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Isolation and characterization of actinophages specific to *Streptomyces rochi* from Sohag Governorate

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Abstract

The objective of this study was to identify and separate *Streptomyces rochei*-specific actinophages. From two different sites of reclaimed soil in the Sohag Governorate region, eight actinophage (*streptomyces* phage) isolates specifically for *Streptomyces rochei* were successfully identified. The samples were taken from reclaimed soil in Alkawmel city and the New Sohag experimental farm of the Faculty of Agriculture, Sohag University, in Sohag, Egypt. All of the isolated phages (the actinophage) looked to be head-and-tail types, according to electron micrographs of the isolated phages. The eight isolates of the phage were given the numbers 1, 2, 3, 4, 5, 6, 7, and 8. Each phage isolate's heat inactivation point was investigated. Although all phage isolates maintained their infectivity at 85 °C during 10 minutes, they all demonstrated resistance to high temperatures. It has been discovered that a pH of 7 is ideal for phage infection, while phage isolates can survive in both alkaline and acidic environments. The susceptibility of the different phage isolates to UV light varies.

Keywords: Actinophages, *streptomyces rochi*, Soahg.

INTRODUCTION

Streptomyces rochi are gram-positive aerobic bacteria belongs to actinobacteria that produce spores and aerial mycelium development (Barke, et al., 2010). Diseases in plants, people, and animals are brought on by some actinomycetes (Babalola et al. 2009).

According to MCIntyre (2002), Berdy (2005), Bibb (2005), Kariminik and Baniasadi (2010), Streptomyces has a strong potential for producing secondary metabolites, including antibiotics, anthelminthic enzymes, herbicides, anti-cancer medications, and immunological modulators (Mann, 2001). Streptomyces species create a variety of specialised metabolites that serve a variety of purposes, such as nutrient availability (for example, siderophores for iron uptake) and antagonistic behavior towards other microbes (Jones *et al*., 2017). One of the species that can attack Streptomyces spp. most frequently is the actinophage. Plants are well-protected from bacterial and fungal diseases by streptomyces (Li *et al*.2019). Actinophages must be separated and described in order to better comprehend the biology of these actinophages, as well as their prevalence and dissemination. The goal of this experiment was to separate *Streptomyces rochi*-specific actinophages from the Sohag Governorate and analyses the various traits of these phages.

MATERIAL AND METHODS

The used bacteria *S. rochei*

The microbiology department of the Faculty of Agriculture at Sohag University in Sohag, Egypt, kindly provided the isolate of *Streptomyces rochi*.

collection of soil samples:

Tomato plants (*Solanum lycopersicum*) rhizosphere soil samples were taken at the Experimental Farm of the Fac. of Agriculture, Sohag University. Actinophages were isolated from the soil sample that was taken.

Separation of actinophages

The liquid enrichment approach of Adams (1966) was used to isolate the Streptomyces rochi phages, with a few minor alterations as indicated by Hammad (1989). A mixture of 40 cc of nutritional broth and 20 grammes of soil was incubated at 30°C for an entire night. After killing any germs with 5 ml of chloroform for 10 minutes, the sample was centrifuged at 4000 rpm for 10 minutes. The supernatant was then put to a liquid culture of *Streptomyces rochi* that was 36 hours old and cultured at 30-33°C for 24–30 h. After shaking for 10 minutes with 5 ml of chloroform, the culture was centrifuged at 4000 rpm for 10 minutes to eliminate any remaining bacteria. Phage detection was done on the supernatant (phage lysate).

detection of phages

The agar double layer plats technique (Adams, 1966) was applied for phage identification. A foundation layer of 20 ml of nutritional agar medium containing 1.5% agar was placed onto Petri plates with a 10 cm diameter. The basal layer was allowed to solidify. The indicator Streptomyces (24–36 h. liquid culture) and 5 ml of nutritional agar melting media containing 0.7% agar were combined on each plate. The phage lysate was spotted with a sterile micropipette on the top layer after the top layer had solidified. The plates were examined for lyses of bacterial lawn where the drops had been applied after 36–48 hours of incubation at 30-33°C. The lysed clear zones were selected and added 1 ml of SM media separately to Eppendorf tubes (Maniatis *et al*., 1982). After adding 200 ml of chloroform per tube, the tubes were maintained at 4 °C.

Purification of isolated actinophages:

As explained by Kiraly *et al*., (1970) the single plaque isolation approach was used to obtain pure single isolates of phages. In the spot test, the clear zones produced phage suspension, which was diluted in SM media (10-4 to 10-6 as needed). A mixture of 3 ml semi-solid media, 300 l of suitable liquid bacterial culture, and 5 l of diluted phage suspension were placed on top of the double layer plates. During 24 to 30 hours, or until an appropriate plaque development was noticed, the plates were incubated at 30-33°C. Eight single plaques with various morphological characteristics were chosen at random using sterile Pasteur pipettes. 500 l of SM medium were put in an Eppendorf tube with each plaque. Each of these phage isolates received 200 ml of chloroform and was kept at 4 °C.

The process of creating high-titer phage suspensions

According to Maniatis *et al*., (1982) the high titer phage suspension was created utilizing the agar double layer plate approach. After being extracted from the single plaques that had been produced, the phage suspensions were diluted (10-4 pfu/ml) in SM medium. Three milliliters of semi-solid nutritional agar medium, three hundred milliliters of liquid indicator bacterium culture, and fifty milliliters of diluted phage suspension made up the top layer of the double layer plates. After 24–30 hours of incubation at 30–33°C, plates showed nearly complete lysis. Five ml of SM media were applied to each plate (Maniatiset al., 1982). Each plate's top agar layer was removed, and the remaining SM was combined with it in a flask. Agar and bacterial detritus were separated by centrifugation at 4000 rpm for 30 minutes after 30 minutes of shaking. Using 3 ml of chloroform, the phage-containing supernatant was stored at 4 °C.

The isolated phages' characteristics:

The ideal pH value:

Several pH levels of SM medium were created. The pH scale went from 4 to 12. Each of the prepared Eppendorf tubes contained 1ml of SM at each pH level. For each isolated phage, a single plaque was added to a tube (one plaque/tube). Five milliliters (5 l) from each tube were spotted onto the double agar layer plates (four replicates) containing the indicator bacteria after 60 minutes of incubation at 30°C. The lysed spots' diameter was measured. The means of the replicates were then determined.

Thermal threshold of inactivity

Each Eppendorf tube received one ml of phage suspension (high titer for each phage isolate). Tubes were cooled under running water after 10 minutes of heating in water baths set at 50, 55, 60, 65, up to 90°C. 10 l from each tube were spotted onto plates with double agar layers and the appropriate indicator microorganisms after being heated. Plates were checked for lysed areas following a 24-hour incubation period at 30-33°C. (Fathy 2004)

Sensitivity to ultraviolet radiation: Petri dishes holding 5 ml of each phage's high-titer suspension were placed 20 cm away from a germicidal UV lamp with a wavelength of 254 nm. By sprinkling 10 l of each irradiation phage over double-layer agar plates containing Streptomyces rochi, phage activity was monitored every 10 min for 90 min. Following 24 to 30 hours at 33°C, the plates were checked for lysed spots (Elsharouny 2007).

Electron microscopy:

Using an electron microscope, grids were created in accordance with instructions.'s to examine the dimensions and morphology of each phage isolate Stacy *et al*., (1984). Grids were examined under a transmission electron microscope after being dyed with 0.5% uranyl acetate pH 4.

RESULTS

***Streptomyces rochei*-specific actinophage isolation:**

Source of the actinophage:

Actinophages unique to *Streptomyces rochei* were obtained from tomato plant rhizosphere soil samples (*Solanum lycopersicum*).

Given that it is widely known that bacteriophages are frequently found in locations with the suitable bacterial host. Similar to this, Othman *et al*. (2008) obtained actinophages specific for Streptomyces Griseofulvin from soil samples.

Streptomyces phage detection: Soil samples were examined for the presence of phages using the spot test. The spot test revealed that *Streptomyces rochei* actinophages were widespread in the soils where the samples had been taken, as shown in Figure (1). Therefore, it is widely known that actinophages are frequently found in locations with the suitable bacterial host. Actinophages peculiar to the investigated bacterial isolate are present, which may indicate that *Streptomyces rochei* predominates in the soils of the Sohag Governorate (the locations from where samples had been collected). Abdelrhim *et al*. (2021), who isolated a novel phage infecting Streptomyces scabies from soil sample taken from El-Minia, Egypt, reported observing similar results.

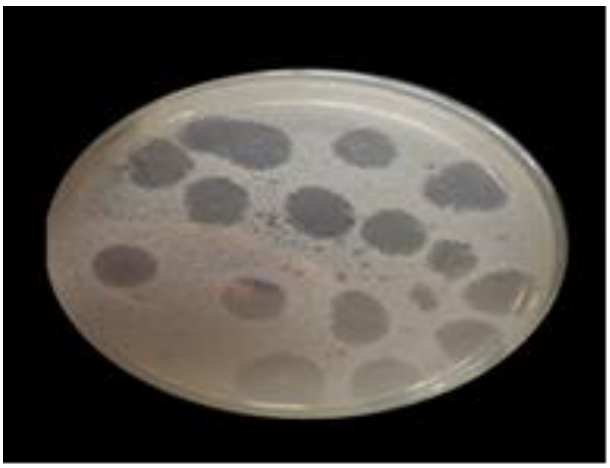


Figure 1: shows the spot test of the produced phage lysate on the indicator bacteria *streptomyces rochei.*

Purification of actinophages:

By using the single plaque isolation approach, phage isolates were refined. Eight single plaques of morphologically distinct S. rochei-specific phages were chosen as pure phage isolates. Elmaghraby *et al.* (2015) and Kiraly, et al. (1970) both utilised the same method to purify phages. The isolated *S. rochei* phages produced round, transparent plaques with a diameter of 1 to 2 mm, as depicted in Figure 2.

According to Fathy (2008), Elsharouny (2014), and Farahat (2016), the morphology of plaques and their diameters are the first characteristics that can be utilised to distinguish between the phages of different bacteria.

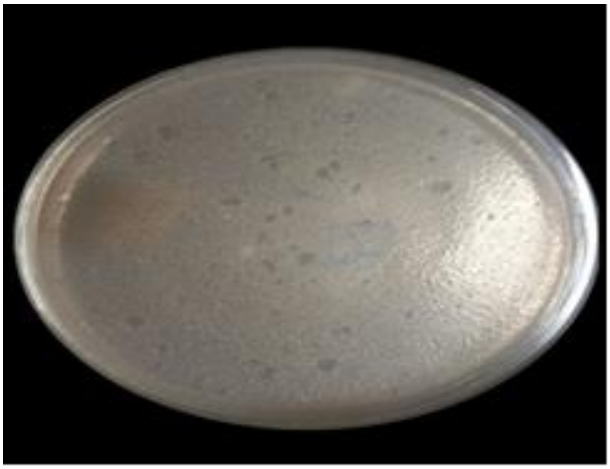


Figure (2): On a double agar layer plate that was made using phage lysate and *S. rocheia* as a bacterial host, the various morphologies of single plaques can be seen.

It was anticipated that each individual phage isolate would represent a particular phage-type because the single plaques of the eight *S. rochei* phage isolates were morphologically distinct. In other words, the isolated phages are probably from eight different phage kinds. The many traits of the isolated phages were researched in order to assess this anticipation.

The isolated phages were characterized using a variety of techniques to learn more about the actinophages of *S. rochei*

The isolated phages' characteristics:

To learn more about the actinophages of *S. rochei,* a variety of characterization techniques were employed.

pH of actinophage isolates:

Actinophage isolates were examined for infectiousness at various pH levels (from 4 to 12). According to Table, all phages produced lysed spots at pH values between 5 and 11. (1). A lysis was not seen at pH 4 or pH 12, however. Our findings suggest that the isolated actinophages of *S. rochei* are acidic and alkaline reaction resistant. There were eight phage isolates, and all of them showed bigger lysed areas at pH 7 than they did at any other pH. For the eight phages being studied, pH 7 is ideal. The alkalinity of the soil where phages were isolated may be responsible for the vast range of phage tolerance to acidic and alkaline conditions.

Thermal inactivation points:

Numerous researchers used the actinophage isolates' thermal inactivation point as a distinguishing factor. Furthermore, according to Abo-Sinna (2004), four Bacillus subtilis phages had thermal inactivation values that ranged from 50 to 80 degrees Celsius.

Effect of UV irradiation on actinophages:

We investigated the sensitivity of isolated Streptomyces rochei actinophages to UV light at 254 nm. The separated phages were rendered inactive by UV light with a 254 nm wavelength over a range of exposure times, according to data in Table 3.

Some of the phage isolates shared similarities in their sensitivity to UV light, as seen in Table 3

below. The most UV-sensitive actinophage isolates were nos. 3, 6, and 8, which lost their ability to infect after 10 minutes of exposure. nos. 2, 4, and 7 also lost their ability to infect after 15 minutes of exposure. The two most UV radiation-tolerant phage isolates, nos. 1 and 5, lost their ability to infect after 20 minutes of exposure.

Such observations could prove that phage isolates of the same UV sensitivity belong to one type, especially if they similar in other characteristics.

Table (1): Effect of different pH levels on S. *rochei* phages.

Phage No.	pH levels								
	4	5	6	7	8	9	10	11	12
	Average size of the lysed areas (mm.)								
1	00	4.4	6.2	7.3	6.5	6.0	5.2	4.8	00
2	00	4.8	5.8	7.0	6.2	5.7	5.0	4.2	00
3	00	5.1	6.3	7.5	6.4	5.3	4.8	3.3	00
4	00	4.9	5.3	6.9	6.8	6.2	5.5	4.1	00
5	00	5.3	6.1	7.2	6.6	6.0	5.2	4.3	00
6	00	5.6	6.3	7.8	7.2	6.4	6.0	4.8	00
7	00	5.7	6.5	7.6	7.5	6.9	5.7	4.2	00
8	00	4.6	5.8	7.3	6.7	6.4	5.7	4.4	00

The lysed spots' diameter is the average of four replicates.

The optimum pH.

Table (2): Thermal stability of actinophages of Streptomyces rochei, exposed to 50 -90°C for 10 min.

phage No.	Tempreture (°C)								
	50	55	60	65	70	75	80	85	90
1	+	+	+	+	+	+	+	+	-
2	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+	-
4	+	+	+	+	+	+	+	+	-
5	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	+	+	-
7	+	+	+	+	+	+	+	+	-
8	+	+	+	+	+	+	+	+	-

+ = Lysis - = no Lysis

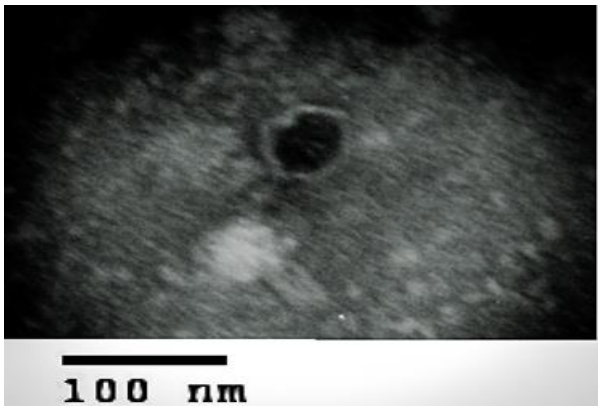
Table (3): Effect of U.V. radiation (254 nm) on actinophages specific to Streptomyces rochei

Phage No.	Exposure time (min.)			
	5	10	15	20
1	+	+	+	—
5	+	+	+	—
2	+	+	—	—
4	+	+	—	—
7	+	+	—	—
3	+	—	—	—
6	+	—	—	—
8	+	—	—	—

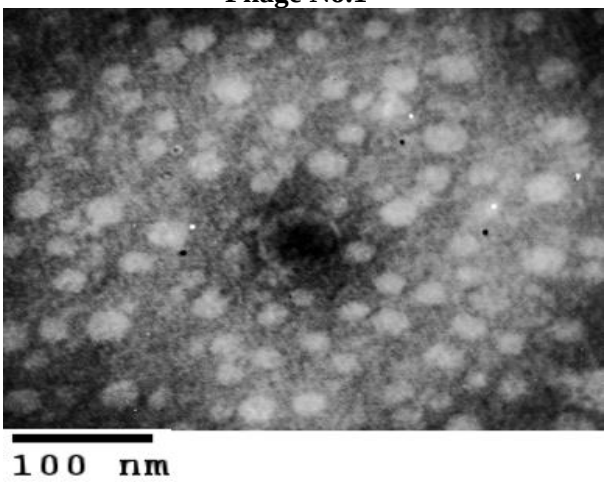
+ = Lysis - = No lysis

Electron microscopy

The morphology and particle size of all eight isolated actinophages were observed using transmission electron microscopy after the isolated actinophages were stained with uranyl acetate. Actinophage isolates differed in their morphologies and sizes, as shown by particle micrographs (Figs. 3 and 4). (table 4).

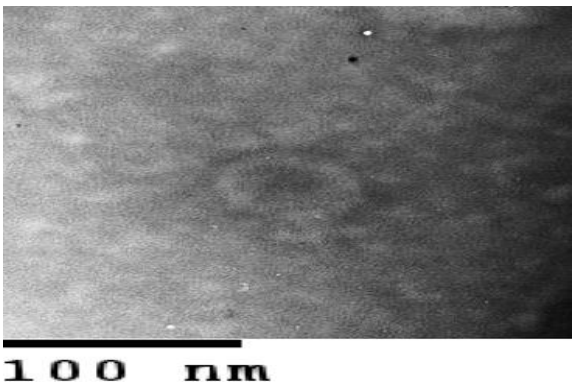


Phage No.1

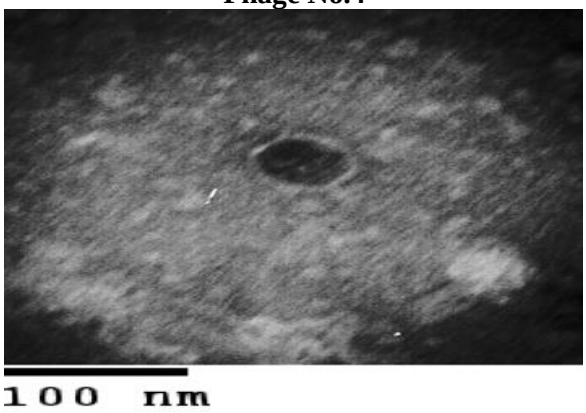


Phage No.2

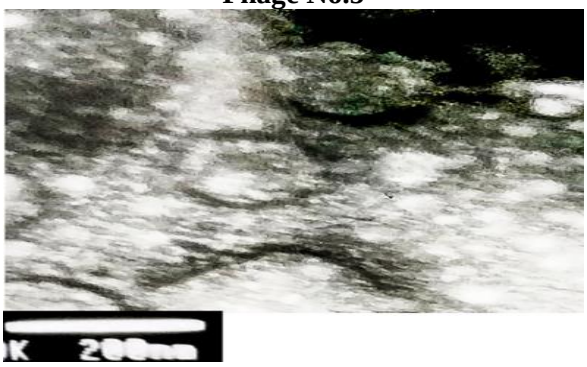
Phage No.3



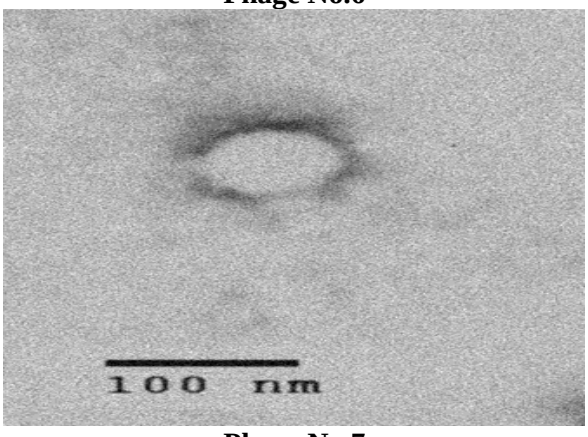
Phage No.4



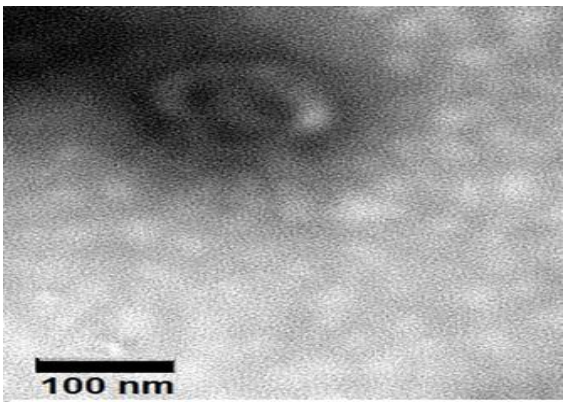
Phage No.5



Phage No.6



Phage No.7



Phage No.8

Figure (4): Micrographs of *S. rochei* phage particles isolate No.7, 3, 6 and 8.

Actinophage isolates 1 and 5 had identical head diameters, measuring 50, 48, and 3 nm in isometric units, respectively, and flexible tail lengths of 45, 4, and 52, 3 nm. It was discovered that all actinophage isolates were of the head and tail types. The morphologies of isolates No. 2, 4, and 7 were discovered to be comparable. Moreover, among all isolated phage particles, actinophage isolates No. 3, 6, and 8 exhibited the biggest diameters.

Phage isolates No. 1, 5, 3, and 6 had morphologies that are consistent with those of the Podoviridae family. Furthermore, the 2, 4, and 7 actinophage isolates' tail types and particle morphology all match those of the Siphoviridae family.

DISCUSSION

In the soil microorganism community, bacteriophages play a significant role in preventing the growth of helpful bacteria like Streptomyces species. In this study, soil from the Sohag Governorate was used to isolate S. rochi phages. For example, S. rochi phages are widespread in soil that is exposed to extreme heat, aridity, salinity, and alkalinity. From Egyptian soils, Streptomyces scabies phages were identified by Abdelrhim et al. in 2021. In addition, two phages unique to Streptomyces griseoflavus were isolated and identified by Othman et al. (2008) from a soil sample. Eight isolated and purified single plaques were found. The isolated plaques were 0.5–1 mm in diameter, transparent, and round. The initial characteristic that was employed to distinguish and classify the phage isolates was plaque morphology. All isolates of actinophages (isolates 1, 2, 3, 4, 5, 6, 7 and 8) were of the head-and-tail variety and varied in size, according to transmission electron micrographs. The form and size of phages 2.4 and 7 fulfil the requirements of the Siphoviridae family (McCorquodale 1999). 1.3.5, 6.8, and 8 phage isolates meet requirements for the podoviridae family (Molineux 1999). It was investigated where phage isolates became thermally inactive. Unexpectedly, all phage isolates are thermo-stable because they remain active for 10 minutes at 85 °C. This might be because the hot, dry atmosphere in Upper Egypt served as the phage isolate's source (Sohag Governorate). The effects of environmental conditions on bacteriophages were discussed by Joczyk et al. (2011) and Santhanam et al. (2012), who also discovered that phage isolates were stable at high temperatures (up to 97 °C). Using various pH levels, the impact of pH and the ideal pH for phage isolates' infectivity were examined (from 4 to 12). At pH ranges of 5 to 11, all phage isolates showed lysed patches. A lysis was not found at pH 4 or pH 12, however. Our findings suggest that the isolated actinophages of S. rochei are acidic and alkaline reaction resistant. Compared to those produced at any other pH evaluated, the eight actinophage isolates formed broader lysed areas at pH 7. This would suggest that for the eight phage isolates being studied, pH 7 is the ideal pH. The alkalinity of the soil where the phages and bacterial host were isolated may be responsible for the vast range of phage tolerance to acidic and alkaline conditions. According to Reddy et al. (2011), S. rochei had an optimal pH of 7 for maximal growth, a pH range of 6 to 10.5, and the bacteria were acidity sensitive (pH 5). Similar to this, researchers Roslycky et al. (1962), Challaghan et al. (1969), Hammad and Ali (1999), and Fathy (2008) showed that phages were stable to a range of pH ranges from pH 5 to 12. Furthermore, Bacillus megaterium phage isolates that belonged to the same phage type were discovered to share the same susceptibility to UV radiation and other features, according to Hafez et al. (2022).

Table (4): Dimensions* of the phage particles specific for *S. rochei*

Phage No.	Head Diameter SD (nm)	Tail	
		Width SD (nm)	Length SD (nm)
1	55□ 3	10□2	44□4
5	48□ 3	10□ 2	52□ 3
2	58□ 3	18□ 3	18□ 2
4	51□2	18□2	17□□
7	56□ 3	15□ 3	22□ 3
3	175□ 3	37□ 2	162□ 4
6	170□3	37□2	150□2
8	168□ 2	26□ 2	147□ 3
SD = Standard deviation			

CONCLUSION

The isolated phages of S. rochei were generally classified and distinguished in this study using various features based on the information stated above. These characteristics (thermal inactivation point, sensitivity to UV radiation, as well as size and morphology of the particles) must be studied collectively to give clear differences between phage isolates tested, as no single method for characterizing phages is sufficient for identification or classification.

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عزل وتوصيف الأكتينوفاج الخاص ببكتيريا الاستربتومايسيس روشي من محافظة سوهاج

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الملخص العربي

كان الهدف من هذه الدراسة هو عزل وتوصيف الأكتينوفاج الخاصة بالبكتريا Streptomyces rochei. تم عزل ثمانية عزلات من الاكتينوفاج الخاص ببكتيريا Streptomyces rochei بنجاح من موقعين مختلفين من التربة المستصلحة في محافظة سوهاج. (تم جمع العينات من التربة المستصلحة في مدينة الكوامل ومزرعة سوهاج التجريبية الجديدة بكلية الزراعة ، جامعة سوهاج ، سوهاج ، مصر أشارت الصور المجهرية الإلكترونية للاكتينوفاجات المعزولة إلى أن جميع عزلات الاكتينوفاجات تبدو وكأنها من أنواع الرأس والذيل. تم توصيف الاكتينوفاجات الثمانية 1 ، 2 ، 3 ، 4.5.6.7 و 8. تمت دراسة نقطة التثبيط الحراري لكل عزلة اكتينوفاج. أظهرت جميع عزلات الاكتينوفاجات تحمل درجات الحرارة المرتفعة، حيث حافظت على العدوى عند 85 درجة مئوية لمدة 10 دقائق. وجد أن الرقم الهيدروجيني الأمثل لعدوى الاكتينوفاج هو الرقم الهيدروجيني 7 ، وأن الاكتينوفاجات تتحمل الظروف القلوية والحمضية. اختلفت عزلات الاكتينوفاجات ت في حساسيتها للأشعة فوق البنفسجية.