Original Research Article

Biotransformation of atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole and penicillin G by the natural microflora

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ABSTRACT

It has been found that natural microorganism have a siginificant role in the production and development of new medicines. The present study aimed to evaluat biotransformation of atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole and industrially compound (penicillin G), arrive in the human body, by some natural microorganism (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6051, *Lactobacillus acidophilus* ATCC 314, *Mycobacterium Bovis* (BCG), *Saccharomyces cerevisiae* ATCC 5052, and *Candida albicans* ATCC 10231), for the first time. The best values for the concentration and required times were evaluated for each metabolites in microbioal biotransformation process. The progress of the formation of the metabolites were monitored by Thin Layer Chromatography (TLC) method. Obtained results showed that only indole, atropine, and 4-hydroxy coumarin could be biotransformed by *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, and *Saccharomyces cerevisiae* ATCC 5052, respectively.

***Keywords*:** Biotransformation, natural flora microorganism, plant secondary metabolites

INTRODUCTION

The history of microbial biotransformation is closely associated with vinegar production that dates back to around 2000 years BC. Vinegar production is maybe the oldest and best-known example of microbial oxidation, which can explain some of the important developments in the

field of microbial biotransformation by active cells [1]. Humans have utilized microbial enzymes for thousands of years to produce and preserve foodstuffs such as cheese, beer, vinegar, and wine [2].

The microbial transformation has been used to produce products that are difficult to produce by chemical methods. In addition, chemical processes can lead to a group of environmentally harmful wastes. Biotechnological production, for example, biotransformation compounds, appears as an interesting alternative to overcome the problems associated with chemical synthesis and it is cheaper to use a microorganism for the synthesized compound [1-5].

Today, products obtained through biotransformation are labeled as natural products [6-7]. Biotransformation is the reaction of one enzyme to several enzymes or the use of the entire contents of a cell. Microorganisms have abundant potential for inducing many changes of innovative and improvised enzyme systems that are capable for converting unfamiliar substrates [7-11].

Microbial biotransformation of biological compounds has useful applications in the synthesis of drugs. Pharmaceuticals are the most important type of fine-chemical products, and this applies to fine-chemical products that are produced by biotransformation. Some other types of fine chemicals, such as dyes, are never produced by biotransformation, but biotransformation is relatively important for food ingredients, and also seems to have found a place in the cosmetics industry. Natural compounds and their derivatives are indeed the prevailing classes of products produced by biotransformation [1,3,4,5,8,12]. The microbial transformation has been studied for centuries. The number of products of microbial biotransformation is almost doubling every decade.

The human body contains important and beneficial microorganisms, each of which in turn is effective in the biotransformation process. Many microorganisms are part of the body's natural flora, but microorganisms that arrive in the body through food are also involved in the microbiological process. The following are the most important microorganisms in the human body: *Escherichia coli*, *Bacillus subtilis*, *Lactobacillus*, *Mycobacterium*, *Saccharomyces cerevisiae*, and *Candida albicans*.

Plant and medicinal substances in the human diet contain metabolites and active substances that can react with microflora and change the process of microbial

biotransformation. Among the important plant metabolites and drugs that are in the human food basket, the following can be mentioned: Atropine, a tropane alkaloid of medicinal interest, was found in plants of the Solanaceae family. Atropine has been widely utilized in clinical for many years as anticholinergic agents and a mydriatic drug in ophthalmology [13-16]. Indole and Quinoline, are polycyclic aromatic nitrogen heterocycles compounds, occur in various products such as oil, creosote, and pharmaceuticals. Quinoline and its derivatives are widely used as raw materials and solvents in the manufacture of dyes, paints, fungicides, and wood-treatment chemicals [17-20]. Indole and derivatives are widely used for the synthesis of pharmaceutical, dyes, and industrial solvents [21-23]. Coumarins, are widely distributed in nature and make up an important part of the human diet. They are found naturally in all parts of plants and microorganisms. Coumarine and derivatives are biologically active. It has a wide range of pharmacological activities and much research has focused on the antibacterial and antifungal activities [24-29].

Penicillin is known as the first antibiotic to be discovered and is still involved in a range of pharmacological studies [30-31]. Since their importance in the food and medicine basket of humans, their change is important and their microbial transformation with natural flora are the basis for research biotransformation.

In this present work, we have reported results of the ability microbial biotransformation of atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole, and penicillin G with Escherichia coli, Bacillus subtilis, Lactobacillus, Mycobacterium, Saccharomyces cerevisiae, and Candida albicans for the first time.

MATERIALS AND METHODS

Atropine was obtained from Minsheng Group Shaoxing Pharma Co.Ltd, China. Indole, 4-hydroxy coumarin and 8-hydroxy quinoline were obtained from Merk, Germany. Penicillin G sodium was obtained from Jaber Pharma, Iran. Organic solvents: Ethanol, n-hexane, chloroform, methanol, tri ethylamine, dichloromethane, acetone, acetic acid, toluene and dimethyl-sulfoxide were of analytical grade and were obtained from Merck, Germany. Ethyl acetate was obtained from Chem lab, Belgium. Silica gel TLC sheets were obtained from Merck, Germany.

Growth medium and condition

Escherichia coli ATCC 25922 and *Bacillus subtilis* ATCC 6051 were grown in culture medium Nutrient broth (was obtained from

HIMEDIA, INDIA), Culture flasks were incubated at 37 ℃ in a shaker rotating at 150 rpm for 24 h under aerobic conditions. *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 5052 were grown in culture medium Sabouraud maltose broth (was obtained from DIFCO, Becton, Dickinson, USA), Culture flasks were incubated at 37℃ in a shaker rotating at 150 rpm for 48 hours under aerobic conditions. *Lactobacillus acidophilus* ATCC 314 was grown in culture medium MRS broth (was obtained from Liofilchem, ITALY), Culture flasks were incubated at 37 ℃ in a shaker rotating at 150 rpm for 24 h under anaerobic conditions. *Mycobacterium Bovis* (BCG) was grown in culture medium Middlebrook 7H9 Broth (was obtained from HIMEDIA, India), Culture flasks were incubated at 37 ºC in a shaker rotating at 150 rpm for 72 h under aerobic conditions. All of the microorganisms were added after dissolving, autoclaving, and cooling broth medium culture.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole, and penicillin G by *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC, *Saccharomyces cerevisiae* ATCC 5052, and *Lactobacillus acidophilus* ATCC 314 were determined by following the broth microdilution method for an antifungal and antimicrobial test based on NCCLS M27-A [32]. Sabouraud maltose broth was used as the growth medium for *Candida albicans* and *Saccharomyces cerevisiae*. The nutrient broth was used as the growth medium for *Escherichia coli* and *Bacillus subtilis*, and MRS Broth was used as the growth medium *Lactobacillus acidophilus*. The absorbance was read 530 nm for fungi and 600 nm for bacteria inoculums to reach the suitable density of test microorganisms. The compounds were dissolved in Dimethyl Sulfoxide (DMSO) at the concentration of 10 mg/ml. Fluconazole and nystatin were used as a reference in the antifungal test, streptomycin was the reference for an antimicrobial test, and DMSO was the solvent control. Wells including serial dilution of DMSO and broth media only were also included as controls. The plates were covered and incubated at 37 ℃ for 24 and 48 h. The MIC value was measured by reading the concentration of the well with no growth.

This test for *Mycobacterium* is different from other bacteria and fungi tested in this study. There is no need to make a microbial suspension. MIC of atropine, 4-hydroxy

coumarin, 8-hydroxy quinoline, indole and penicillin G by *Mycobacterium Bovis (BCG)* was performed using broth microdilution method as the standard procedure for RGM [33]. Middlebrook 7H9 broth was used as the medium in this assay. Ethambutol was reference for antimicrobial test and DMSO as solvent control. The microplate was incubated for 72 h in 30˚C. After addition of Alamar Blue and incubation, the MIC was determinate as the lowest sample concentration at which no pink color appeared.

Microbial transformation

According to MIC, some of the compounds were used for transformation that does not have the power to remove microorganisms during the biotransformation. The compound's concentration was below the MIC in the water phase. Biotransformation by growing microorganisms was carried out as follows: microorganisms were first incubated in 250 ml flasks (37 ℃, 150 rpm) containing 100 ml medium culture broth. Then, substrates (atropine, indole, 8-hydroxy quinoline, and penicillin G dissolved in ethanol, 4-hydroxy coumarin dissolved in methanol) were added into this system. Meantime, three kinds of control were performed as follows: (1) transformation broth with microorganisms but no addition of substrates, (2) transformation broth with substrates but no addition of microorganisms, (3) medium culture broth souls. The transformation was terminated by the addition of ethyl acetate. After further cultivation at 30 °C for 14 days, samples were taken daily and were extracted thrice with an equal volume of ethyl acetate. The sample liquid was evaporated in a rotary evaporator under reduced pressure, and the residue was dissolved in methanol. The controls were done in the same way.

Thin Layer Chromatography (TLC) separation of produce biotransformation

All the crude ethyl acetate extracts were monitored by TLC on percolated silica gel sheet $60F_{254}$ .Compounds were visualized by UV light at 254 nm and 366 nm in the TLC chamber. The appropriate mobile phase was selected according to the point resolution. Control flasks were also extracted using the same procedure and analyzed by TLC. The *Rf* value of each band was calculated as the distance traveled by the solute separated by the distance traveled by the solvent.

RESULTS

Antimicrobial activity

The inhibitory effect of compounds on microorganisms was tested. The antimicrobial activities, as given by the

MIC values, of the six compounds are shown in Table 1. As previously mentioned, for biotransformation, some substrates are used that do not affect the growth of microorganisms during the process.

Biodegradation

For 14 days, sampling was performed regularly and daily during a specified hour. The ability to biotransformed or not biotransformed compounds were tested and those that can be biotransformed with the day of the beginning of transformation are listed in Table 2 and schematically

represented in Figure 1. Within 3 days for Indole and Atropine, and 8 days for 4-hydroxy coumarin, compounds were disappeared from the culture medium and new compounds appeared in the supernatant. This metabolite was not detected in the extract of the supernatant of the control. The microbial transformation of Atropine by Escherichia coli K12, Indole by Candida albicans ATCC 10231, and 4-hydroxy coumarin by Saccharomyces cerevisiae ATCC 5052 gave novel compounds.

Table 1. Antimicrobial activity of compounds against natural microflora, expressed as µg/ml, using broth dilution method

Compound	**Microorganism**											
	E.coli k12 ATCC 29922		*Bacillus ubtilis* ATCC 6051		*Lactobacillus acidopillus* ATCC 314		*Mycobacterium Bovis* (BCG)		*Candida albicans* ATCC 10231		*Saccharomyces cerevisiae* ATCC 5052	
	MIC µg/ml											
	24h	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
Indole	>1000	>1000	>1000	>1000	1000	1000	1000	1000	250	250	250	500
Atropine	>1000	>1000	>1000	>1000	1000	1000	1000	1000	500	250	500	500
8-hydroxy quinoline	125	125	<7.93	7.93	31.75	31.75	<7.93	<7.93	15.78	15.78	<7.93	<7.93
4-hydroxy coumarin	500	250	500	1000	500	500	31.25	<7.93	250	125	125	125
Penicillin G	62.5	62.5	500	250	<7.93	<7.93	62.5	62.5	250	500	500	500
DMSO	>1000	>1000	>1000	>1000	1000	1000	>1000	>1000	500	500	250	250
Fluconazole	-		-		-		-		-		62.5	
Streptomycin	125		62.5		500		-		-		-	
Nystatin	-		-		-		-		<7.93		-	
Ethambutol	-		-		-		<7.93		-		-	

(-) Not tested

Table 2. Ability of microorganism for biotransformed compounds with the day of beginning biotransformation

Compound	Microorganism	Time (Days)
Indole	*Candida albicans ATCC 10231*	3
Atropine	*Escherichia coli* ATCC 25922	3
4-hydroxy coumarin	*Saccharomyces cerevisiae* ATCC 5052	8

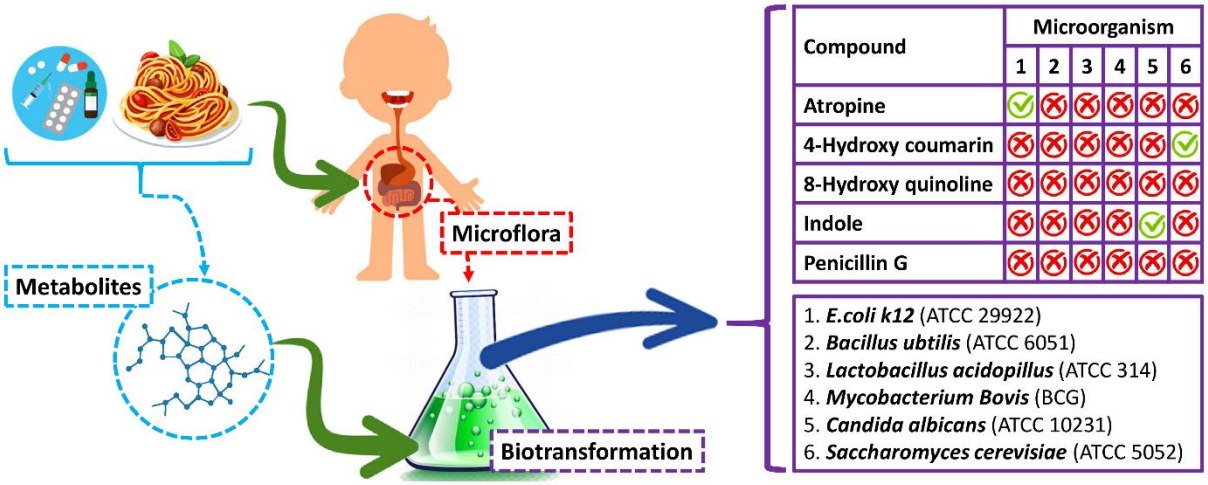

Figure 1. Schematically representation of the ability of microorganism for biotransformed compounds.

Thin Layer Chromatography (TLC) analysis

Qualitative analysis of the reaction products was carried out by TLC on glass plates, using a 0.2-mm layer of silica gel. The best mobile phase for purifying the ethyl acetate fraction of the biotransformation was selected and was listed in Table 3. According to TLC results, the spot appeared in biotransformation but not in the original was selected.

DISCUSSION

The present study aimed to evaluat the biotransformation of atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole, and penicillin G. Bacteria, fungi, and yeasts were tested for their ability to biotransform substrate. The critical difference between fermentation and microbial transformation is that there are several catalytic steps between the substrate and the product in fermentation while there are only one or two in biotransformation. The distinction is also in the fact that the chemical structures of the substrate and the product resemble one another in biotransformation, but not necessarily in fermentation. There are many potential pharmaceutical applications of the biological compounds along with their different chemical structures have to lead to researches and identifying new compound from microbial biotransformation [1-12]. Microorganisms are capable to produce an abundant variety of enzymes in a short period of time as a result of their natural characteristic to multiply and aqueous media are generally compatible with enzymes and growing whole cells [10-35]. Atropine, 4-hydroxy coumarin, 8-hydroxy quinoline, indole, and penicillin G have not been applied as a substrate for the biotransformation by microorganism were mentioned. Indole, atropine, and 4-hydroxy coumarin ability to transform by *Candida albicans* ATCC 10231, *Escherichia coli* K12, and *Saccharomyces cerevisiae* ATCC 5052. Indole is an important heterocyclic system because it is built into proteins in the form of amino acid tryptophan and is used for the synthetic pharmaceuticals [36-38]. The study of indole derivatives by yeast biotransformation was very limited. Atropine, a tropane alkaloid of medicinal importance, however, little data is available on the in vitro metabolism and metabolites of atropine [14]. Coumarins produce a major group of secondary plant metabolites and it is normally in human food. Coumarine biotransformation is very important and is used for new pharmaceuticals [28].

The most broadly exploited and commercially significant yeasts are the related species and strain *Saccharomyces cerevisiae* and *Candida albicans* which have been particularly shown to catalyze compounds with carbonyl groups or carbon-carbon bonds [39]. The microbial biotransformation products were loaded on thin layer silica gel plates and separated on TLC plates through different solvent systems. This study reports novel

compounds from microbial biotransformation. In future investigations, it would try to purify and identify the mentioned metabolites with spectroscopy methods such as NMR and Mass spectroscopy.

Table 3. Mobiles phase systems studied and Rf value in new compound

Compounds	Solvent system	V:V	$Rf_{compound}$	$Rf_{new\ compound}$
Indole	n-hexane: Ethyl acetate	5:5	0.93	0.26
Atropine	Toluene: Ethyl acetate: Tri ethylamine	7:2:1	0.82	0.62
8-hydroxy quinoline	Dichloromethane: Methanol	9:1	0.76	-
4-hydroxy coumarin	n-hexane: Ethyl acetate	2.5:7.5	0.37	0.42
Penicillin G	Acetone: Acetic acid	9.5:0.5	0.94	-

Rf: retardation factor

CONCLUSION

This study described the biotransformation of natural and commercial compounds by microflora. This work, introduced new compounds from bioconversion of indole, atropine, and 4-hydroxy coumarin. *Candida albicans* ATCC 10231, *Escherichia coli* K12, and *Saccharomyces cerevisiae* ATCC 5052 with high capacity for microbial degradation were identified. The whole-cell of microorganisms were used to biotransform and the ability of microorganism this regard were assessed. In this research, three new compounds were introduced which are the basis for further microbial biotransformation research.

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