Evaluation of the effects of pigments produced by environmental bacteria on cancer cells

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Abstract

Cancers are a collection of incapacitating diseases in which cells initiate to divide and spread to adjacent tissues in an uncontrolled manner. Many researches demonstrated the potential of bacterial pigments as promising anticancer agents. Therefore, in this study, the cytotoxic effects of bacterial pigments were evaluated in breast and colon cancer cell lines. In this study, a total of 90 samples were collected from air, water, and soil from 28 different geographical areas of Iran. Forty isolates were selected based on differences in colony color and geographic regions. The MTT assay was used to evaluate the effect of pigment on MCF-7 and SW-48 cells. Bacteria whose pigment had the highest cytotoxic effect on cell lines were selected. Accurate identification was performed using PCR, and their relative purity was measured using TLC. Bacteria isolated from any three ecosystems are capable of producing pigments. Pigment-producing bacteria are more abundant in the soil than air and water. Among pigment-producing bacteria, 3 isolates had the highest cytotoxicity on MCF-7 cells, and 3 isolates had the greatest effect on SW-48 cells. The results of sequencing of isolates at the BLAST site showed that 6 isolates with cytotoxic effects were identified (*Micrococcus xinjiangensis*, *Dietzia*, *Arthrobacter agilis*, *Exigubacterium mexicanum*, *Bacillus beijingensis*). Chromatography shows that these pigmented bacteria have different pigment components. Pigment extraction from bacteria can be used as a complementary therapy or other therapies for breast and colon cancer in the future.

Introduction

Cancers are a collection of incapacitating diseases in which cells initiate to divide and spread to adjacent tissues in an uncontrolled manner. Various factors cause cancer, including chemical contaminants, diet, hormones, and other unknown factors[1]. Cancer develops in the body of people in a long and dynamic way[2]. One of the leading causes of death worldwide is cancer. Increased use of carcinogens along with lifestyle changes and increased life expectancy has led to the identification of cancer as a threat[3]. Chemotherapy is one of the most popular cancer treatments and is defined as the use of chemicals to treat cancer. On the other hand, old surgical methods and radiotherapy increase adverse consequences[4]. Due to the increasing resistance to chemotherapy drugs, the search for a new drug is considered a priority goal. Many anticancer compounds are natural products or their derivatives, mostly produced by microorganisms[4, 5]. The main sources of natural pigments are microorganisms and plants. These pigments are not only used in industry but also have other biological properties, such as antimicrobial, antiviral, antioxidant, and anticancer agents[6, 7]. Today, many natural pigments have been extracted from microorganisms that have many benefits. The advantages of these pigments include the stability of the produced pigments, the availability of cultivation technology, easy processing, and low cost[8]. Bacteria can be used as an excellent alternative source for pigment production due to their short life cycle and ease of genetic modification[9]. In addition, bacterial pigments are easily degraded and have fewer environmental hazards[9, 10]. Bacteria can produce a variety of pigments, including melanin, carotenoids, pyocyanin, bacteriochlorophylls, violacein, prodigiosin, and monastrols[11]. Pigment-producing bacteria can be easily isolated from various sources, such as the sea and various soil sources

(desert sand and saline areas)[12, 13]. Some bacteria produce large amounts of pigment. These bacteria include *Achromobacter*, *Serratia*, *Sarcina*, *Thiobacillus*, and *Bacillus* sp. However, most pigment-producing bacteria are from the Actinobacter family, which includes the genera *Streptomyces*, *Nocardia*, *Micromonospora*, *Thermomonospora*, *Actinoplanes*, *Microbispora*, *Stereosporangium*, *Actinomyces*, *Rhodococcus*, and *Kittasporia*. *Streptomyces* is the largest genus capable of producing a variety of color pigments [14]. Recent research on anticancer drugs has focused on newer and more effective chemotherapy agents that have fewer toxic effects. Various studies on bacterial pigment as an anticancer agent have been performed on different types of cancer. This research demonstrates the potential of bacterial pigments as promising anticancer agents. Given the above issues and the importance of cancer as a common disorder in society, this study aimed to evaluate the cytotoxic effects of environmental pigments on colon and breast cancer cell lines.

Material And Methods

Isolation and identification of bacterial pigment

A total of ninety different soil, water, and air samples were collected from 28 geographic areas. The details of which are shown in Figure 1 and Table 1. The samples were processed based on standard procedures. Water and soil samples were transported at 4°C to the laboratory and processed within a maximum period of 24 h.

Table 1
Scattering of pigment-producing bacteria from soil, water, and air samples by location

sample	Sample location	Sample number	Colony count	Colored colony count	Selected color colony	Color of selected colony
Soil sample						
1	Shiraz	1	27	3	1	Pale orange
2	Marvdasht	3	29	3	1	light brown
3	Doroudzan	1	41	-	-	-
4	Larestan	1	36	-	-	-
5	Arsanjan	1	30	-	-	-
6	Fasa	1	36	6	1	Red Pomegranate
7	Qalat	1	42	-	-	-
8	Estahban	1	23	-	-	-
9	Maharlu	1	13	-	-	-
10	Kouhmareh	1	41	3	1	pale pink
11	Kazeron	1	15	-	-	-
12	Jahrom	1	22	-	-	-
13	Bandar Dayer	1	19	-	-	-
14	Bandar Kangan	1	42	6	1	dark brown
15	Bandar Parsian	1	31	-	-	-
16	Bandar Siraf	1	17	-	-	-
17	Bandar Abbas	1	43	-	-	-
18	Gachsaran	1	13	2	1	Lemon green
19	Mahshahr	1	43	5	1	pale pink
20	Ramhormoz	1	33	4	1	Pea color
21	Shadegan	1	38	4	1	Bright yellow
22	Andimeshk	1	18	-	-	-
23	Dezfol	1	31	5	1	Peach color

sample	Sample location	Sample number	Colony count	Colored colony count	Selected color colony	Color of selected colony
24	Arak	1	29	4	1	Peach color
25	Shahrekord	1	18	3	1	Peach color
26	Isfahan	1	16	-	-	-
27	Shahinshahr	1	41	7	1	Bold yellow
28	Mashhad	1	39	6	1	dark pink
Total		30	826	61	14	
Water sample						
1	Shiraz	1	16	-	-	-
2	Marvdasht	3	29	3	1	Peach color
3	Doroudzan	1	17	2	1	Pale orange
4	Larestan	1	23	3	1	dark brown
5	Arsanjan	1	40	6	1	Red
6	Fasa	1	14	-	-	-
7	Qalat	1	25	-	-	-
8	Estahban	1	19	-	-	-
9	Maharlu	1	29	-	-	-
10	Kouhmareh	1	27	-	-	-
11	Kazeron	1	18	-	-	-
12	Jahrom	1	30	-	-	-
13	Bandar Dayer	1	22	5	1	Pale orange
14	Bandar Kangan	1	15	-	-	-
15	Bandar Parsian	1	17	2	1	Pea color
16	Bandar Siraf	1	19	3	1	Mustard color
17	Bandar Abbas	1	15	3	1	dark brown
18	Gachsaran	1	13	2	1	Bold yellow

sample	Sample location	Sample number	Colony count	Colored colony count	Selected color colony	Color of selected colony
19	Mahshahr	1	28	-	-	-
20	Ramhormoz	1	31	-	-	-
21	Shadegan	1	16	-	-	-
22	Andimeshk	1	20	3	1	Lemon green
23	Dezfol	1	31	-	-	-
24	Arak	1	24	-	-	-
25	Shahrekord	1	18	3	1	Pale orange
26	Isfahan	1	22	3	1	light yellow
27	Shahinshahr	1	19	-	-	-
28	Mashhad	1	20	-	-	-
Total		30	617	38	12	
Air sample						
1	Shiraz	1	27	3	1	Lemon green
2	Marvdasht	3	29	3	3	light yellow
3	Doroudzan	1	17	2	1	pale pink
4	Larestan	1	23	-	-	-
5	Arsanjan	1	22	-	-	-
6	Fasa	1	11	-	-	-
7	Qalat	1	39	4	1	Bold yellow
8	Estahban	1	35	5	1	Bold yellow
9	Maharlu	1	41	5	1	Peach color
10	Kouhmareh	1	45	-	-	-
11	Kazeron	1	40	4	1	Bold yellow
12	Jahrom	1	42	3	1	dark orange
13	Bandar Dayer	1	22	5	1	dark green
14	Bandar Kangan	1	35	-	-	-

sample	Sample location	Sample number	Colony count	Colored colony count	Selected color colony	Color of selected colony
15	Bandar Parsian	1	49	-	-	-
16	Bandar Siraf	1	30	-	-	-
17	Bandar Abbas	1	29	-	-	-
18	Gachsaran	1	32	-	-	-
19	Mahshahr	1	43	5	1	Red
20	Ramhormoz	1	23	-	-	-
21	Shadegan	1	28	-	-	-
22	Andimeshk	1	48	-	-	-
23	Dezfol	1	31	-	-	-
24	Arak	1	29	4	1	Bold yellow
25	Shahrekord	1	19	-	-	-
26	Isfahan	1	22	3	1	dark brown
27	Shahinshahr	1	41	7	1	dark green
28	Mashhad	1	39	6	1	dark pink
Total		30	891	59	14	

To isolate pigmented bacteria, sampling was performed from the soil, water of different geographical areas and culture on nutrient agar medium and then incubated at 37°C for 48 to 72 hours.

For air samples, first, the plates containing the culture medium (nutrient agar) were randomly placed in different geographic areas, and after a period of 1 to 5 min, the plates were inoculated in an incubator at 37°C for 48 to 72 hours[15].

The pigmented colonies of bacteria were selectively isolated and transferred to nutrient agar medium with the help of the loop inoculum method. The plates were incubated at 37°C for 24 hours in an inverted position to screen for pigment-producing strains. The pigmented colonies were purified by the pure culture method. The isolates thus obtained were then subjected to morphological and biochemical analysis to identify the organism.

pigment extraction:

Colonies with different appearances and colors from different geographical areas were selected and cultured in nutrient agar (10 micrograms of nalidixic acid and 25 micrograms of nystatin were added to prevent the growth of another microorganism), incubated at 28 to 30°C for 4 to 7 days and then recultured to extract the pigment. Bacterial cells were grown in nutrient broth medium and kept in a shaker incubator for 48 hours at 120 rpm at 28°C. The bacterial cells were precipitated following centrifugation at 3500 rpm for 15 minutes from the culture medium. The extraction was performed by adding 5 ml of 99.8% (w/v) methanol and mixing at room temperature on a shaker at 200 rpm for 24 hours. This operation was repeated several times until finally, a white pellet remained at the end of the tube, and all the pigments were separated from the cell. The colored centrifuge was performed at 3500 rpm for 15 minutes, poured into separate tubes and kept at 30°C in an oven until complete removal of solvents[16].

UV/Visible absorption of extract

The biomass was homogenized using solvents and filtered. The concentration of pigments extracted from bacteria was determined by UVD3200 spectrophotometry (Labomed Company). For this purpose, the adsorption rate of each pigment was recorded at wavelengths of 900-200 nm, and the maximum amount of adsorption for each pigment was considered the concentration[17].

Separation and Purification of bacterial pigment

Thin-layer chromatography (TLC) was developed using precoated silica TLC sheets (60 F 254, Merck). The concentrated pigment was dissolved in a methanol solution, spotted half a centimeter above the bottom edge, and allowed to run until the solvent system reached a half-centimeter below the top edge on a silica gel sheet and developed with a mobile phase of 80% ethyl acetate and 20% hexane at room temperature. The separated fractions were detected by observing the TLC sheet under UV light.

Screening for anticancer activities

The breast cancer cell line (MCF-7) and colon cancer cell line (SW-48) were obtained from the collection of cell lines of Shiraz University of Medical Sciences. Bot cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Scotland) supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin and grown at 37°C in an atmosphere of 5% CO₂ in air.

Cell viability assay:

The cytotoxicity of the isolated pigments was evaluated based on cell proliferation using an MTT assay. The test was performed on a 96-cell plate. Each well contained 90 µl of cells with 10,000 and 10 µl of an enzyme. First, 40 extracted pigments with a concentration of 2 mg/ml were repeated three times on breast and colon cancer cells separately. After determining the results, the pigments that had the highest cytotoxic effect were concentrated on colon and breast cancer cells at concentrations of 10^{-7} - 10^{-1} mg/ml. It should be noted that the plates were incubated for 24 hours in an incubator at 37°C and containing 5% carbon dioxide. After incubation, 10 µl of 5 MTT mg/ml was added to all wells, and the

plates were incubated at 37°C for 3 hours and then centrifuged for 10 minutes at 1800 rpm. Then, the supernatant was discarded, and the cells were dried in an incubator at 37°C for several minutes. Finally, the formazan dye crystals were precipitated. In the cytoplasm, the cells were dissolved by adding 100 µl of dimethyl sulfoxide and shaken for 30 minutes in light, and the color intensity was recorded with an ELISA reader at 595 nm. The percentage of cell viability and the amount of toxicity generated were calculated using the following formula[18]:

$$\text{Viability} = \frac{[(AT)/AC] \times 100}{}$$

Molecular identification of pigment-producing bacteria that have the most cytotoxic effects on cancer cell lines:

Chromosomal DNA from pigment-producing bacterial isolates was extracted using a DNA extraction kit (Genet Bio, South Korea). PCR analysis of 16S rRNA and phylogenetic analysis were performed by the method described by Makhtumi et al (17). Sequencing was performed at Bioneer Company (South Korea). The obtained sequences in the current study were aligned manually with all existing sequences of the closely related microorganisms retrieved from the GenBank database, compared with the relevant sequences and analyzed using the jPhydit program version 1.0

Statistical analysis

All tests were repeated at least three times, and the results are reported as the mean ± S.D. Statistical analysis was performed using SPSS-18 software. Equal variances between treatments were measured using Levene's test for equality of variance. A two-tailed P-value less than 0.05 was considered statistically significant.

Results

From 90 soil, water, and air samples, 40 isolates were selected based on color differences and different geographical areas. The results of Fig. 2 show that the soil samples with 7.6% pigment-producing bacteria had the highest frequency, and the water samples with 5.6% pigment-producing bacteria had the lowest frequency among the samples. The results of pigment extraction from 40 selected isolates are shown in Fig. 3. The concentration of different pigments was measured using a spectrophotometer at wavelengths between 200 and 900 nm for use in cell culture.

The cytotoxicity of the pigments was examined on human breast cancer cells and human colon cancer cells (MCF-7 and SW-48) by exposing cells to 2 µg/ml pigment for 24 hours. The reduced MTT formazan was dissolved in DMSO, and the absorbance was read in a 96-well plate reader (Fig. 4, Fig. 5). Three pigments that had the most cytotoxicity in the bot cell line were selected and used to affect the cell line at various concentrations from 10^{-1} to 10^{-7} for 24 hours. The graph was plotted as % inhibition against the concentration of the pigments (Fig. 6, Fig. 7). The analysis indicates that greater concentrations of the 3 examined pigments cause increased cytotoxicity and anticancer properties. In addition, the maximum

cytotoxicity potential among the three metabolites related to the pigment extracted from Arsanjan air (0.1 µg/ml) was not significant according to the nonparametric Mann-Whitney test ($p = 0.32$). The effects of pigments on human breast cancer cells and human colon cancer cells after 24 and 48 hours are shown in Figures 8 and 9. In the present study, PCR was used to molecularly identify pigment-producing isolates with the highest cytotoxic properties. The phylogenetic analysis results based on the 16S rRNA gene sequence are shown in Table 2 and Figures 10, 11, 12, 13, and 14. The relative purity of the effective pigments extracted from the identified bacteria was determined using thin-layer chromatography, and the results are shown in Figure 15.

Table 2

Results of blasting sequences of selective pigment-producing bacteria identified by molecular methods

samples	Isolated bacterial	Pct(%)	Accession number
Shiraz air sample	<i>Micrococcus xinjiangensis</i> SRDD42	99%	U 714900.1
Shahrekord soil sample	<i>Dietzia</i> sp. A14101	99%	KF 923451.1
Shahrekord water sample	<i>Exiguobacterium mexicanum</i> strain OZK24	100%	KT 630833.1
Arsenjan water sample	<i>Arthrobacter agilis</i> strain DSM 20550	99%	NR 026198.1
Bandar Dayer water sample	<i>Exiguobacterium mexicanum</i> strain OZK24	99%	KT 630833.1
Bandae Kangan soil sample	<i>Bacillus beijingensis</i> strain ge10	99%	NR 117988.1

Discussion

In recent years, much attention has been given to the pigments of microorganisms and their biosynthetic pathways (18). Considering the importance of pigments isolated from different strains of bacteria in various industries and even medical sciences, as well as the existence of several studies on the anticancer effect of pigments isolated from bacteria, in this study, the anticancer activity of pigments extracted from *Micrococcus xinjiangensis* SRDD42, *Dietzia* sp. A14101, *Exiguobacterium mexicanum* strain OZK24, *Arthrobacter agilis* strain DSM 20550, and *Bacillus beijingensis* strain ge10 were investigated. The results of this study showed that bacteria isolated from water, soil, and air can produce pigments. It was also found that the highest abundance of pigment-producing bacteria was related to soil, air, and water. The *Micrococcus xinjiangensis* SRDD42 isolated in this study had the ability to produce yellow carotenoids that had a significant lethal effect on MCF-7 cells. Our findings were consistent with the findings of Zohari et al.[19] These results showed that the MCF-7 cell line had a significant reduction in living cells after treatment with pigments isolated from bacteria of this genus. Various organisms have the ability to produce pigments in the red and orange color spectra that are classified as carotenoids. Among these bacteria, *Dietzia* sp., which in the present study was isolated from

Shahrekord soil and has the ability to produce orange pigment. Various studies have examined the antioxidant effects of pigments obtained from these organisms[20, 21]. However, to date, no study has been done on the lethal effect of the pigments of this organism on cancer cells. In this study, we first examined the effect of these pigments on MCF-7 cells and showed that these pigments have a good lethal ability in this cell line. Another bacterium that was identified by molecular methods in this study was *Exiguobacterium mexicanum strain OZK24*, which was isolated from Deir and Shahrekord seals. The orange pigment of this organism in the MTT assay showed a high ability to kill cancer cells in both MCF-7 and SW-48 cell lines. In a study conducted by Gajaraj et al., pigments isolated from another species of this genus were studied on a variety of cancer cells, and it was shown that these pigments reduce cell proliferation and increase apoptosis[22]. The genus *Arthrobacter* is a heterogeneous group of bacteria that were first identified by Conn and Dimmik [23]. Bacteria of this genus are isolated from various environments, such as soil, water, and air [24]. *Arthrobacter agilis strain DSM 20550* isolated in this study has the ability to produce red carotenoid pigment, which has anticancer activity on the SW-48 cell line. In a study by Afra et al., pigments extracted from this bacterium were shown to have the ability to kill cancer cells, which confirms the results of our study[1]. There have been many reports of the anticancer activity of bacterial pigments, including prodigiosin, violacein, astaxanthin, pyocyanin, and β -carotene[25]. Among these pigments, *Bacillus spp* pigments belong to the carotenoid group[26]. In the present study, *Bacillus beijingensis strain ge10* was isolated from Bandar Kangan soil and had the ability to produce brown pigments. The pigment effects on cancer cells were studied for the first time and showed that it has the ability to destroy SW-48 cells.

Conclusion

Exertions made in the current study have been efficacious and are reinforced by evidence from recent reports on the isolation and characterization of novel bacteria from researched environments. Therefore, it can be supposed that the screening of available regions that possess unexplored pigmented bacteria will intensify the chances of discovering new pigments to be used as a resource for treating cancer. From a biotechnological perspective, the current study showed that pigments produced in bacteria have cytotoxic effects on cancer cells and can be a useful and inexpensive source of these compounds used to treat cancer. Ordinary environments that are simply reachable and pigmented bacteria isolated from these environments could readily be selected so that we can evaluate this bacterial species to determine their biological potential. Afterward, pigmented bacteria from these surroundings are largely present. Thus, the group of bacteria from these emerging habitats have yet to be discovered and deserve special mention.

References

1. Afra S, Makhdoumi A, Matin M, Feizy J (2017) A novel red pigment from marine *Arthrobacter* sp. G20 with specific anticancer activity. *J Appl Microbiol* 123(5):1228–1236

2. Pan M-H, Ho C-T (2008) Chemopreventive effects of natural dietary compounds on cancer development. *Chem Soc Rev* 37(11):2558–2574
3. Chang C-C, Chen W-C, Ho T-F, Wu H-S, Wei Y-H (2011) Development of natural anti-tumor drugs by microorganisms. *J Biosci Bioeng* 111(5):501–511
4. Prashanthi K, Suryan S, Varalakshmi KN (2015) In vitro anticancer property of yellow pigment from *Streptomyces griseoaurantiacus* JUACT 01. *Brazilian Archives of Biology and Technology* 58:869–876
5. Berdy J (2005) Bioactive microbial metabolites. *J Antibiot* 58(1):1–26
6. Samanta AK, Chaudhuri S, Dutta D (2016) Antioxidant efficacy of carotenoid extract from bacterial strain *Kocuria marina* DAGII. *Materials Today: Proceedings* ;3(10):3427-33
7. Ravikumar S, Uma G, Gokulakrishnan R (2016) Antibacterial property of Halobacterial carotenoids against human bacterial pathogens.
8. Manikprabhu D, Lingappa K (2013) γ Actinorhodin a natural and attorney source for synthetic dye to detect acid production of fungi. *Saudi journal of biological sciences* 20(2):163–168
9. Venil CK, Aruldass CA, Dufossé L, Zakaria ZA, Ahmad WA (2014) Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry—an incisive evaluation. *RSC Adv* 4(74):39523–39529
10. Venil CK, Zakaria ZA, Ahmad WA (2013) Bacterial pigments and their applications. *Process Biochem* 48(7):1065–1079
11. Azman A-S, Mawang C-I, Abubakar S (2018) Bacterial pigments: the bioactivities and as an alternative for therapeutic applications. *Nat Prod Commun* 13(12):1934578X1801301240
12. Rashid MM, Fakruddin M, Mazumdar RM, Kaniz F, Chowdhury MA (2014) Anti-bacterial activity of pigments isolated from pigment-forming soil bacteria. *Journal of Pharmaceutical Research International* :880–94
13. Samyuktha S, Mahajan SN (2016) Isolation and identification of pigment producing bacteria and characterization of extracted pigments. *International Journal of Applied Research* 2(7):657–664
14. Lin YB, Wang XY, Fang H, Ma YN, Tang J, Tang M et al (2012) *Streptomyces shaanxiensis* sp. nov., a blue pigment-producing streptomycete from sewage irrigation soil. *Int J Syst Evol MicroBiol* 62(Pt8):1725–1730
15. Moazamian E, Emami A, Mehdizadeh H, Pirbonyeh N (2018) Bactericidal activity of pigments isolated from Fars province (Iran) environmental bacteria on MDR clinical isolates of *Acinetobacter*. *International Journal of Molecular and Clinical Microbiology* 8(1):922–930
16. Mayuran S, Radhakrishnan M, Balagurunathan R (eds) (2006) proceeding of the National Symposium on "Recent Trends in Microbial Biotechnology" Sri Sankara Arts and Science College;
17. Velmurugan P, Kamala-Kannan S, Balachandar V, Lakshmanaperumalsamy P, Chae J-C, Oh B-T (2010) Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. *Carbohydr Polym* 79(2):262–268

- ## Figures

Figure 1

Geographical map of provinces collecting soil, water and air samples

Figure 2

The number of bacteria in terms of pigment production according to the type of sample

Figure 3

Total pigments extracted using methanol

Figure 4

Evaluation of the effect of extracted pigments at a concentration of 2 µg/ml on a breast cancer cell line

Pigment extracted from water sample -
Shahrekord - after 48 hours

Pigment extracted from air sample - Shiraz
- after 24 and 48 hours (200%)

Pigment extracted from soil sample -
Shahrekord - after 48 hours

Figure 8

Cytotoxic effects of treated colon cancer cells after 24 and 48 h

Figure 9

Figure 11

Bacterial phylogeny tree with code Sh s related to Dietzia sp. A14101. The phylogenetic tree of this bacterium also showed that the closest species to this isolate is Dietzia maris starin DSM 43672, which has a 23% probability of having a common ancestor with the desired isolate. Additionally, isolated Dietzia sp. probably 24% related to Nocardia Globerula strain DSM 44596, Rhodococcus qingshengii strain djl-6, Nocardia arthritidis strain IFM10035 and Rhodococcus soil strain DSD51 W.

Figure 13

Bacterial phylogeny tree with code Bd w and code Sh W related to Exiguobacterium mexicanum strain OZK24. This isolated phylogenetic tree showed that this bacterium primarily and with a probability of 77% has a common ancestor with the bacterium Exiguobacterium mexicanum strain OZK24. Additionally, the second degree, with a probability of 57%, was related to Exiguobacterium alkaliphilum strain 1.12 and Exiguobacterium aquaticum strain IMTB-3094.

Figure 15

Thin layer chromatography (TLC) of ethanolic extract of pigments isolated from bacteria A (Exiguobacterium mexicanum, B (Exiguobacterium mexicanum), C (Bacillus beijingensis strain ge10), D (Dietzia sp. A14101), E (Micrococcus SRDD42), and F (Arthrobacter agilis strain DSM 20550) exposed to UV light from left to right, respectively.