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Research Article

SCREENING OF ACTIVE ANTIMICROBIAL AND BIOLOGICAL ENZYMES OF MICROBIAL ISOLATED FROM SOIL IN THAILAND

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ABSTRACT

Objective: The objectives of this study were to isolate microorganisms and screen for potential antimicrobial activities from the soil.

Methods: In this study, a total of 425 isolates were isolated from 100 soil samples. The preliminary screening for antimicrobial activities of these isolates was performed by modified cross-streak, agar diffusion, and modified microdilution technique against 16 pathogenic bacteria and fungi.

Results: In the anti-microbial activity, there were three isolates, namely, 277, 303, and 307 exhibited inhibitory activity against methicillin-resistant *Staphylococcus aureus* and *Salmonella typhimurium* respectively. This study also examined the various enzymes producing from soil microorganisms including chitinase, chitosanase, amylase, cellulose, caseinase, gelatinase, esterase, and lipase production of different selective media for 24 and 48 hrs using the direct spot method. The results revealed that 28 isolates could produce various enzymes with strong activity. Most of them produced gelatinase (5.65%) and caseinase (5.18%). There were four isolates that produce broad-spectrum enzyme. In addition, the investigation of selected microorganism identification showed that they can be divided into three groups: *Burkholderia* spp., *Pseudomonas* spp., and *Rhodococcus* spp.

Conclusion: This study demonstrated that the microorganisms from soil are capable of producing potential, antibacterial, and bioactive enzymes.

Keywords: Antimicrobial activity, Extracellular enzyme, Soil microbial, Drug-resistant bacteria.

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INTRODUCTION

Most bacteria can be product many microbial defense systems, including broad-spectrum classical antibiotics, metabolic by-products such as organic acids, and lytic agents such as lysozyme. This biological product is remarkable in its diversity and natural abundance. Some substances are restricted to some bacterial groups while others are widespread produced [1]. Due to the emergence of new infectious diseases and antibiotic resistances in infectious microbial strains that represent a serious problem for human life by making it very serious problem in the treatment of pathogenic microbes [2,3], the need for new therapeutic agents through natural, synthetic, or semisynthetic sources is still urgent.

Nowadays, the majority of industrial enzymes used come from a microbial origin and have hydrolytic activity with quite diverse potential applications in different areas. Microbial enzymes are relatively more stable and have properties more diverse than other enzymes derived from plants and animals [4]. Screening of microorganisms from natural environments for enzyme production has already been published, especially in unconventional and extreme environments [5-8].

Soil represents a promising habitat for discovering and isolating new natural products [9] and as <1% of soil bacterial species are currently known [10]. Discovery of new antibiotic agents and bioactive enzymes in natural environments such as soil involving all the cultivable microorganisms in these habitats is still missing.

Thailand is an investigated biological system which is situated in the tropical rainforest range. This is a result of tropical zones giving a great environment to development and is an extraordinarily assorted quality of living beings. Therefore, it may be a source of new microorganisms producing new compounds.

In an ongoing work on natural products from microorganisms and due to our interest in isolating soil bacterial strains with the ability to produce biologically active metabolites, the present work, an attempt to screen bacterial strain isolated from different soil of Thailand and assess their antimicrobial activity as well as enzyme production capacity, has been established.

METHODS

Isolation of microbial from soil

One hundred soil samples were gathered from different parts of rural range in Thailand. As per topographical assortment, six sections of Thailand were gathered. There were 35 samples from the southern section, 27 samples from the northeast section, 18 samples from the central section, 8 samples from the eastern section, 7 samples from the northern part, and 5 samples from the western part. Microorganisms from the examples were confined by the dilution plate technique. Soil tests were serially 10-dilution. A volume of 100 µl from serial dilution of 10^{-3}–10^{-5} was pipetted and spread on basal mineral salt agar (BSA) by spread plate technique and incubated at room temperature for 24-48 hrs. Single colonies were chosen from plate which delivered 30-300 colonies for each plate and streaked on BSA and identified. In the identification tests of strains, colony morphology and Gram strains were analyzed. The pure culture was kept up in BSA, put away at 4°C, and initiated in the same media for 24 hrs before test use.

Test microbial pathogen

Test bacterial pathogen used in this experiment was received from the Department of Medical Sciences, Faculty of Science, Rangsit University. It was composed of 14 strains of pathogenic bacteria: *Escherichia coli* extended-spectrum beta-lactamases, *Klebsiella pneumoniae*, *Salmonella typhimurium*, methicillin-sensitive *Staphylococcus aureus* (MSSA) (ATCC25923), and 10 strains of methicillin-resistant *S. aureus* (MRSA); MRSA 4, MRSA 5, MRSA 6, MRSA 14, MRSA 18, MRSA 28, MRSA 29, MRSA 31, MRSA 34, and MRSA 36. Test fungi pathogens were composed of two strains of pathogenic fungi: *Candida albicans* and *Cryptococcus neoformans*.

Preliminary antimicrobial assay

Determinations of the antimicrobial activities of pure bacterial cultures were modified according to the cross-streak method from Fernando [11]. In brief, the Mueller–Hinton agar (MHA) was inoculated with 24 hrs pure isolate cultures by a single streak in the center of the plate and incubated at room temperature for 2 days.

The plates were seeded with test pathogen by streaking perpendicular to the line of selected bacterial growth. The cross-streak method was done by keeping the distance between the test pathogen streaks fixed and kept for 7 days. Antagonism was observed based on the inhibitory interaction between the selected bacterial growth, and test strains were measured in millimeter using a scale. The experiment was carried out in triplicates.

Semi-quantitative antimicrobial assay

Antimicrobial activity was evaluated by agar well diffusion method. Isolated colonies of the tested bacteria or yeast pathogens were suspended in sterile 0.85% NaCl solution and the turbidity was measured using spectrophotometry (optical density [OD]=0.08-0.1 at 625 nm for bacteria and OD=0.12-0.15 at 530 nm for yeast). A volume of 100 µl of test organism was spread over the surface of the MHA using a 3-way swab. Six millimeter diameter wells in agar were made using a sterile cork borer. Isolated colony of antibiotic-producing bacteria was suspended in a sterile nutrient broth and the turbidity was measured using spectrophotometry (OD=0.08-0.1 at 625 nm). An aliquot of 20 µl of antibiotic-producing bacterial suspension was added to each well. Nutrient broth was used as negative controls. The plates were incubated at 35±2°C for 24 hrs for test bacteria pathogen and *C. albicans* and 48 hrs for *C. neoformans*. The diameter of inhibition zone around the well was measured using a scale.

Preparation of extracellular filtration

Isolated colony of each antibiotic-producing bacteria was suspended in sterile 0.85% NaCl solution and standardized by measuring spectrophotometry turbidity (OD=0.08-0.1 at 625 nm). Then, 5% (v/v) of this standardized inoculum was inoculated into 15 ml centrifuge tube containing 5 ml of nutrient broth and incubated at room temperature on static condition for 2 days. Next, the culture medium was centrifuged at 5000 g at 4°C for 10 minutes. The supernatant was gathered and filtered by 0.45 µm utilized as an extracellular filtration for quantitative screening of antibiotic-producing bacteria.

Determination of the quantitative antimicrobial assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracellular filtration were determined using the standard broth microdilution method and as recommended by the NCCLS and CLSI methodologies [12-14] with a few modifications. The serial 2-fold dilutions were made in a concentration ranged from undiluted to 1:512. The test was done in a 96-well plate by 50 µl of test sample combined with 50 µl of standardized tested bacteria or fungi suspension containing 1×10^6 CFU/ml was added into each well. Gentamicin and ketoconazole (initial concentration 1000 µg/ml) were used as positive control for bacteria and fungi, respectively. Mueller–Hinton broth was used as a negative control. Microtiter plates were incubated at 37°C, 24 hrs. After the incubation period, 10 µl of the 0.18% sterile resazurin indicator solution was added to each well according to modified resazurin microtiter plate assay. The positive result was blue or purple color (oxidized form). The negative result was purple to pink or colorless (reduced form). The lowest concentration at which the blue or purple color occurred was taken as the MIC value. All tests were carried out in triplicate.

The MBC was determined by inoculating 1 loop of the sample from wells that showed no apparent growth from the MIC assays onto MHA plates and incubated at 37°C, 24 hrs. The plates were examined for growth or lack of growth for each dilution subculturing. The lowest concentration showing no visible growth on agar subculture was taken as MBC value.

Extracellular enzyme production of selected bacterial strains

The productions of the following enzymatic activities were evaluated: Amylolytic activity (starch hydrolysis), esterase activity, lipolytic activity, proteolytic activity (protease, gelatinase), cellulolytic activity, chitinase, and chitosanase by the spot agar method. The enzymatic activities were investigated after inoculated colony within 2-14 day incubation on the specific culture media, according to specific methodologies for each investigated enzyme. All assays were done in triplicate.

For the detection of amylolytic, activity was applied from Hankin and Anagnostakis [15]. The isolates were heavily inoculated in the basal mineral salts medium (BMSM) with 0.01% of soluble starch, pH 6.0. After incubation at 24 and 48 hrs, the cultures were treated under Lugol's iodine solution, which allowed the visualization of clear halos around the colonies.

To verify the esterase and lipolytic activity, the isolates were heavily inoculated in the BMSM containing 1% (v/v) Tween 80 for esterase and 1% (v/v) Tween 20 for lipolytic activity, respectively, together with 0.1 g/L $CaCl_2$ and 0.1 g/L phenol red. The esterase and lipolytic activity was detected by the presence of clear halos around strain growth after 24 and 48 hrs of incubation.

For the detection of the casein hydrolysis and gelatin hydrolysis, the isolates were heavily inoculated in the BMSM containing skim milk (10 g/L) for casein hydrolysis and bacteriological gelatin (4.0 g/L) for gelatin hydrolysis. The casein hydrolysis-producing isolate showed a clear halo around the bacterial colony after 2 ml of 0.1 mol/L hydrochloric acid (HCl) was added to the plate. Gelatin hydrolysis-producing isolates demonstrated a clear halo around strain growth after covering with Frazier's revealers (distilled water 100 ml, HCl 20.0 ml, and mercury dichloride 15.0 g) after 24 and 48 hrs of incubation [16].

To verify the cellulolytic activity, the isolates were heavily inoculated in the BMSM-containing carboxymethyl cellulose (10 g/L). After incubation at 30°C for 24-48 hrs, the presence of clear halos around the colonies was observed after covering with 0.2% Congo red and destained with 1 M NaCl for 15 minutes [17].

For the detection of the chitinase and chitosanase activity, the isolates were heavily inoculated in the BMSM-containing colloidal chitin (10 g/L) for chitinase activity and colloidal chitosan (10 g/L) for chitosanase activity. The detection of chitinase- and chitosanase-producing isolates was observed in the presence of halos observed after incubation at 30°C for 2-14 days.

Statistical analysis

All experimental results were carried out in triplicate and were expressed as an average of three analyses±standard deviation using the SPSS version 22.

RESULTS AND DISCUSSION

The expanding recurrence of multi-resistant pathogenic bacteria is made an urgent demand in the modern world for additional methodologies and strategies to the screening of new antibiotic agents with a broad spectrum of activity that can oppose the inactivation processes exploited by microbial enzymes [18,19]. It is necessary to search new microbes and novel metabolites to counter the dangers postured by the fast-emerging phenomenon of antibiotic resistance.

In the present work, the potential of microbial isolates from soil samples for extracellular antibiotic and enzyme production was characterized. Of the 100 soil samples analyzed, 477 isolates were obtained, including bacteria and fungi. Among the isolates, 13 isolates (2.73%) exhibited inhibitory activity against at least 6 of the tested microbial pathogens; MRSA, MSSA, C. albicans, and C. neoformans in modified cross-streak method. The spectra of inhibition varied among the isolated bacteria. As observed in Table 1, isolates 17, 191, and 225 were the most efficient isolates which can inhibit 13 isolates of the tested microbial pathogen

Table 1: Antimicrobial activity of the microbial strains against test microbial pathogen at 48 hrs by modified cross-streak method

S.No.	ID number	Inhibition zone of antimicrobial activity at 48 hrs (mean±SD, mm)								
		MRSA4	**MRSA5**	**MRSA6**	**MRSA14**	**MRSA18**	**MRSA28**	**MRSA29**	**MRSA31**	**Total inhibition**
1	10	9.0±1.1	-	11.5±2.2	11.5±0.1	5.0±1.4	11.0±1.4	1.5±0.2	-	10
2	17	11.0±1.4	11.5±0.7	10.0±1.4	15.5±0.1	17.0±1.4	14.5±1.1	14.0±1.1	14.0±1.8	13
3	38	10.5±0.1	10.0±1.1	13.5±2.1	15±1.4	15.5±0.1	15.0±0.1	13.5±0.7	9.0±0.6	12
4	69	7.5±0.7	5.0±1.4	6.0±1.1	-	9.0±1.4	4.5±1.1	9.0±1.3	8.5±1.2	10
5	70	9.0±1.4	9.5±0.1	14.0±1.2	18±1.1	9.5±0.1	14.0±0.7	7.0±1.5	12.0±1.3	11
6	191	11.0±1.2	10.5±2.2	10.5±0.7	15.5±0.7	17.0±1.1	14.5±1.4	14.0±1.4	14.5±1.4	13
7	225	10.5±0.7	9.5±2.1	13.5±2.1	15.0±1.1	15.0±1.4	14.0±1.4	13.0±1.1	9.5±1.1	13
8	277	7.0±1.4	5.5±0.7	6.0±1.4	-	9.5±0.1	4.0±0.1	9.5±0.1	8.5±01	10
9	278	9.5±0.1	9.0±1.1	14.0±1.4	17.5±2.1	8.5±2.2	14.5±2.1	7.0±1.4	12.5±1.1	11
10	303	8.5±2.1	-	12±1.41	11±1.1	5.5±0.1	11.0±0.1	1.5±0.7	-	9
11	307	6.0±1.0	-	-	5.0±0.0	-	7.0±1.4	5.5±2.12	-	9
12	367	-	6.0±1.4	-	-	-	-	9.5±0.71	12.0±0.1	7
13	401	-	9.0±1.3	8.5±2.1	6.5±2.1	-	-	-	-	6
		Inhibition zone of antimicrobial activity at 48 hrs (mean±SD, mm)								
		MRSA34	**MRSA36**	***E. coli* (ESBL)**	***S. typhimurium***	***K. pneumoniae***	**MSSA (ATCC 25923)**	***C. albicans***	***C. neoformans***	
1	10	9.5±0.1	2.5±0.3	-	9.0±1.4	-	-	-	14.0±1.1	10
2	17	15.0±0.7	19.0±1.4	4.5±0.6	4.5±0.2	-	-	-	21.0±1.4	13
3	38	19.0±1.4	7.5±0.1	5.5±0.2	-	-	-	-	21.5±0.7	12
4	69	6.0±1.3	-	5.0±1.3	-	-	-	-	12.5±0.1	10
5	70	12.0±1.4	4.5±0.7	-	-	-	-	-	19.0±1.2	11
6	191	14.0±1.1	19.0±1.2	4.0±1.4	4.0±1.41	-	-	-	21.0±1.6	13
7	225	19.0±1.4	7.0±1.3	5.5±0.7	4.0±1.4	-	-	-	20.5±2.1	13
8	277	6.5±0.1	-	5.0±1.1	-	-	-	-	12.0±1.5	10
9	278	12.0±1.1	4.0±1.1	-	-	-	-	-	19.0±1.1	11
10	303	9.0±1.1	2.5±0.1	-	-	-	-	-	14.5±0.1	9
11	307	-	9.0±1.41	4.5±0.1	22.0±7.8	7.5±0.1	-	-	24.0±6.3	9
12	367	-	16±1.4	-	9±1.1	-	6±1.41	-	14.5±2.2	7
13	401	-	-	-	6.5±2.2	9.5±3.4	-	-	20.0±2.3	6

Inhibition zone from modified cross-streak method in the table is derived from three experiments and given in mean±SD (n=3). SD: Standard deviation, MRSA: Methicillin-resistant *Staphylococcus aureus,* ESBL: Extended-spectrum beta-lactamases, *E. coli*: *Escherichia coli*, *S. typhimurium: Salmonella typhimurium*, *K. pneumoniae: Klebsiella pneumoniae*, MSSA: Methicillin sensitive *Staphylococcus aureus, C. albicans: Candida albicans, C. neoformans: Cryptococcus neoformans*

(Table 1). It was noticed that the spectra of inhibition varied among the isolated bacteria. Agar well diffusion method was used to assess the production of antimicrobial compounds by bacteria isolated from soil samples against 16 pathogenic strains of the tested microbial pathogen. Of the 13 isolates tested, 6 isolates were found to exhibit antibacterial activity against as least 1 tested microbial pathogen (Table 2).

As shown in Table 3, the MIC and MBC values of the extracellular filtration were evaluated by the broth microdilution method. MIC and MBC values for isolates 303 have shown inhibition against MRSA 29 (dilution 1:512) and (dilution 1: 256) while other isolates (191, 225, 277, 278, and 307) shown MIC against MRSA 36, MRSA 29, MRSA 28, and *S. typhimurium* with undiluted filtrated and no MBC (Table 3).

It indicates that antibiotic-producing bacteria which we isolated have more ability to inhibit the growth of most MRSA and only 1 stain of *S. typhimurium* even we use only non-concentrated extracellular filtration. This is because Gram-negative bacteria usually have better protection to other antimicrobial compound rather than Gram-positive bacteria because both kinds of bacteria have different cell wall components. The cell wall of Gram-positive bacteria contains peptidoglycan while cell wall of Gram-negative bacteria contains peptidoglycan and lipopolysacaride. Zuhud *et al.* [20] and Ajizah *et al*. [21] stated that the cell walls of Gram-positive bacteria contain very thick peptidoglycan to protect the bacteria. Moreover, cell walls of Gram-negative bacteria, besides peptidoglycan, also contain lipopolyssacaride to protect the bacteria from antibiotics [22].

Even if some isolates have only MIC but not have MBC, it leads to the conclusion that even the bioactive compounds from extracellular filtration are diffuse throughout the medium. There may be a small amount of these compounds which are not enough to inhibit the growth of microbial pathogen when diluted and compared to the commercial drug. It may relate to external factors such as the incubation period, amount of antibiotic-producing bacteria cell biomass, temperature, and amount of microbial pathogen biomass. There is a need to test the bioactive compound extraction when using the suitable solvent extracting in this case.

Although these bacteria were not yet identified to the species level, morphological and biochemical characteristics indicate that they belong to the genus *Burkholderia* spp. (2 isolates; isolate 191, and 278), *Pseudomonas* spp. (3 isolates; isolate 225, 277, and 303), and *Rhodococcus* spp. (1 isolates; isolate 307).

This outcome was correlated with the result of Mashoria *et al.* that isolated bacteria from soil and tested for antimicrobial activities to the seven pathogenic bacteria stains (*Salmonella typhi*, *Serratia ficaris*, *Streptococcus faecalis*, *Pseudomonas vesicularis*, *Staphylococcus cohni*, *E. coli*, and *Pseudomonas aeruginosa*). It was found that only one stain was effective and characterized as *Pseudomonas* spp. [23]. Moreover, Kaur *et al*. revealed that two microorganisms isolated from soil in Punjab, India, can inhibit the growth of *S. aureus* and *Proteus vulgaris* indicator strains verified by the disc diffusion method, agar well method, and streak agar method [24].

The importance of the microorganisms in enzyme production has been an area of constant research due to their high production capability, low cost, and susceptibility to genetic manipulation. In reality, the enzymes of microbial origin have high biotechnological interests, including the industrial, agricultural, biological, pharmacological, and environmental fields [25]. The soil microorganisms occupy a relatively unexplored site with respect to enzyme production and so they can represent a new source in obtaining more enzymes with different potentialities.

In this study, the potential of microbial isolates from soil samples for extracellular enzyme production was characterized. Of the 477 isolates analyzed, 28 bacteria isolated were obtained. It was revealed that many

Table 2: Antimicrobial activity of microbial strains against test microbial pathogen at 24 hrs and 48 hrs by agar well diffusion

S.No.	Isolate	Gram's-stain	Identification	Inhibition zone of agar diffusion at 24 hrs and 48 hrs (mean±SD)							
				MRSA28		MRSA29		MRSA36		*S. typhimurium*	
				24	48	24	48	24	48	24	48
1	191	Gram-negative bacilli	*Burkholderia* spp.	-	-	-	-	17.0±0.7	18.0±0.1	-	-
2	225	Gram-negative bacilli	*Pseudomonas* spp.	-	-	11.0±2.2	13.0±2.1	-	-	-	-
3	277	Gram-negative bacilli	*Pseudomonas* spp.	-	-	10.0±0.0	10.0±0.0	-	-	-	-
4	278	Gram-positive bacilli	*Burkhoderia* spp.	-	-	11.0±0.0	11.0±0.0	-	-	-	-
5	303	Gram-negative bacilli	*Pseudomonas* spp.	14.0±1.1	14.0±1.4	-	-	-	-	-	-
6	307	Gram-positive coccobacilli	*Rhodococcus* spp.	-	-	-	-	-	-	9.0±0.5	10.0±0.3

Inhibition zone by agar well diffusion in the table is derived from three experiments and given in mean±SD (n=3). SD: Standard deviation, MRSA: Methicillin-resistant *Staphylococcus aureus, S. typhimurium: Salmonella typhimurium*

Table 3: MIC and MBC of extracellular filtration from different isolates of microbial strains against test microbial pathogen determined by broth microdilution

No.	Isolate	Identification	Tested microorganisms							
			MRSA28		MRSA29		MRSA36		*S. typhimurium*	
			MIC dilution	MBC dilution	MIC dilution	MBC dilution	MIC dilution	MBC dilution	MIC dilution	MBC dilution
1	191	*Burkholderia* spp.	-	-	-	-	undiluted	G	-	-
2	225	*Pseudomonas* spp.	-	-	Undiluted	G	-	-	-	-
3	277	*Pseudomonas* spp.	-	-	Undiluted	G	-	-	-	-
4	278	*Pseudomonas* spp.	-	-	Undiluted	G	-	-	-	-
5	303	*Burkholderia* spp.	512	256	-	-	-	-	-	-
6	307	*Rhodococcus* spp.	-	-	-	-	-	-	Undiluted	G
control	Gentamicin (µg/ml)		62.50	62.50	1.95	1.95	62.50	62.50	62.50	62.50

*G: Growth, ND: Not done. These data in the table are derived from three experiments and given in mean ± SD (n = 3). MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, SD: Standard deviation, *S. typhimurium: Salmonella typhimurium*

Table 4: Biological enzyme activities of microbial strains

S.No.	ID number	Identification	Clear zone of interested biological enzyme at 2-14 days (mm)								Total enzyme production per isolated
			Chitin	Chitosan	Starch	Cellulose	Skim milk	Gelatin	Tween 20	Tween 80	
1	162	*Rhodococcus* spp.	-	7.0±0.0	9.0±0.0	7.0±1.4	21.5±0.1	17.5±2.12	-	-	5
2	295	*Rhodococcus* spp.	-	8.0±1.1	17.0±1.2	18.0±0.0	49.0±1.4	32.0±1.41	7.0±0.0	7.0±1.4	7
3	297	*Rhodococcus* spp.	-	11.0±1.4	15.5±0.4	28.0±1.1	43.5±0.7	40.0±1.41	11.0±1.1	5.5±0.7	7
4	304	*Rhodococcus* spp.	-	10.5±0.1	10.5±0.1	18.5±0.7	33.0±1.1	37.5±2.12	6.5±2.2	10.5±0.7	7
5	306	*Rhodococcus* spp.	-	-	16.0±1.4	18.5±0.1	63.5±0.1	45.5±0.71	10.0±0.0	13.0±0.0	6
6	307	*Rhodococcus* spp.	-	-	16.5±0.1	12.0±0.0	71.0±0.1	34.0±0.71	10.0±0.1	17.0±0.1	6
7	319	*Rhodococcus* spp.	11.5±2.1	-	22.5±0.6	130±1.4	78.5±0.7	46.0±1.41	8.5±0.7	20.5±0.1	7
8	323	*Rhodococcus* spp.	-	-	17.5±0.4	16.5±0.1	-	37.0±1.41	7.5±0.1	6.0±0.0	5
9	331	*Rhodococcus* spp.	-	14.5±0.1	28.5±0.2	17.0±1.4	43.5±0.1	-	8.0±0.0	11.5±2.2	6
10	333	*Rhodococcus* spp.	-	-	40.0±0.0	24.0±1.4	33.0±1.1	-	-	-	3
11	226	*Burkholderia* spp.	-	10.5±0.1	9.0±1.1	20.0±0.0	27.5±3.5	-	26.5±2.2	-	5
12	293	*Pseudomonas* spp.	-	31.0±1.1	19.0±1.4	21.0±1.4	52.5±3.5	33.5±2.12	-	-	5
13	310	*Pseudomonas* spp.	-	13.0±1.4	25.5±0.1	15.5±0.1	74.0±1.1	36.5±2.12	-	9.5±0.1	6
14	353	*Pseudomonas* spp.	-	-	9.0±0.0	-	-	19.0±1.41	-	-	2
15	356	*Pseudomonas* spp.	-	-	-	-	24.0±5.6	37.5±3.54	15.5±0.1	-	3
16	357	*Pseudomonas* spp.	-	-	-	-	26.5±2.2	29.0±1.41	11.0±1.1	-	3
17	360	*Pseudomonas* spp.	-	-	-	-	27.0±2.8	36.5±4.95	-	-	2
18	361	*Pseudomonas* spp.	-	-	-	-	25.0±1.4	37.0±4.24	-	-	2
19	362	*Pseudomonas* spp.	-	-	-	-	25.5±0.1	35.0±1.41	-	-	2
20	364	*Pseudomonas* spp.	-	-	-	-	24.5±2.2	45.0±7.07	-	-	2
21	366	*Pseudomonas* spp.	-	-	-	-	28.5±0.1	51.0±1.41	16.0±1.4	-	3
22	367	*Pseudomonas* spp.	-	-	-	-	27.0±1.4	60.5±0.71	-	-	2
23	369	*Pseudomonas* spp.	-	-	-	-	28.5±2.2	70.0±0.00	-	-	2
24	378	*Pseudomonas* spp.	-	-	-	11.0±1.1	60.0±0.0	-	-	-	2
25	385	*Pseudomonas* spp.	-	-	-	2.5±0.1	-	15.5±0.71	-	-	2
26	393	*Pseudomonas* spp.	-	-	-	-	-	27.5±0.71	-	-	1
27	413	*Pseudomonas* spp.	-	-	-	6±1.4	-	25.0±0.00	-	-	2
28	420	*Pseudomonas* spp.	-	-	-	-	-	32.0±1.41	-	-	1
		Total effective isolate (%)	1 (0.24)	8 (1.88)	14 (3.29)	16 (3.76)	22 (5.18)	24 (5.65)	12 (2.82)	9 (2.12)	

Clear zone of interested biological enzyme in the table is derived from three experiments and given in mean±SD (n=3). SD: Standard deviation

isolates produced more than one of the tested enzymes. There were four isolates producing 7, 6, 5, and 3 enzymes, respectively (14.29%), ten isolates producing 2 enzymes (7.14%), and two isolates producing only 1 enzyme (3.57%) as mentioned in Table 4.

As shown in Table 4, gelatinase was the enzyme most produced in all among samples (24 isolates; 5.65%), while the enzyme caseinase (22 isolates; 5.18%) and CM-cellulase (16 isolates; 3.74%) were found with the second and third ranges, respectively. In particular, the isolate which produced the widest diameter hydrolysis zone in gelatin, skim milk, and cellulose were 369, 319, and 297, respectively. The present study indicates that some of these might produce good amounts (high activity) of the biological enzyme. Some of the isolates have shown a significant positive result in terms of the zone of clearance around the bacterial colony, whereas some of them showed less area indicating the different rate of enzyme production by different isolates.

Morphological and biochemical characteristics indicated that most active enzyme production bacteria belong to the genus *Rhodococcus* spp. (10 isolates), *Pseudomonas* spp. (17 isolates), and *Burkholderia* spp. (1 isolates).

Our results were similar to that of Alves *et al*. who found that all isolated microbial enzymes in natural environments possess extracellular enzymes caseinase and esterase [26]. While, other reports have found esterase, cellulase, and protease activities in soil [27-29]. Soil is a complex system that includes a range of microhabitats with different physicochemical characteristics and discontinuous environmental conditions. Therefore, in the present report, most of the isolates from soil sample produced many tested enzymes. Organisms that share the same microhabitat may contribute to nutrient availability on the site; hence, the production of various enzymes is important.

CONCLUSION

In the present work, numerous microbial isolates from soil were screened for the production of extracellular antibiotic substance and also hydrolytic enzymes. We reported here a soil bacteria isolate capable of inhibiting the growth of MRSA and *S. typhimurium*. Determination of the biological enzyme in preliminary test revealed that most of enzyme-producing bacteria produced more than one analyzed enzyme. Further, the interactions between these microorganisms and modifying culture conditions such as pH, growth media, and stimulant supplement in large scale might be studied to help in getting better production of the particular bioactive compound and enzyme.

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