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An Analysis study on Soil Bacterial Metabolites and their Antimicrobial Anticancer and Molecular Docking

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Abstract - Soil microbial communities are one of the most complex, diverse, and important assemblages of organisms in the biosphere because of the many biological activities they support. The soil is the earth's "living organ," performing a wide range of functions essential to maintaining life on our planet. Until recently, cultures both big and little all across the globe acknowledged this reality and gave spiritual expression to it by cultivating close ties to the land. Several studies conducted over the course of many years have provided conclusive evidence of soil's vital role as the location of numerous processes essential to maintaining life. One gramme of soil may hold up to 10 billion microorganisms of potentially thousands of distinct species, making soils the most diverse ecosystems on Earth in terms of microorganisms and even more so than eukaryotic creatures (Rosello 2001).

Keywords - Analysis, Soil Bacterial Metabolites, Antimicrobial Anticancer, Molecular Docking

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INTRODUCTION

The smallest living things on Earth are also the most numerous. Although most bacteria are invisible to the naked eye, we cannot ignore them since life would not be possible without them. One of the locations they may be hiding is in the digestive tract of an animal, or else in the deepest undersea vents, where sophisticated life forms die. Microbial variety makes a substantial contribution to the emergence of life in all of its many forms as well as to the maintenance of life by participating in the fundamental energy and element cycle. They have a long history of advancing the existing environment for humans, from producing various bioactive chemicals to acting as hosts for extraterrestrial DNA. The unicellular dynasty has a close connection to the growth of the modern medical industry and bio-based businesses. History reveals the crucial role bacteria have played in controlling and curing many human illnesses, as well as their influence on other crucial agricultural and economic sectors. Microbes work as the tiniest form of factories for the mass production of a variety of pharmaceuticals, including enzymes, antibiotics, and anticancer drugs. In the fermentation and enzyme-based industries, there is a variety of microorganisms with special qualities that make them amenable to technological improvement.

Total biomass cell numbers (6 x 1030 bacteria, 1.3 x 1028 archaea, and 3.1 x 1029 eukarya), cell biomass (6 x 1018 kg bacteria, 1.3 x 1016 kg archaea, and 3.1 x 1017 kg eukarya, assuming an average weight of 1 pg per cell), and species diversity all point to the predominance of these unicellular organisms (Beloqui et al 2008). Only 0.001-0.1% of all microorganisms in sea water, 0.25% in freshwater, 0.25% in sediments, and 0.30% in soil microorganisms are suitable for lab growth conditions, according to scientific research (Amann et al 1995; Singh et al 2008). Traditional and commercial studies of microbes have only explored a small fraction of the about 6,500 microbial species that exist. The genomes of 762 microorganisms have been painstakingly unravelled from the previously described set of bacteria, and around half of them are of use to the biotech industry. There have been 2,773 efforts so far to sequence the whole microbial genome, and some of them could provide new information on genes and enzymes. Antibiotics, vitamins, pharma chemicals, sweeteners, and performance materials are just some of the many examples of high-value molecules that have been synthesised using microbial activity. The vast majority of these methods rely on one hundred fifty large-scale industrial processes (Ana Beloqui et al., 2008, Woodley et al., 2006). Today, antibiotics, cancer drugs, and industrial enzymes generated from soil microorganisms account for a disproportionate share of global sales. Soil bacteria grown using conventional agricultural techniques are the primary source of these bioactive chemicals, which are essential to human comfort and health.

Since the germ hypothesis of illness, which said that bacteria and other germs were responsible for the aetiology of many different diseases, gained popularity in the late 17th century, researchers have been on the lookout for effective antibiotics ever since. Therefore, researchers have put in a lot of hours looking for medications that might perhaps eliminate these harmful bacteria. Unlike previous therapies for infections, which included poisons like arsenic, antibiotics solely operated on the infection and did not damage the host, giving them the nickname "magic bullets." Antibacterial drugs produced from microorganisms were made possible by a 1928 work by British scientist Alexander Fleming. Fleming lost one of his bacterial culture agar plates because it became tainted. He saw a clean area surrounding a mould colony on the dirty plate and reasoned that the mould must be secreting something that inhibited the spread of bacteria. To more effectively demonstrate the chemical's effects, Fleming first withdrew the compound from plates. Since the mould was found to be a kind of Penicillium, the chemical was given that name. Infections in wounds became more common when World War II began in the late 1930s. A concerted effort was made to collect all available resources in order to further investigate and examine the purification of penicillin. Howard Walter Florey effectively led the squad to victory. The purified active component may be mass-produced in usable amounts and rapidly evaluated in clinical settings. In its raw form, the medicine miraculously treated animals and people who were near to death from bacterial diseases. Penicillin manufacturing was boosted by international cooperation from the start of the 1940s. Since practically all of Britain's industrial capability was being deployed for the war effort, there were no facilities for the bulk manufacture of the medicine. Together, British and American researchers developed penicillin. A major and continuing search for antibiotics was sparked by the early achievement of eliminating a chemically essential component from a bacterium. Extensive studies of microorganisms for bioactive chemicals have led to the development of new antibiotics, anticancer medicines, and enzymes.

Microbes that are part of complex consortia or can adapt, survive, and reproduce in environments that cannot be replicated in vitro generate a large number of pharmacologically active macromolecules. Cloning and heterologous expression of biosynthetic genes that encode secondary metabolites (typically present in gene clusters) is the simplest and most repeatable approach to accessing their biosynthetic potential, although there are reports on how to circumvent this general issue of microbial cultivation by simulating natural habitats or by allowing for interspecies communication after single cell micro-encapsulation (Lorenz 2005). Finding novel bacteria with the ability to produce antibiotics can help in finding solutions to problems now facing agriculture and medicine. New medications will be required to treat primary human ailments that were formerly treated with efficient antibiotics in order to preserve and enhance the health of the human population (Lederberg et al. 1992). The same is true for fungicides, which have been widely used to combat plant disease, but whose use will eventually be limited due to safety concerns and the evolution of drug-resistant pathogen populations. More study is needed to discover novel antimicrobial medications and cure infectious diseases that affect plants and animals.

Historically, agriculture has relied on soil bacteria because of their role in biogeochemical cycles. Plant health and soil fertility are both influenced by interactions in the rhizosphere between plants and microbes. Plant growth-promoting rhizobacteria (PGPR) are helpful soil bacteria that may be found in the soil and may stimulate plant growth if they colonise the plant's root. Rhizobacteria, also known as PGPR, are beneficial to plants because they promote nodule growth and plant health (NPR). The rhizosphere, an important biological setting in the soil where plants and bacteria interact, is related to these. Crop nutrition provisioning, plant growth promotion (through hormone synthesis), plant disease management or inhibition, soil structure improvement, bioaccumulation or microbial leaching of inorganics, and so on are only some of the key roles attributed to these bacteria by Davison (1988). (Brierley 1985; Ehrlich 1990). Bioremediation of contaminated soils often involves the employment of microorganisms to break down organic contaminants (Middledrop et al. 1990; Burd et al. 2000; Zhuang et al. 2007). For plants to realise their full genetic potential in the age of sustainable agricultural production, interactions between plants and microorganisms in the rhizosphere are critical for converting, mobilising, solubilizing, etc. nutrients from a restricted nutrient pool. Biological techniques are increasingly being used as part of an integrated plant nutrition management system alongside chemical fertilisers. Sustainability in agricultural production systems may be improved with the help of PGPR in this case (Sturz et al. 2000;Shoebitz et al. 2009). Chemicals have been used in farming for a long time. Because of their widespread usage, environmental systems have suffered and pollution has been produced. These chemicals have the potential to enter the food web and kill off important soil invertebrates and rhizosphere microorganisms. More importantly, their efficacy decreases when infections grow resistant to them. The employment of biological control microorganisms has been the subject of a number of research over the last 25–30 years as a potential alternative control strategy. Microorganisms may be used as a non-polluting alternative to traditional pesticides for the management of soil-borne plant diseases. Facilitating direct and indirect plant growth and development is possible. Another need for encouraging indirect plant development is reducing the impact of phytopathogenic organisms. Siderophores, or metal-binding small molecules, may be synthesised for this purpose. It has been discovered that many different types of bacteria produce antibiotics and biologically combat soil-borne plant diseases. Enzymes that break down fungal cell walls and/or hydrogen cyanide might be another way in which PGPR suppresses

phytopathogens (HCN). Based on their mechanisms, biocontrol strategies may be classified as either root colonisation, degradative parasitism, antibiosis, or competitive antagonism. Fungal pathogens in plants may be biologically controlled in a number of ways. Hormones such abscisic acid, auxins, cytokinins, ethylene, and gibberellins are produced by symbiotic and non-symbiotic PGPR and stimulate plant development directly. It has been determined which bacterial taxa generate the auxins indole-3-ethanol and indole-3-acetic acid (IAA).

Less than 1% of the microbial diversity was recognised, recognised, and employed for human purposes, while the remainder of the pie is still unexplored, according to a trip through the history of the human-microbial cooperation. Metagenomics is the latest scientific weapon to probe the microbial world beyond the tip of the iceberg. Metagenomics enables the genome analysis of non-culturable bacteria with the help of a host organism that can be grown in a lab. Since the great majority of microbial environments consist of non-cultured microorganisms, metagenome searches will always uncover previously undiscovered genes and proteins. Due to the chance of finding unique sequences, this approach is thus preferred over searches in previously farmed microbes.

MATERIAL AND METHODS

Chemicals

Common suppliers were enlisted to procure bioassay necessities such glassware, solvents, and culture media (Hi-Media, Merck, and Sigma).

Sterilization

Culture media and distillated water were autoclaved for 20 minutes at 15 lbs/in2 of pressure, while glassware was sterilised in a hot air oven at 160°C for three hours. The laminar airflow chamber (LAF) underwent 15 minutes of ultraviolet (UV) irradiation before to use.

Collection of soil samples

The soil samples were collected from the Sagar state. The depth of the soil at which the soil sample was taken ranged from 10 to 20 cm. The laboratory received soil samples aseptically and in sterile plastic bags.

Isolation of soil bacteria

By using the serial dilution and spread plate approach, bacterial colonies were isolated and purified (Elliah, et al., 2004). An orbital shaker was used to shake a one gramme soil sample at 100 revolutions per minute for ten minutes at room temperature in 10 millilitres of sterile, deionized water. On the Nutrient Agar (NA) medium, the serial dilution aliquots (0.1ml) of 10-2, 10-3, 10-4, and 10-5 were applied. For one to two days, the plates were incubated at 37oC and routinely checked. A pure bacterial isolate was acquired by repeatedly sub-culturing the bacterial colonies that displayed antagonistic behaviour against other colonies. The nutrient agar slants with the purified bacterial isolates were kept at 4°C for future research.

Characterization of the bacterial isolates

According to Bergey's handbook, bacterial isolates were first distinguished based on morphological and Gram's staining features (Bergey, 1984).

Bacterial bioactive metabolites may be extracted by fermentation

During 72 hours at 37°C, 100 ml of Nutrient Broth (NB) media was added to 250 ml Erlenmeyer flasks containing the soil bacterial strains, and the flasks were agitated at 130 rpm in a rotary shaker. The bacteria were incubated, and then centrifuged for 15 minutes at 10,000 rpm. In order to extract the intracellular metabolites, the pellets from the extract were collected and dried at 40°C in the oven. Using a pestle and mortar, the dried pellets were ground before being extracted with ethyl acetate. Whatman filter paper was used to filter the extract. Extracellular metabolites were extracted using the supernatant. Ethyl acetate was added in equal parts to the supernatant (1:1) and vigorously stirred for 30 minutes. After that, a separating funnel was used to separate the ethyl acetate phase. An incubator was used to concentrate the unprocessed bacterial intracellular and extracellular metabolites. Dimethyl sulfoxide (DMSO) was used to dissolve the concentrated extracts, which were then kept at 4^0C for future research.

Pathogenic strains

Two pathogenic Gram-positive (Ve+) bacteria, E. lenta (ATCC 43055) and Enterococcus faecalis (MTCC 3104), and three pathogenic Gram-negative (Ve-) bacteria, *E. aerogenes* (MTCC 111), *A. baumannii* (MTCC 1425), and A. faecalis (MTCC 3104), were obtained from the Institute of Microbial Technology, Chandigarh, India.

Antimicrobial activity of bacterial metabolites

Kirby-Bauer disc diffusion was used to test the crude bacterial extract for antibiotic activity (Bauer, et al., 1996).

Molecular identification

Genomic DNA from bacteria that generate medicinal chemicals was extracted using a Nucleospin tissue kit (Macherey-Nagel). We centrifuged a fresh bacterial culture at 12,000 rpm for two minutes. Protein lysing was accomplished

by heating a solution comprising 25 litres of proteinase K and 180 litres of T1 buffer to 56 degrees Celsius in a water bath. RNase A (100 mg/ml) was added after cell lysis, and the mixture was permitted to incubate for five minutes at room temperature.

The purity of the extracted DNA was checked using agarose gel electrophoresis. Five litres of DNA were added to one litre of 6X gel-loading buffer with 0.25% bromophenol blue, 30% sucrose, and TE buffer pH-8.0. A 0.8% agarose gel containing 0.5g/ml ethidium bromide in a 0.5X Tris-Borate-EDTA solution was used to run the samples. The bromophenol dye was lowered to the bottom of the gel by electrophoresis at 75 V in a 0.5X TBE electrophoresis buffer. The Gel documentation system was used to capture a photograph of the gels in a UV transilluminator (Bio- Rad).

The bromophenol blue front reached almost the bottom of the gel after 1.5 hours of electrophoresis at 75V power supply and 0.5X TBE as the electrophoresis buffer. A 2-log DNA ladder was used due to the need of a molecular standard (NEB). Gels were photographed using the Gel documentation system after inspection with a UV transilluminator.

Optimization of growth conditions for enhancing quantity of thebioactive metabolites

To determine whether nutrient and environmental culture conditions encouraged the highest synthesis of the most bioactive metabolites by specific bacterial strains of interest, a series of experiments was conducted. The study considered the optimal medium, pH, temperature, incubation time, and circumstances (Static and Shaker). By progressively adjusting one variable while maintaining the status quo of the others, metabolite synthesis was optimised. We evaluated the highest bioactive metabolite synthesis by chosen bacterial strains against the test pathogens using a disc diffusion experiment.

Determination of Minimum Inhibitory Concentration (MIC) of crude extracts against test pathogens

Minimum inhibitory doses of microorganisms were obtained using macro broth two-fold serial dilution techniques and optimised crude extracts (NCCLS, 1993). The best crude bacterial extracts were diluted to six different concentrations, and they were used to treat cultures of bacteria and fungi grown in sterile Nutrient broth (NB) and Sabouraud dextrose broth (SBD), respectively. Concentrations of 200, 100, 50, 25, 12,5, and 6.25 g/ml were employed. It is possible that the pathogenic cultures' cell count increased to 1.5 x 108 CFU/ml overnight at 37 degrees Celsius and turbidity of 0.5 McFarland. We placed 100 l of the infectious cultures into tubes containing different amounts of crude extracts. Both a culture without crude extracts and an empty broth medium with tubes served as negative and positive controls, respectively. Each tube of bacteria was maintained at 37 degrees for 24 hours, whereas each tube of fungus was kept at 30 degrees for 48 to 96 hours. We measured the optical density of the crude extract using a UV visible spectrophotometer and discovered that the MIC value, which was thought to indicate its lowest concentration, did not cause a substantial rise (OD600). Using this approach, we were able to determine the crude extract's percentage of inhibition against the various infections we tested it against.

$$\text{Percentage}(\%)\text{ of inhibition} = \left(\frac{[\text{Control OD} - \text{Test OD}]}{\text{Control OD}}\right) \times 100$$

Biofilm forming ability assay for tested pathogens

To determine whether or not a microbe is likely to cause the specified bacterial and fungal illnesses, the crystal violet test may be used. Put 10 ml of confirmed pathogenic cultures grown overnight in 190 ml of broth medium in each well of 96-well microtitre plates. Nutrition broth (NB) and Sabouraud dextrose broth were used to cultivate the bacterial pathogens (SDB). The cells of the dangerous bacteria were cultured for 48 and 72 hours at 30 °C and for one night at 37 °C. After discarding the incubation culture media, the 96-well plates were washed with phosphate buffer saline (PBS). The wells were stained for 5-10 minutes at room temperature with a 1% crystal violet solution. The plates were washed with water and then dried upside down on a cotton towel. Afterwards, acetic acid (at a dosage of 30%) was poured into each well to neutralise the discoloration. All of the wells were made to look the same by shaking or swirling the mixture. Using an Eliza microtitre plate reader, we measured the absorbance of each well at 630 nm. Identify the studied pathogens as having either low (OD = 0.100) or high biofilm-forming potential (OD = 0.100 to 0.300).

Anti-biofilm formation assay

In order to battle the pathogens under research, we first ensured optimal circumstances for bacterial growth, and then examined the influence of bacterial crude extracts on biofilm formation. The 96 well microtitre plates were inoculated with 190 l of the appropriate medium and 10 l of the tested pathogenic cultures after overnight growth. Sub-lethal concentrations (minimum bactericidal/minimum fungicidal) of the crude extract were applied to culture wells at 100 g/ml, 6.25 g/ml, and 100 g/ml, respectively. We employed a culture devoid of crude extracts as a positive control and plate wells containing just broth medium as a negative control. The fungal plate was kept at 30 degrees Celsius for 48-72 hours, whereas the pathogenic bacteria plate was

kept at 37 degrees Celsius for 24-48 hours. When the 96-well plates had been incubated for the appropriate time, we washed the wells in phosphate buffer saline (PBS) and stained them for 5-10 minutes at room temperature with 1% crystal violet solution. In order to remove the staining, the plates were treated with 30% acetic acid in the wells after being washed with water and dried upside down on cotton towels. All of the wells were made to look the same by shaking or swirling the mixture. To determine the extent to which the crude extracts inhibited biofilm formation, we measured the absorbance at 630 nm in each well using an Eliza microtiter plate reader.

$$\text{Percentage}(\%)\text{ of biofilm inhibition} = \left(\frac{[\text{Control OD} - \text{Test OD}]}{\text{Control OD}}\right) \times 100$$

Gas Chromatography -Mass Spectrometry analysis

Beneficial compounds in bacterial crude extracts were analysed using Gas Chromatography-Mass Spectrometry. A Perkin-Elmer GC Clarus 500 system equipped with a fused silica column filled with Elite-1 (100% dimethyl polysiloxane, 30 nm 0.25 nm ID 1 m df) performed the GC-MS study. Helium with a purity of 99.999% is used as the carrier gas at a constant flow rate of 1 ml/min for compound separation. The Turbo gold mass detector picked up the presence of the 2 l of optimised bacterial extract that had been introduced to the device. Temperatures were raised from 110 degrees Celsius in the oven, 250 degrees Celsius in the injector, 200 degrees Celsius in the ion source, and finally 280 degrees Celsius in the ion source at a rate of 10 degrees Celsius per minute, where they stayed isothermally for nine minutes. At a scan time of 0.5 seconds and an energy of 70 eV, a mass spectrum was successfully recorded. As a matter of fact, the GC-MS was running for a whole 36 minutes. Each compound's concentration was determined by dividing the sum of its peak areas by its average peak area. The chromatograms and mass spectra were generated using Turbo-Mass v5.2 finding the potential bioactive substances by searching a database provided by the National Institute of Standards and Technology.

Cell lines for anticancer and cytotoxicity analysis

The National Centre for Cell Sciences (NCCS), Pune, graciously contributed human MCF-7, Hep-2, HepG2, A375, and skin cancer cell lines in addition to the healthy Vero cell line. To keep them alive, the cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS). In addition, they were housed in an incubator at 37 degrees Celsius and 5% carbon dioxide. A combination of penicillin and streptomycin (100 g/ml) was employed for sterilisation.

Statistical Analysis

Graph Pad Prism 7.0 was used for all statistical analyses. Data from three separate investigations were used to calculate means and standard deviations. At the P 0.05 level of significance, the results were deemed reliable.

RESULT

Isolation and characterization of bacterial isolates

In all, 27 different types of bacteria were selected for the antimicrobial experiments, including 16 Gram-positive and 11 Gram-negative strains.

Table 1: Morphological characteristics of the selected bacterial strains isolated from soil samples

Isolate number	Colour	Surface	Margin	Consistency	Gram Staining
3	Light Orange	Smooth	Entire	Smooth	(+Ve), Rod
4	Pink	Glistening	Entire	Smooth	(-Ve), Rod
10	Milky White	Smooth	Lobate	Sticky	(-Ve), Cocci
11	White	Glistening	Entire	Dry	(-Ve), Rod
12	Light Yellow	Dry	Lobate	Dry	(+Ve), Cocci
15	Yellow	Glistening	Filamentous	Smooth	(+Ve), Rod
19	Yellow	Wrinkled	Entire	Smooth	(+Ve), Cocci
20	Dark Yellow	Glistening	Entire	Smooth	(-Ve), Rod
22	White	Smooth	Undulate	Dry	(+Ve), Rod
25	Milky White	Smooth	Filamentous	Dry	(-Ve), Rod
29	White	Wrinkled	Lobate	Dry	(+Ve), Rod
30	Creamy White	Dry	Lobate	Buttery	(-Ve), Rod
31	Light Brown	Rough	Undulate	Dry	(+Ve), Rod
33	White	Smooth	Entire	Sticky	(+Ve), Cocci
36	Cream	Rough	Entire	Smooth	(-Ve), Rod
39	White	Smooth	Undulate	Sticky	(-Ve), Rod
44	Light Pink	Dry	Entire	Smooth	(-Ve), Rod
50	Dark Orange	Dry	Entire	Sticky	(-Ve), Rod
55	Milky White	Dry	Undulate	Smooth	(+Ve), Cocci
60	White	Smooth	Undulate	Dry	(+Ve), Rod
66	Creamy White	Rough	Lobate	Buttery	(-Ve), Rod
69	White	Smooth	Undulate	Smooth	(+Ve), Rod
79	White	Dry	Filamentous	Buttery	(+Ve), Rod
99	Milky White	Rough	Lobate	Dry	(+Ve), Rod
107	Light Yellow	Wrinkled	Entire	Dry	(+Ve), Rod
123	White	Smooth	Lobate	Sticky	(+Ve), Rod
127	White	Smooth	Entire	Smooth	(+Ve), Rod

Molecular identification of the selected bacterial strains

The two bacterial strains employed in this study were identified by 16S rRNA analysis and PCR amplification. In a PCR thermal cycler, bacterial strains were sequenced. The identified sequences

were then submitted to an NCBI blast search to detect closely related sequences. *B. amyloliquefaciens* (NR117946) and *B. subtilis* (NR102783) sequences from GenBank showed 100% and 99% similarity with the selected bacterial strains, isolates #29 and #60, respectively. MH198041 (*B. amyloliquefaciens*) and MH198042 (*B. subtilis*) were assigned to the sequences after they were deposited in NCBI GenBank. The neighbor-joining strategy was employed to create the phylogenetic tree of both bacterial strains, which demonstrated the relationship between closely related species (Figure- 1).

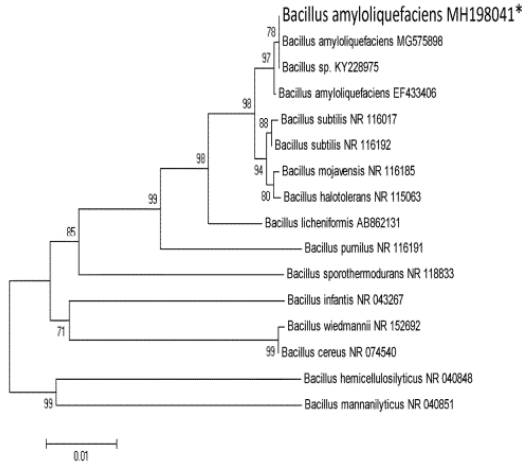

Figure 1: *B. amyloliquefaciens* (MH198041) Phylogenetic tree

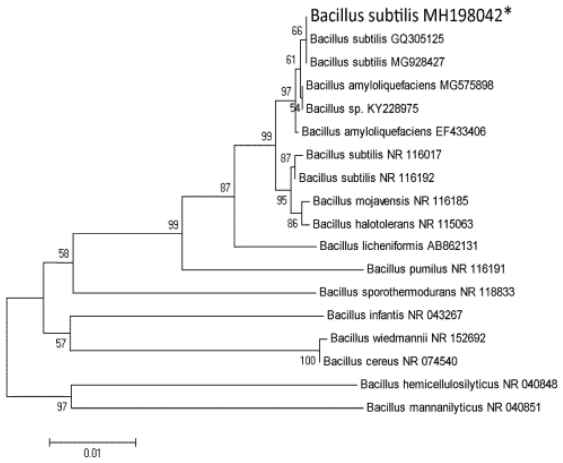

Figure 2: *B. subtilis* (MH198042) Phylogenetic tree

Antimicrobial activity of *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198042) of extracellular and intracellular metabolites

From *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198041), extracellular and intracellular metabolites were tested for antibacterial activity against all of the tested pathogens. Zones of inhibition of between 13.15 and 15.40 mm were recorded for *B. amyloliquefaciens* (MH198041) and between 13.80 and 15.3 mm were recorded for *B. subtilis* (MH198042). *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198042) had zones of inhibition for their respective internal metabolites spanning 8.70–10.35 and 9.30–11.3, respectively (Table 2).

Table 2: Antimicrobial activity of *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198042) of extracellular and intracellular extracts

Bacterial strains	Tested pathogens	Extracellular extract	Intracellular extract
B. amyloliquefaciens (MH198041)	*A. baumannii*	-	-
	A. faecalis	13.15±0.07	10.35±0.07
	E. aerogenes	15.05±0.35	09.10±0.14
	E. faecalis	13.75±0.21	-
	E. lenta	14.25±0.35	08.70±0.21
	A. niger	15.40±0.14	-
	C. albicans	14.50±0.42	-
B. subtilis (MH198042)	*A. baumannii*	-	-
	A. faecalis	13.85±0.07	10.20±0.14
	E. aerogenes	14.10±0.14	-
	E. faecalis	14.75±0.21	11.60±0.56
	E. lenta	15.30±0.14	10.70±0.28
	A. niger	14.40±0.42	09.30±0.14
	C. albicans	15.30±0.42	-

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of crude extracts of *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198042) against bacterial pathogens

For this study, the MIC and LC50 values of crude extracts of *Bacillus amyloliquefaciens* (MH198041) and *Bacillus subtilis* (MH198042) were determined against bacterial pathogens. The potential development of pathogens may have been halted by crude extracts from two bacterial strains. Against bacterial pathogens, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *B. amyloliquefaciens* (MH198041) and *B. subtilis* (MH198042) crude extracts are 50–100 g/ml and 100–200 g/ml, respectively (Table- 3).

Table 3: Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)of crude extract of *B. amyloliquefaciens* (MH198041) against bacterial pathogens

	Bacterial pathogens	Concentrations of the crude extract (µg/ml)					
		200µg	100µg	50µg	25µg	12.5µg	6.25 µg
	A. baumannii	-	-	+	+	+	+
	A. faecalis	-	-	-	+	+	+
MIC	*E. aerogenes*	-	-	+	+	+	+
	E. faecalis	-	-	+	+	+	+
	E. lenta	-	-	-	+	+	+
		200µg	100µg	50µg	25µg	12.5µg	6.25µg
	A. baumannii	98.82±0.26	98.65±0.36	77.27±0.62	59.25±0.36	33.92±0.62	14.56±0.35
Percentage (%) of inhibition	*A. faecalis E. aerogenes*	98.37±0.85 98.78±0.01	97.96±0.56 98.26±0.39	97.35±0.63 75.04±0.35	77.43±0.29 53.81±0.96	57.62±0.26 33.70±0.98	17.17±0.52 13.34±0.85
	E. faecalis	98.22±0.36	98.10±0.85	74.82±0.26	50.47±0.85	27.42±0.56	06.85±0.39
	E. lenta	98.44±0.29	98.18±0.52	97.66±0.01	68.48±0.52	38.42±0.29	10.62±0.23
		200µg	100µg	50µg	25µg	12.5µg	6.25µg
	A. baumannii	-	+	+	+	+	+
	A. faecalis	-	-	-	+	+	+
MBC	*E. aerogenes*	-	+	+	+	+	+
	E. faecalis	-	-	+	+	+	+
	E. lenta	-	-	+	+	+	+

(+) = *Growth present*, (-) = *Growth absent*Positive control: Bacterial suspension without crude extract

CONCLUSION

In the current work, a sum of 132 bacterial disengages were disconnected from soil tests gathered. These two strains were recognized to be *B. amyloliquefaciens* and *B. subtilis* by 16S rRNA examination and the successions were kept in the NCBI Genbank and promotion numbers were gotten. The antimicrobial capability of intracellular and extracellular concentrates of *B. amyloliquefaciens* (MH198041) and *B. subtilis* not entirely settled against test microorganisms. From the outcomes, obviously the extracellular concentrate of both the bacterial strains showed better antimicrobial movement contrasted and the intracellular concentrate. Subsequently, to work on the amount of bioactive mixtures, a streamlining study was completed with the extracellular concentrates of the chose bacterial strains.

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