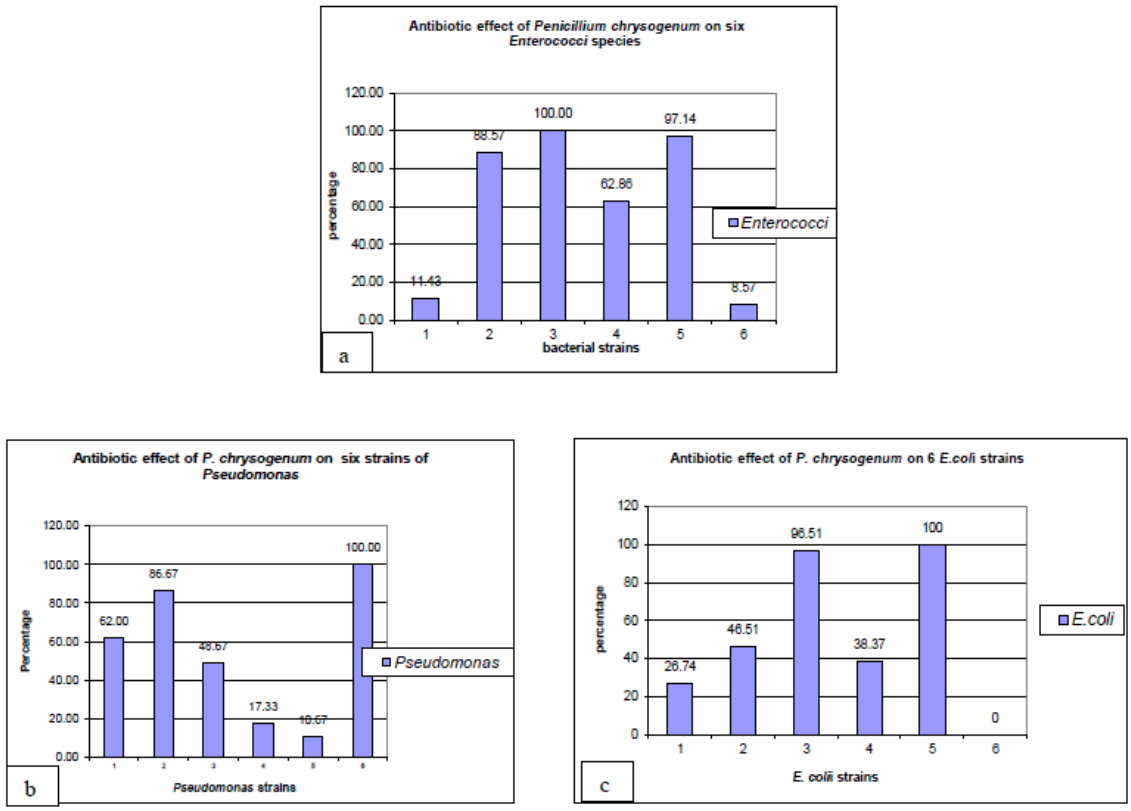

Figure 4. Growth inhibiting effect of *P. chrysogenum* on (a), *Enterococci* , (b) *Pseudomonas* and, (c) *E. coli*

Inhibition levels for the other two Enterococci sp. test strains were 62.86 % and 88.57%, respectively (Figure 4). *P. chrysogenum* suppressed two *Pseudomonas* test strains up to 82.67% and 89.33%. Three species of *E. coli* were inhibited in the test strains to a lesser than 53.49%, and one test strain of *E. coli* did not exhibit any growth, as shown in figure 4c. Figure 5 shows that *A. repens* inhibited 1 strain by roughly 82.8% and 2 strains by 71.42% of the six test strains of *Enterococci* species. *Pseudomonas* sp. test strains revealed one test strain that was completely inhibited, four strains that were less than 38% inhibited, and one strain that did not grow when exposed to *A. repens*. The most effective of the antibiotic fungus studied was *P. chrysogenum*. The majority of the gram-positive and gram-negative bacteria were resistant (Figure 4).

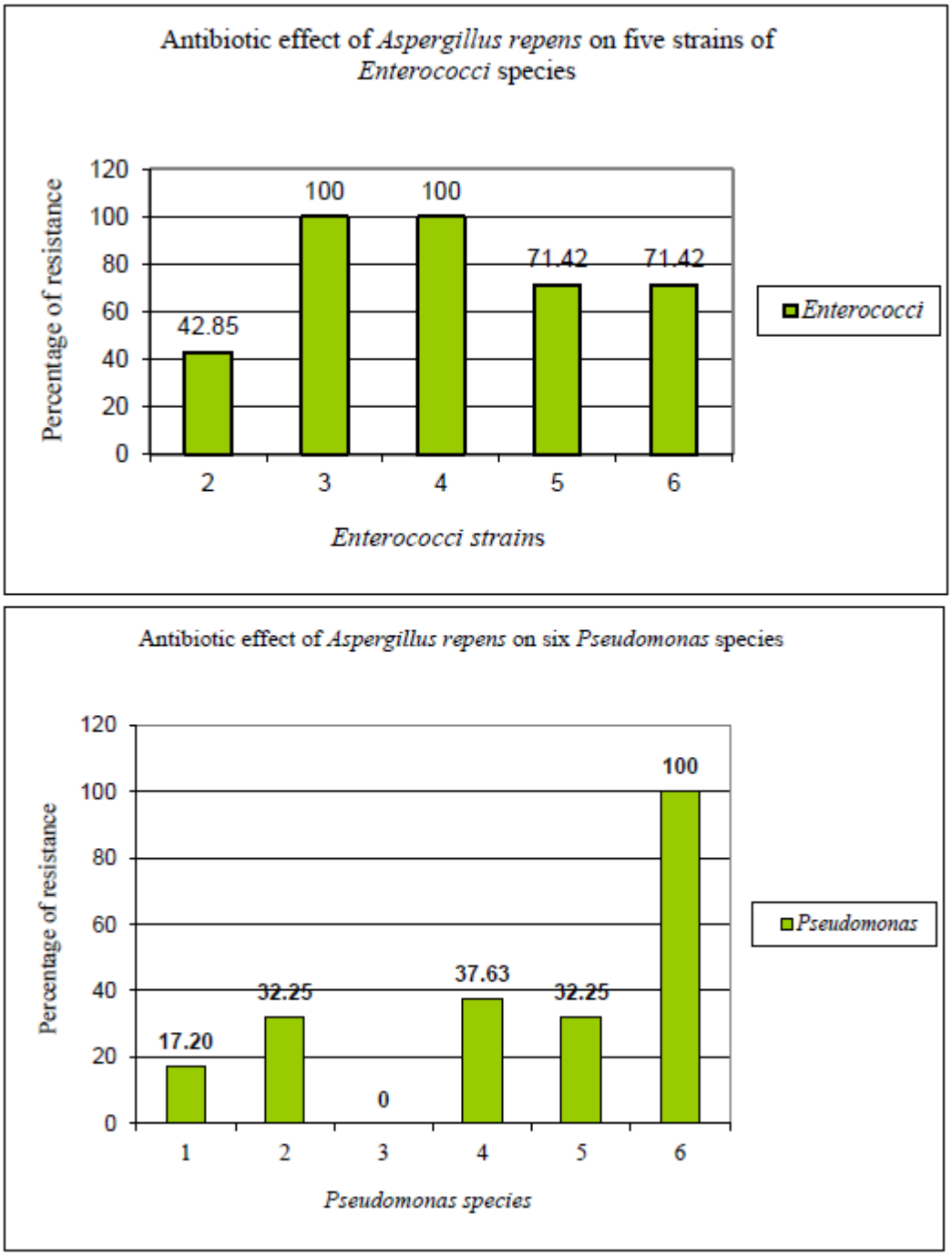

Figure 5. Growth inhibiting effect of *A. repens* on *Enterococci* (a), *Pseudomonas* (b)

P. chrysogenum and *P. notatum* were excellent antibacterial agents when the environment was anaerobic. Anaerobic conditions resulted in extremely sluggish growth rates for A. repens and A. fumigatus. A. repens had completely covered the petriplate after a week, and there was an increase in growth rate. Table 5 lists the useful antibiotic-producing chemicals in order of their impact on bacteria. Figure 6, shows that *P. chrysogenum* significantly suppressed *E. coli* while *Pseudomonas* sp. had little effect on *Fusarium* sp. *Fusarium* had a pink colour on

Sabouraud agar and a white colour on Nutrient agar. *P. notatum* did not prevent growth of bacteria streaks under aerobic conditions, although it did so somewhat under anaerobic conditions. *P. funiculosum* was ineffective because the bacteria on Sabouraud agar were covered by the fungi, and in anaerobic environments, the bacteria proliferated while the fungi remained small.

Table 5. Order of effect of useful antibiotic producing substances on bacteria

Enterococcus	***Pseudomonas***	***E. coli***	***Bifidobacterium***
P. chrysogenum	*P. chrysogenum*	*P. chrysogenum*	*P. chrysogenum*
A. repens	*A. fumigatus*	*A. flavus*	*P. notatum*
A. flavus	*A. repens*	*A. fumigatus*	*P. meleagrinum*
A. fumigatus	*A. flavus*	*P. meleagrinum*	*A.repens*
			A. fumigatus

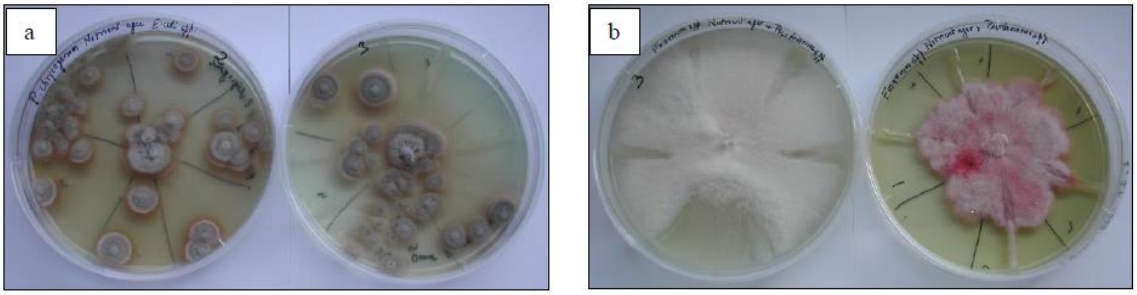

Figure 6.a) *Penicillium chrysogenum* showing antibiotic affect on *E.coli* and b) *Fusarium poae* non antibiotic producing with *Pseudomonas* species

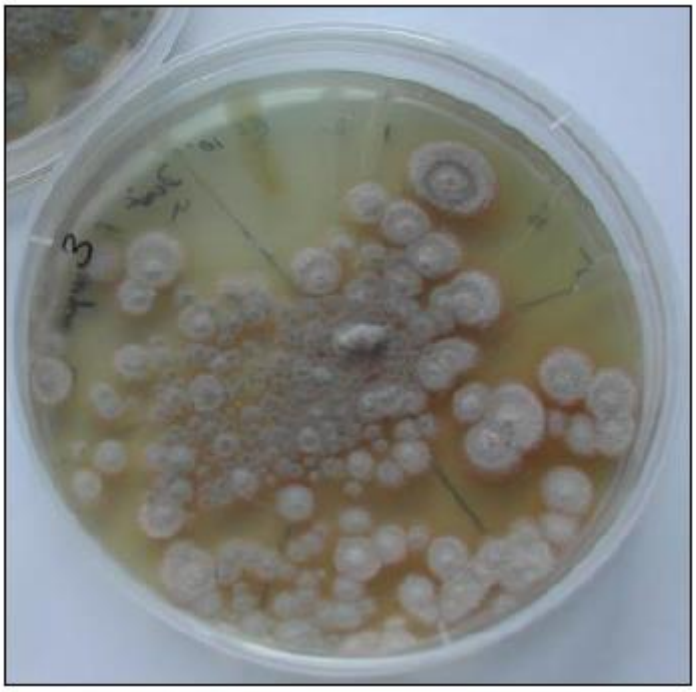

Figure 7. Resistant bacteria against *P. chrysogenum*

4. Discussion

Fungi were isolated from sewage under aerobic and anaerobic conditions. The fungi were identified as P. chrysogenum, P. notatum, P. meleagrinum, P. funiculosum, A. fumigatus, A. repens A. flavus, F. poae, F. sporotrichoides and T. harzianum in various agars (and checked for characteristic features in Czapek and Sabouraund agar). Many beneficial fungi were found to have antibiotic activity against Gram positive aerobic Enterococci, Gram negative pseudomonas and E. coli, and Gram-positive anaerobic Bifidobacterium (aerobic). The effectiveness of different naturally occurring antibiotics against resistant and non-resistant bacteria was tested. P. chrysogenum, which was obtained from raw sewage in a 10^{-1} dilution under anoxic (anaerobic) conditions, was the best antibiotic producing fungus against Enterococci, Pseudomonas, and E. coli. E. coli, a Gram-negative bacteria, hindered P. chrysogenum's strongest effects. Only one Penicillium species, P. chrysogenum, outperformed the other Penicillium species in terms of effectiveness. Apart from P. chrysogenum, additional fungus species that shown antibiotic efficacy reducing bacterial growth were A. fumigatus, A. repens, and A. flavus. P. meleagrinum had very little impact on Enterococci, Pseudomonas, and E. coli species (Table 4).

Because every bacterial strain was shown to be resistant to *P. funiculosum*, growth was unaffected in the culture plate containing the fungus and bacteria. Only Sabouraud agar plates showed an increase in *P. funiculosum* colony and covered the bacterial strip when the culture was old. *P. funiculosum* has only mild antibiotic activity against bacteria, according to the literature (Domsch et al., 1980). The highest results with Bifidobacterium were produced by *P. chrysogenium* and *P. notatum*, whereas other fungal species did not show significant antibiotic activity.

There are resistant strains of bacteria within genera and species that are susceptible to penicillin (or they are formed during penicillin action). There may be 50% or more non-sensitive strains in diverse circumstances, according to study. A few penicillin-resistant bacterial strains produce penicillinase, an enzyme that is frequently seen in penicillin-resistant microbes. This investigation revealed that *P. chrysogenum* had the best activity of *Penicillium spp.* on *Enterococci, E. coli, Bifidobacterium spp.*, and to some extent on *Pseudomonas spp*., in contrast to Bilai 1963, who found that some species, including *E. coli* and *Pseudomonas*, were ineffective against penicillin (in vitro and vivo). Penicillin is particularly effective against the bulk of Gram-positive bacteria that are hazardous to people, animals, and plants. The primary function of penicillin is bacteriostatic. It interferes with several processes involved in bacterial metabolism as well as how the bacterial cells process glutamic and other amino acids. Other research indicates that penicillin prevents young, developing bacteria from generating membranes. As in this investigation, *P. chrysogenium* shown the best antibiotic action in aerobic conditions when compared to other *P. funiculosum* or *P. meleagrinum* and *P. notatum*, whereas *P. chrysogenium* and *P. notatum* were most effective in anaerobic conditions with gram positive bacteria. Conclusions can only be made when the fungus has been identified at species and employed to combat bacteria. Numerous terrestrial mushrooms are unmistakably facultative anaerobes, and some species are more tolerant to low oxygen levels than others. There are several of these, including the

Penicillium, Mucor, *F. oxysporum*, *F. solani*, *T. viride*, and *F. solani* species (Tabak and Cooke, 1968; Curtis, 1969). When tested anaerobically with Bifidobacterium, Aspergillus species demonstrated robust development, displaying white mycelium under anaerobic circumstances and green under aerobic settings.

Fungi in raw sewage and those that had passed through soil columns differed in terms of their general population and type. Population of fungi isolated from wastewater ranked in *Penicillium, Aspergillus, Fusarium, Trichoderma* and other fungal types, indicating their spores to be present in sewage. Most of the fungi isolated from sewage were antibiotic producing and have some significant antibiotic activity on Gram positive as well as Gram-negative bacterial types. Population of fungi in aerobic conditions was more compared to anaerobic conditions, though the fungi grew well under anaerobic conditions as observed with fungal species and *Bifidobacterium* bacteria.

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