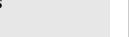


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Original article

Cloning, sequencing, and characterizing of soil antibiotic active-producing *Streptomyces* species-specific DNA markers



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ABSTRACT

Association of the antibiotic activity of the soil *Streptomyces* isolates to their genetic profiles analyzed through RAPD-PCR fingerprints prompted us here in this study to use the most common bands as specific markers to identify homologous proteins within these isolates by cloning, sequencing, and characterizing these markers. Six out of twelve DNA bands ranged between 600 and 1350 bp previously obtained by RAPD-PCR analysis were purified out of the RAPD gels, and then cloned into pGEM-T Easy vector system. Success of the cloning process was confirmed by digesting purified plasmids with *Eco*RI. The clones namely No. 54, 55, 20, 56, 57, and 58 were sequenced using the DNA BigDye Terminator Sequencing System utilizing the M13 primer. Results indicated that the size of the inserted sequences is 599, 566, 522, 870, 857, and 254 bp, in clones No. 54. 55, 20, 56, 57, and 58, respectively. Homologous proteins of the six cloned sequences generated by DNA blast software indicated that the highest score of protein homology was scored for clone No. 54 with 87 % homology to putative secreted pectate lyase [*Streptomyces coelicolor* A3(2)]. The other clones showed less homology with 77 % homology for the clones No. 55 and 56, 73 md 58. The association of homologous proteins to the reported RAPD pattern is confirmed here for the first time, and the resulting DNA cloned fragments deserve further molecular analysis.

1. Introduction

Streptomyces spp. are filamentous Gram-positive bacteria that play a remarkable role in medical, veterinary, agricultural, and environmental fields (Lee et al., 2019; Hamed et al., 2020; Butt et al., 2023). They are very important sources of antibiotics and novel bioactive compounds such as probiotics, antitumor, immunosuppressants, herbicides, and pesticides (Alam et al., 2022; Cuozzo et al., 2023; Shepherdson et al., 2023). Moreover, *Streptomyces* spp. are widely distributed in soil (Mahasneh et al., 2021; Saadoun et al., 2023; Shepherdson et al., 2023) and in a variety of terrestrial, aquatic, and extreme environments (Aryal et al., 2021; Donald et al., 2022; Moore et al., 2023). Furthermore, *Streptomyces* spp. have a significant role in the realm of biotechnology, industry, and drug discovery (Alam et al., 2022) and they are the biggest

producer of novel secondary metabolites and various biological products (Lee et al., 2020; Donald et al., 2022) as well as diverse and valuable natural products (Moore et al., 2023; Wan et al., 2023) that promote good health and improve life quality. Therefore, isolation and identification of different species of the genus *Streptomyces* offer significant advantages to explore novel pharmaceutical compounds (Foulston, 2019), overcome antibiotic resistance (Viswapriya and Saravana, 2022), expand biotechnological applications (Alam et al., 2023).

The discovery of new products as well as the efficiency of bioactive compounds produced by *Streptomyces* could be accelerated through cloning (Amore et al., 2012; Fazal et al., 2020). The ability to clone and manipulate *Streptomyces* DNA has revolutionized the process of natural product discovery, enabling scientists to unlock the vast chemical

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Available online 30 October 2023

Abbreviations: BLAST, The Basic Local Alignment Search Tool; BSA, Bovine Serum Albumin; LB, Lauria Broth; IPTG, Isopropyl β-D-1-thiogalactopyranoside; RAPD, Random Amplified Polymorphic DNA; S.O.C., Super Optimal broth with Catabolite repression.

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Table 1

DNA bands purified out of the gel of the RAPD reactions (Mahasneh et al., 2021).

Serial No*.	Band (clone) No. (Isolate No.)	Size (bp)
1	54 (H116)	1350
2	55 (C15)	870
4	20 (J133)	600
5	56 (J17)	870
9	57 (A39)	870
10	58 (S144)	1000

Represent the lane No. of the tested clones in Fig. 1.

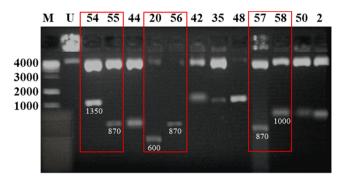


Fig. 1. 2 % agarose gel electrophoresis of purified plasmids digested with *Eco*RI restriction enzyme. Lane M: 1000 bp marker, lane U: uncut plasmid, No. from 54 to 2 represent the purified plasmids digested with *Eco*RI.

diversity encoded within these organisms (Wan et al., 2023). Besides, Streptomyces clones offer a promising avenue for the discovery of novel bioactive chemicals with therapeutic potential (Foulston, 2019; Fazal et al., 2020) and foster the production of a variety of natural products in efficient and environmentally friendly procedures (Yamanakaa et al. 2014). Moreover, Wan et al. (2023) pointed out that, exploring of the genetic diversity within Streptomyces spp., facilitates the identification of unexplored biosynthetic gene clusters (BGCs) and assists the prediction of the chemical structures of the compounds they encode. In addition, microbial cloning is a useful genetic tool that has many advantages. For example, Behrouzpour and Amini (2019) stated that the advantages of producing enzymes through microbial cloning include: (i) a quick fermentation process, (ii) cost-effectiveness, (iii) straight forward screening techniques, and (iv) the ability of microbes to grow rapidly. In brief, Streptomyces cloning holds exciting prospects for enhancing the understanding of Streptomyces genetics, improving the isolation and characterization of novel chemicals, and expanding avenues for drug discovery for biotechnological and pharmaceutical applications.

In our recent study (Mahasneh et al., 2021), similarities and differences of the predominant gray and white antibiotic-producing *Streptomyces* isolates based on their RAPD-PCR fingerprints were analyzed. We reported and for the first time in literature, the correlation between RAPD clustering and the antibiotic activity of the dominant white and gray aerial mycelium-bearing *Streptomyces* isolates. The results showed that there was one common band of ~600 bp shared by ~85 % of the isolates. Building on the findings from that research, the current study aims to utilize these common bands as specific markers to identify homologous proteins in the dominant soil *Streptomyces* isolates by cloning, sequencing, and characterizing these markers.

2. Materials and methods

2.1. Streptomyces isolates

Six out of the twelve pure isolates that represent the predominant antibiotic-producing *Streptomyces* soil isolates in Northern Jordan recovered in a previous study (Mahasneh et al., 2021) were tested here.

2.2. Purification of RAPD-PCR DNA bands

In our previous study (Mahasneh et al., 2021) of RAPD-PCR analysis of *Streptomyces*, 12 DNA bands that ranged between 600 and 1350 bp were purified out of the gel of the RAPD reactions using a commercial PCR preps DNA purification kit. Table 1 shows the size of six (No. 54, 55, 20, 56, 57, and 58) out of 12 bands that were selected to be the most common bands among the different isolates for each random primer used.

The most common bands among samples were selected and purified out from the gels using the Wizard PCR preps DNA purification system (Promega, USA) as follows: The DNA bands were excised using a clean, sterile razor blade and transferred to 1.5 ml Eppendorf tubes. A volume of 1 ml of resin was added and incubated at 65 °C in water bath for 5 min. One Wizard Minicolumn (Promega, USA) attached to 3 ml disposable syringe was prepared for each PCR product. The resin/DNA mix was pipetted into the syringe barrel and with syringe plunger gently pushed into the minicolumn. The columns then were washed using 2 ml of 80 % isopropanol and centrifuged for 2 min at 10,000 xg to dry the resin. Fifty µl of nuclease free water were then added to the minicolumns, and then centrifuged for 20 s at 10,000 xg to elute the DNA. The eluted DNA was stored at -20 °C.

2.3. Cloning of the RAPD-PCR DNA fragments

The pGEM-T Easy vector system (Promega, USA) was used for the cloning of the DNA fragments as follows:

2.3.1. Ligation reaction

Reactions of 10 μl were prepared by adding 3 μl of each purified DNA fragment to 5 μl 2X rapid ligation buffer, 1 μl (50 ng) pGEM-T Easy vector, and 1 μl (3 Weiss units) T₄ DNA ligase. The reactions were incubated overnight at 4 °C.

2.3.2. Transformation of the JM109 competent cells

Ligation reactions were centrifuged briefly and 2 μ l of each ligation reaction was added to a sterile 1.5 ml micro-centrifuge tube on ice. Fifty μ l of *E. coli* JM109 High Efficiency Competent Cells (Promega, USA) were transferred carefully into each tube and heat-shocked for 50 s in a water bath at 42 °C and then transferred immediately to ice. After that, 950 μ l of room temperature S.O.C. medium (ThermoFisher Scientific) was added and incubated for 90 min at 37 °C in a shaker incubator at 150 rpm. One hundred μ l of each transformation culture were plated onto LB/Ampicillin plates containing IPTG and X-Gal and incubated overnight at 37 °C in an ordinary incubator (Memmert, Germany).

2.3.3. Plasmid preparation

A single, well-isolated white colony from LB/Ampicillin/IPTG/X-Gal plates was inoculated in 15 ml LB-Ampicillin broth and incubated overnight at 37 °C in a shaker incubator (Jeio Tech, Korea). Five ml of each bacterial culture were centrifuged for 5 min at 10,000 xg and pellets were re-suspended in resuspension solution (Promega, USA) and then cell lysis solution (Promega, USA) was used to lyse the cells. The whole mixtures were centrifuged for 10 min at 14,000 xg. Plasmids were prepared by using a centrifugation protocol involving spin columns (Promega, USA). Briefly, centrifuged cell lysates were washed twice with column wash solution (Promega, USA). Plasmids were eluted using nuclease free water and kept at -20 °C.

2.3.4. Digestion with restriction enzyme

To confirm that each plasmid has the inserted DNA, a digestion reaction was performed using the *Eco*RI restriction enzyme (Promega, USA). Five μ l of plasmid prepared were incubated with 0.5 μ l *Eco*RI, 1 μ l buffer, 0.2 μ l BSA, and nuclease free water to final volume of 10 μ l for 2 h at 37 °C. Gel electrophoresis was then run, and bands visualized under U.V.

A

	GAATTCGATTAGGTG	ACCGTCAAGA	ACGTCAAGA	AGTCGGGCTC	GCCCACGTCC	AACGGCGGCG	ACGCCA Majority
	10	20	30	40	50	60	70
1	GAATTCGATTAGGTG	ACCGTCAAGA	ACGTCAAGAA	AGTCGGGCTC	GCCCACGTCC	AACGGCGGCG	ACGCCA 54F.SEQ
Ţ	GAATTCGATTAGGTG	ACCGTCAAGA	ACGTCAAGAA	AGTCGGGCTC	GCCCACGTCC	AACGGCGGCG	ACGCCA 54RC.SEQ
	TCGGCACGGAGAGCG	ACGTCCGCAP	CGTCTGGGT	GACCACACC	ACCCTCGAGG	CGTCGGGCGG	TGAGTC Majority
	80	90	100	110	120	130	140
71 71	TCGGCACGGAGAGCG	ACGTCCGCAP	CGTCTGGGT	GACCACACC	ACCCTCGAGG	CGTCGGGCGG	TGAGTC 54F.SEQ
1	TCGGCACGGAGAGCG						
	GGAGGGCTACGACGG	CCTCTTCGAC	ATGAAGGACA	ACACCCAGT	ACGTGACCTT	GTCCTACAGC	ACCCTG Majority
	150	160	170	180	190	200	210
14 14	G G A G G G C T A C G A C G G G G A G G G C T A C G A C G G	CCTCTTCGAC	ATGAAGGACA ATGAAGGACA	ACACCCAGT	ACGTGACCTT	GTCCTACAGC	ACCCTG 54F.SEQ
	CGCAACTCCGGCCGC	GGAGGCCTCA	TCGGGTCCAG	CGAGACCGAC	GCTCTCCAACO	GGCTTTGTGA	CCTACC Majority
21	220	230	240	250	260	270	280
21	C G C A A C T C C G G C C G C C G C A A C T C C G G C C G C	GGAGGCCTCA GGAGGCCTCA	TCGGGTCCAG TCGGGTCCAG	CGAGACCGA (CGAGACCGA (GCTCTCCAAC(GCTCTCCAAC(G G C T T T G T G A G C T T T G T G A	CCTACC 54F.SEQ
	ACCACAACCTGTACG 290	•	1	1			GTACAA Majority
28	ACCACAACCTGTACG	300	310	320	330	340	350
28	ACCACAACCTGTACG	AGAACATCGA	CTCCCGTGCG	CCCCTGCTGC	GCGGCGGCA 1	CGCCCACAT	G T A C A A 54F.SEQ G T A C A A 54Rc.SEQ
	CAACTACTACCTGAA	GCTCAACGAG	TCCGGGATCA	ACTCCCGGGG			
	360	370	380	390	400	410	GALAAC Majority
351	C A A C T A C T A C C T G A A				1		
351	CAACTACTACCTGAA	GCTCAACGAG	TCCGGGATCA	ACTCCCGGG	CGGGGCTCGC	GCCAAGGTG	GACAAC 54Rc.SEQ
	AACTACTTCAAGGAC	TCCAAGGACG	TCCTCGGCAC	CTTCTACACO	GACGCGGCGG	GGCTACTGGC	A G G T C G Majority
	430	440	450	460	470	480	490
421	AACTACTTCAAGGAC	TCCAAGGACG	TCCTCGGCAC	CTTCTACACO	GACGCGGCGG	GCTACTGGC.	AGGTCG 54F.SEQ
421	AACTACTTCAAGGAC	TCCAAGGACG	TCCTCGGCAC	CTTCTACACC	GACGCGGCGG	GCTACTGGC	A G G T C G 54Rc.SEQ
	CGGGCAACATCTTCG	ACAACGTGAC	GIGGICCGCC	CCCGGTACCO	ACACCAACCO	CGCCGGGCC	CAACGT Majority
	500	510	520	530	540	550	560
491	CGGGCAACATCTTCG	ACAACGTGAC	GTGGTCCGCC	CCCGGTACCG	ACACCAACCO	CGCCGGGCC	CAACGT 54F.SEQ
491	CGGGCAACATCTTCG	ACAACGTGAC	GTGGTCCGCC	CCCGGTACCG	ACACCAACCO	CGCCGGGCC	CAACGT 54Rc.SEQ
	GCAGTCCACGACCAC	GGTCACCTAA	ТСАСТАСТСА	ATTC			Majority
	570	580	590				majority
561	GCAGTCCACGACCAC			1			
	GCAGTCCACGACCAC						54F.SEQ 54Rc.SEQ
				· · · ·			DAKC. 2FQ

Fig. 2. Pairwise alignment of clone No. 54 (A); clone No. 55 (B); clone No. 20 (C); clone No. 56 (D); clone No. 57 (E); clone No. 58 (F) M13 forward and reverse complement sequences using DNA STAR software.

2.4. Sequencing of the inserted DNA clones and sequence analysis

The sequencing reaction of the 6 DNA fragments inserted in clones No. 54, 55, 20, 56, 57, and 58 cloned into pGEM-T Easy vector system (Promega, USA) was made by using the DNA Bigdye Terminator Sequencing kit (Applied Biosystems, USA). Ten μ l volume sequencing reaction tubes were set up as follows: 2 μ l of the BigDye Terminator RR Mix (Applied Biosystems), 1 μ l template DNA (plasmids), 2 μ l of the pUC/M13 forward or reverse forward primer, and nuclease free water to the final volume of 10 μ l. PCR amplification was carried out in PCR tubes (Trefflab, Switzerland) using iCycler Thermocycler (BioRad, USA) programmed as follows: 25 cycles of 96 °C for 10 s, annealing at 50 °C for 5 s, extension at 60 °C for 4 min. After that, tubes were held at 4 °C for direct use, or stored at -20 °C until usage. The contents of each extension reaction were then pipetted into sterile 1.5 ml micro-centrifuge tubes for the precipitation process. Nuclease free water and absolute ethanol were added to the content of each micro-centrifuge tube to the final concentration of 60 % for the absolute ethanol. The micro-centrifuges were vortexed briefly, left at room temperature for 1 h, and then spun for 20 min at maximum speed in a micro-centrifuge (Hermel, Germany). The supernatants were then aspirated carefully with a separate pipette tip for each sample and discarded. Another precipitation process was then carried out using 250 μ l 70 % ethanol. Samples were then dried in a vacuum concentrator (DNA mini,

-

B	G A A T T C G A T T A G G T G A C C G T T G A G T A C T G C A T G A A G A A C C C G C G C T G G A C C C T C T C T C T C T C	Maiority
	10 20 30 40 50 60)
1 1	GAATT C GATT A G G T G A C C G T T G A G T A C T G C A T G A A G A A C C C G C G C T G G A C C C T C T C T C T C G A A T T C G A T T A G G T G A C C G T T G A G T A C T G C A T G A A C C C G C G C T G G A C C C T C T C T C T C	
	C A G A C G C A C A A G T A T C T G G C A A T T C A C T A A T G G A A A T C T ' T C G C G A G T T C A C C T T C G A A G	
	70 80 90 100 110 12	
61 61	C A G A C G C A C A A G T A T C T G G G A A T T C A G T A A T G G A A A T C T T C G C G A G T T C A C C T T C G A A G C A G A C G C A C A A G T A T C T G G G A A T T C A G T A A T G G A A A T C T T T C G C G A G T T C A C C T T C G A A G	
	C T G C A C A T C G C C T G C C C A A C G T G C C C G A G G G T C A C A A G T G C G C C C G T C T G C A C G G T C A C T	Majority
	130 140 150 160 170 18	0
121 121	C	
	CCTATAAGGTCATTGTCCACGTCGAGGCTCCGGCCCGGGACCGGCTGGGTCATGG	Majority
	190 200 210 220 230 241	0
181 181	C C T A T A A G G T C A T T G T C C A C G T C G A G G C T C C G G G T C G A C C C T G G G A C C G G C T G G G T C A T G G C C T A T A A G G T C A T T G T C C A C G T C G A G G C T C C G G T C G G C C C G G C T C G G G T C A T G G	
	A C T T C G G C G A C C T C A A G A A G G C C T T C A A G C C T C G A A G C A C G G C T C G A C C A C T A C T A C	Majority
	250 260 270 280 290 300	• • •
	A C T T C G G C G A C C T C A A G A A G G C C T T C A A G C C T C G A A G C A C G G C T C G A C C A C T A C T A C T A C C A C T T C A A G C C T C C A A G C A C G G C T C G A C C A C T A C T A C C A G C A C G G C T C G A C C A C T A C T A C C A G C A C G G C T C G A C C A C T A C T A C C	55f.SEQ 55Rc.SEQ
	T C A A C G A C A T C G A G G G C C T G G A G A A C C C G A C T A G C G A G G T C C T C G C T C G C T G G A T C T G G G	
	310 320 330 340 350 36	
301 301	T C A A C G A C A T C G A G G G C C T G G A G A A C C C G A C T A G C G A G G T C C T C G C T C G C T G G A T C T G G G T C A A C G A C A T C G A G G G C C T G G A G A A C C C G A C T A G C G A G G T C C T C G C T C G C T C G A T C T G G G	
	AGCGGCTGCAGCCGACCCTGCCTGCACTCTCGGCCCTCACCGTCCGCGAGACGTGCACGT	Majority
	370 380 390 400 410 420	0
	A G C G G C T G C A G C C G A C C C T G C C T G C A C T C T C G G C C C T C A C C G T C C G C A G A C G T G C A C G T A G C G G C T G C A G C C C A C C C T G C C T G C A C T C T C G G C C C T C A C C G T C C G C G A G A C G T G C A	55f.SEQ 55Rc.SEQ
	C T G G C T G C A C C T A T C G G G G C G A G T G A G C G A T G C A C G A T A T T C A G A A C G A G A C A G A C T C T C	Majority
	430 440 450 460 470 48	0
	C	
421	<u>C T G G C T G C A C C T A T C G G G G C G A G T G A G C G A T G C A C G A T A T T C A G A A C G A G A C A G A C T C T C</u>] 55Rc.SEQ
	<u>G C G G C A T C G A G C T C G A C G A T G T C G G C A T C A G T G G T C T G C G G T A T C C C C T G T A C T T C G A G G</u>	Majority
	490 500 510 520 530 54	0
481	G C G G C A T C G A G C T C G A C G A T G T C G G C A T C A G T G G T C T G C G G T A T C C C C T G T A C T T C G A G G	55f.SEQ
481	G C G G C A T C G A G C T C G A C G A T G T C G G C A T C A G T G G T C T G C G G T A T C C C C T G T A C T T C G A G G	55Rc.SEQ
	A C G G T C A C C T A A T C A C T A G T G A A T T C	Majority
	550 560	
541	ACGGTCACCTAATCACTAGTGAATTC	55f.SEQ
541	A C G G T C A C C T A A T C A C T A G T G A A T T C	55Rc.SEQ

Fig. 2. (continued).

Denmark) for 15 min.

Preparation and denaturation of samples prior to loading on the ABI prism instrument were then carried out as follows: a volume of 100 μ l of Hi-Di TM Formamide was added to the tube containing the lyophilized sequenced samples, mixed and heated for 2 min at 95 °C. The samples were then chilled on ice, vortexed thoroughly, and then spun briefly on a micro-centrifuge. After that, the samples were held on ice until ready to load on the 310 Genetic Analyzer (Applied Biosystems, USA).

The sequence data were analyzed with DNA Star program and homologies and identities to previously characterized genes were determined with a Blast search (https://www.ncbi.nlm.nih.gov/blast).

3. Results

3.1. Cloning of the DNA fragments

Using a commercial DNA cloning kit, the 6 DNA fragments, out of the 12, were purified from the gels and cloned into the indicated plasmids. The blue-white script selection method using LB/ampicillin/x-Gal/IPTG plates was applied to show the positive–negative selection of the *E. coli* JM109 competent cells used in the transformation process. Whit colonies represent the positive colonies (colonies having insert), whereas blue colonies represent the negative colonies (colonies not bearing insert).

C GAATTCGATTGTGATCGCAGGTAAAGATAAAGGTAAAAG	A Majority
10 20 30 4 1 GAATTCGATTGTGATCGCAGGTAAAGATAAAGGTAAAAA 1 GAATTCGATTGTGATCGCAGGTAAAGATAAAGGTAAAAA	
<u>GGAACAGTTTTAGCTGCGTTCCCTAAAGAAAAACCGCGCGTG</u>	
50 60 70 8 41 GGAACAGTTTTAGCTGCGTTCCCTAAAGAAAACCGCGTG	г 0 L C 20F.SEQ
41 <u>GGAACAGTTTTAGCTGCGTTCCCTAAAGAAAACCGCGTG</u>	20Re.SEC
<u>TGGTTGAAGGGATCAACATGGTGAAGAAACACACCAAGC</u> 90 100 110 12	
81 TGGTTGAAGGGATCAACATGGTGAAGAAACACACCAAGC 81 TGGTTGAAGGGATCAACATGGTGAAGAAACACACCAAGC	
AAACCCTAACAATCCTCAGGGCGGTATCGTAACTCAAGAZ	-
130 140 150 16 121 A A A C C C T A A C A A T C C T C A G G G C G G T A T C G T A A C T C A A G A F 121	20F.SEQ
121 AAACCCTAACAATCCTCAGGGCGGTATCGTAACTCAAGAA GCTTCTATCCATGCTTCTAACGTAATGATAGCTGATCCTA	-
170 180 190 20 161 GCTTCTATCCATGCTTCTAACGTAATGATAGCTGATCCTF	-
161 <u>GCTTCTATCCATGCTTCTAACGTAATGATAGCTGATCCT</u>	20Rc.SEQ
AGACTGGAGAACCTACACGTATTGGTTCCAAAGTTTTGG 210 220 230 24	
201 A G A C T G G A G A A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A 201 A G A C T G G A G A A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G T T C C A A A G T T T T G G A G A C C T A C A C G T A T T G G A G A C C T A C A C G T A C G T A C C T A C A C C T A C A C C T A C A C	
AAATGGTAATAAAGTACGTTACGCGAAAAATCTGGCGA	
250 260 270 28 241 A A A T G G T A A T A A A G T A C G T T A C G C G A A A A A T C T G G C G A A 260 270 28 241 A A A T G G T A A T A A G T A C G C T A A C G C G A A A A A A T C T G G C G A A 260 270 28	20F.SEQ
241 AAATGGTAATAAAGTACGTTACGCGAAAAATCTGGCGAA GTTCTTGATAAGTAGTCAGGTGAGAAAGGAGGGCCAAGT	
290 300 310 3:	20
281 G T T C T T G A T A A G T A G T C A G G T G A G A A A G G A G G G C C A A G T 281 G T T C T T G A T A A G T A G T C A G G T G A G A A A G G A G G G C C A A G T A	
AATGGCAGCTAGATTAAATGATTTGTACAGAGACAAAAT 330 340 350 3:	C Majority F 50
321 A A T G G C A G C T A G A T T A A T G A T T T G T A C A G A G A C A A A A T G 321 A A T G G C A G C T A G A T T A A T G A T T T G T A C A G A G A C A A A A T G	20F.SEQ
ACTCCTGCTTTAGTATCTAAGTTCGGATACTCTTCCATT	
370 380 390 40 361 ACTCCTGCTTTAGTATCTAAGTTCGGATACTCTTCCATT	20F.SEQ
361 <u>ACTCCTGCTTTAGTATCTAAGTTCGGATACTCTTCCATT</u>	
410 420 430 44	10
401 T G C A G G T T C C T A A A G T A G A A A A G A T C G T C A T C A A C A T G G 401 T G C A G G T T C C T A A A G T A G A A A A G A T C G T C A T C A A C A T G G G	
TGTTGGTGAAGCTGTAGCAAACGCGAAAGCGTTAGACGG 450 460 470 48	r -
441 T G T T G G T G A A G C T G T A G C A A A C G C G A A A G C G T T A G A C G G G 441 T G T T G G T G A A G C T G T A G C A A A C G C G A A A G C G T T A G A C G G G	20F.SEQ
<u>GCTGTAGCTGACCTAACTGCGATCACAATCACTAGTGAA</u>	Majority
490 500 510 52 481 GCTGTAGCTGACCTAACTGCGATCACAATCACTAGTGAAT	20F.SEQ
481 <u>GCTGTAGCTGACCTAACTGCGATCACAATCACTAGTGAA1</u> TC	20Rc.SEQ Majority
521 T C 521 T C	20F,SEQ 20Rc.SEQ

Fig. 2. (continued).

ATTCGATTAGGT	20	30	40	50	,	ACTACC M
ATTCGATTAGGT				1	60 	70 A C T A C C S
						<u>ACTACC</u> 56
AGCGAACATGGC	ACAGCACCTO	CCGACGCCT	GCACTTTATC	GGCACCACCC	TGGTAATAGU	саттот ма
80	90	100	110	120	130	140
AGCGAACATGGCA	ACAGCACCTO	CCGACGCCT	GCACTTTATC	1		
						56
CGTACGCCATCG	CAGAGGCTCA	TTGGTTCTG	TGCTGGCGT	TGGCCCTGGC	CAGGGTACAG	CTTTGC Ha
150	160	170	180	190	200	210
CGTACGCCATCGC	CAGAGGCTCA	TTGGTTCTG	TGCTGGCGT	TGCCCCTGGC	AGGCTACAG	CTTTGC 56
				TGGCCCTGGN		
GGATTGGCCACTT	'	AAAAGAACCO	CCCCGCCAC	CTTTTCAGCA	CCCGTTCTAC	AGCTTG Ma
220	230	240	250	260	270	280
G G A T T G G C C A C T T G G A T T G G C C A C T T	TTTTCTTNGA	AAA GAACCO	CCCCCGCCAC	CTTT-CAGCA	C C C G T T C T A C C C C G T T C T A C	AGCTTG 56 AGCTTG 56
GGGGGATTTGTG						
290	300	310	320	338	340	TCATGT Ma
GGGGGATTTTGTG						350 TCBTCT 56
GGGGGATITIGTG	ATGTACCGCG	ACATGATCCT	GGGCAAGGT	<u>SCCGTTCTAG</u>	<u>G G T G T G A C C</u>	TCATGT 56
CTTGCGCCTGAAC	CATTAAAAAC	TGTGCATCTG	TGGAGATAA	AGTCCTTTT	STCATTTACG	GIGCTG Ma
360	370	380	390	400	410	420
CTTGCGCCTGAAC	CATTAAAAAC	TGTGCATCTG	TGGAGATAA	AGTCCTTTT	GICATTTACG	GTGCTG 56
CTTGCGCCTGAAC	CATTAAAAAC	TGTGCATCTG	TGGAGATAA	AGTCCTTTT	<u>GTCATTTACG</u>	GTGCTG 56
TCCTGCCAAGGCT	TTTTGAGAGC	GCGGAATCAC	CTGCGATTG	AAAAACAGAG	CTTGCGAGG	AGCGAC Ma
430	440	450	460	470	480	490
T C C T G C C A A G G C T T C C T G C C A A G G C T	T T T T G A G A G C T T T T G A G A G C	GCGGAATCAC	CTGCGATTGA	AAAAACAGAG	CTTGCGAGG	AGCGAC 56
		<u> </u>		CAARAA CAGA	<u>ctrocose</u>	MOLGAC 56
CACGATGCACGAC.	ATACCTAATCT	CCCCTTCCCA	AGUCTGCACO	CAACTGAGCA	GCCAACACCG	CAGCAG Ma
500	510	5 20	530	540	550	560
C A C G A T G C A C G A C C A C G A T G C A C G A G		CCCCTTCCCA	AGCCTGCACC	CAACTGAGCA	GCCAACACCG	CAGCAG 56 CAGCAG 56
GCCAGCGACAAGC	580	590	600	610	CGAGACTGAC	GCCGTAMa
GCCAGCGACAAGC	AGCCTGAGGCO	AAAGAAGCAA	AACCAGTCGA	CCCCCARAGC	CGAGACTCAC	GCCGTA: 56
GCCAGCGACAAGC	AGCCTGAGGCC	AAAGAAGCAA	AACCAGTCGA	GCGCGAAAGC	CGAGACTGAC	GCCGTA 56
CCAGACCGTGTGG	AAGCGCGCCT	TTGAGCGCTT	TCCACACTÓC	CIGAATTAIC	CGAGACCTTC	АСТАТБ Ма
640	650	660	670	680	690	700
CAGACCGTGTGG	AAGCGCGCCT	TIGAGCGCTT	TCCACACTGC	CTGAATTATC	CGAGACCTTC	ACTAT G 56
C A G A C C G T G T G G I	A A GCGCCC	TTGAGCGCTT	TCCACACTGC	CIGAATTATC	CGAGACCTTC	ACTATS 56
	CGACACGCCG	GACGCCGCTG	TACTCGACGC	TGCCTTGCAG	ATGGTCGAAG	TCTGGA Ma
710	720	730	740	750	760	770
A C C A C T T C C C C T T C A C C A C T T C C C C T T C	CCGACACGUCG CCGACACGCCG	GACGCCGCTG GACGCCCCCTG	TACTCGACGC TACTCGACGC	T G C C T T G C A G T G C C T T G C A G	A T G G T C G A A G A T G G T C G A A G	TCTGGA 56
ACCGCCTCAGTGU						
780	790	800	•			
CCGCCTCAGTGCC			810 CAAGCGCTTC	820	830	840
CCGCCTCAGTGCC	GACAAGCAAG	CAGCACTGCT	CAAGCGCTTC	GGCACCCAGG	AAAA-CGCCC	7 G G C G G 56
CCTGGTGACCACO	CAATTGCTCG	CCCCCGXXXX	*******	* * * * * * * * * * * *	* * * * * * * * * * *	X X X X X Ma ⁺
850	860	870	880	890	900	910
CCTGGTGACCACO	CAATTGCTGG	CCCCCG		l		
CCTGGTGACCACC						
****	******	<u> </u>	******	* * * * * * * * * * * * *	* * * * * * * * * * * *	XXXXXX Maj
920	930	940	950	960	970	980

Fig. 2. (continued).

E	TGACCCGTGGT	GAGGCCGGGG	CAGCAGGGG	CTGATTCCAC	CGTTCCAGGT	CCCGCA Majority
10	20	30	40	50	60	
1 G AATTCGATTAG 1 G G AATTCGATTAG	STGACC-GTGGT STGANCCCTGGT	G A G G C C G G G C G A G G C C G G G C	CAGCAGGGG CAGCAGGGG	C T G A T T C C A C C T G A T T C C A C	C G T T C C A G G T C G T T C C A G G T	CCCGCA 57F.SEQ CCCGCA 57Rc.SEQ
GGTCAAGATGCAC	TGACGGGGCTG	ATTTCCACAG	TTCCAGGTC	CAACCGGGCC	GCGGGTCAG	GACGGT Majority
80	90	100	110	120	130	140
69 GGTCAAGATGGACC 71 GGTCAAGATGGACC	T G A C G G G G C T G I T G A C G G G G G C T G I	ATT - CCACAG ATTTCCACAG	TTCCAGGTC TTCCAGGTC	CAACCGGGCC CAACCGGGCC	F G C G G G T C A S F G C G G G T C A G	GACGGT 57F.SEQ GACGGT 57Rc.SEQ
AAAGACTCCACTG	TCCAGGTCCTG	TGGACAGGA	TGGAAAGGA	CGGTGTTGGC	GTGGCTTAG	ATGAGA Majority
150	160	170	180	190	200	210
138 AAAGACTCCACTG 141 <u>AAAGACTCCACTG</u> 1	TCCAGGTCCTG TCCAGGTCCTG	C T G G A C A G G A C T G G A C A G G A	T G G A A A G G A G T G G A A A G G A G	C G G T G T T G G C I C G G T G T T G G C I	G T G G C T T A G G T G G C T T A G	A T G A G A S7F.SEQ A T G A G A S7RC.SEQ
TEGETETTOGETAT	ACCAACGCTTC	TAAGACGACA	GCAGAGCTG	CGGGTGCCG	TGGAACACG	AGTCAG Majority
220	230	240	250	260	270	280
208 T G G C T G T T C G C T A T 211 T G G C T G T T C G C T A T	ACCAACGCTTC ACCAACGCTTC	AAGACGACA AAGACGACA	GCAGAGCTGC GCAGAGCTGC	CGGGTGCCGC CGGGTGCCGC	T G G A A C A C G T G G A A C A C G	AGTCAG 57F.SEQ AGTCAG 57RC.SEQ
CAACGTCTCAAACG	GACGCATCGCA	AAGGATCTA	CGGACGCGG	GAACGGCGG	CAAGTGTGG	CAGTTG Majority
290	300	310	320	330	340	350
278 СААС G Т С Т С А А А С G 281 <u>С А А С G Т С Т С А А А С G</u>	GACGCATCGCAC	AAGGATCTA	CGGACGCGG1	GAACGGCGG	CAAGTGTGG	CAGTTG 57RC.SEQ
CAGCAAGACTTGAA	CGACAGATGCA	TACGACCAA	CCAACGAATI	GATCGGTTGC	AAGGACGTA	TGAACG Majority
360	370	380	390	400	410	420
348 C A G C A A G A C T T G A A 351 <u>C A G C A A G A C T T G A A</u>	CGACAGATEGAR	TACGACCAA	CCAACGAATI	CATCGGTTGG	AAGGACGTA	TGAACG 57RC.SEQ
ACCGGATGAACCCT	ATCGGTGCGAAG	ACAGCAGCT	ATCAGTCAAC	TCAATGTAGG	AGGCTCATA	TTTGGA Majority
430	440	450	460	470	480	490
418 A C C G G A T G A A C G C T 421 <u>A C C G G A T G A A C G C T</u>	ATCGGTGCGAAG ATCGGTGCGAAG	ACAGCAGCT ACAGCAGCT	ATCAGTCAAG ATCAGTCAAG	TCAATGTAGG TCAATGTAGG	A G G C T C A T A A G G C T C A T A	T T T G G A 57F.SEQ T T T G G A 57Rc.SEQ
AGTCGGTGAAGGCG	AGTTCGGAATGT	CCCCTGGCT	TCTCTTCAAA	CAAAGCAGCC	CTTGCAGCAG	GCTAT Majority
500	510	520	530	540	S50	560
488 A G T C G G T G A A G G C G 491 A G T C G G T G A A G G C G	AGTTCGGAATGT	CCCCTGGCT	TCTCTTCAAA	CAAAGCAGCC	CTTGCAGCAG	GCTAT 57RC.SEQ
CGTTTCAGAACAAG	CGAAAAGTGGTC	ATTCAGTGG	CGCTATTTCA	GICGGTGGCG	GCTCTCCTGC	GATGG Majority
558 C G T T C & G & D C A A C	580	590	600	610	620	630
558 C G T T T C A G A A C A A G 561 <u>C G T T T C A G A A C A A C</u>	CGAAAAGTGGTC CGAAAAGTGGTC	ATTCAGTGGG ATTCAGTGGG	CGCTATTTCA CGCTATT T CA	GTCGGTGGCG GTCGGTGGCC	GCTCTCCTGC GCTCTCCTGC	GAIGG 57F.SEQ GAIGG 57RC.SEQ
GIGCAATCGGGGTG	CACTACAAGTIC	AGCAGATAA	SAGGTTCAAT	GACGTGGAGC	TATCCATGAA	ACGTA Majority
628 GTGCAATCGGGGTG	650	660	670	680	690	700
628 G T G C A A T C G G G G T G 631 <u>G T G C A A T C C G G G T G</u>	CACTACAAGTTC CACTACAAGTTC	A G C A G A T A A (A G C A G A T A A (GAGGTTCAAT GAGG <u>TTCAAT</u>	GACGTGGAGC GACGTGGAGC	T A T C C A T G A A T A T <u>C C A T G A A</u>	A C G T A, S7F.SEQ A C G T A, 57Rc.SEQ
ACACCGGAAGGGGC	CGAAAGGCCCCT	TCTTCTTGG	ICGATGCAGT	CTCGTATGAT	GAGCGACATC	ATGCT Majority
710	720	730	740	750	760	770
698 A C A C C G G A A G G G G C 701 A C A C C G G A A G G G G C	C G A A A G G C C C C T C G A A A G G C C C C T	TCTTCTTGG1 TCTTCTTGG1	T C G A T G C A G T T C G A T G C A G T	CTCGTATGAT CTCGTATGAT	G A G C G A C A T C G A G C G A C A T C	A T G C T 57F.SEQ A T G C T 57Rc.SEQ
GIGCCCTAGGCACC 780	GCACAAGCCTCA 790	ACCGACTGTC 800		1		
768 G T G C C C T A G G C A C C 771 G T G C C C T A G G C A C C	GCACAAGCCTCA	ACCUACTUT	810 	B20 CACCATGGAC CACCATGGAC	830 A A G G A T A C G G A G G A T A C G G	840 T C A C C 57F.SEQ T C A C C 57Rc,SEQ
TAATCACTAGTGAA						Majority
838 TAATCACTAGTGAA	1 2 4					
B41 TAATCACTAGTGAA						57F.SEQ 57Rc.SEQ

Fig. 2. (continued).

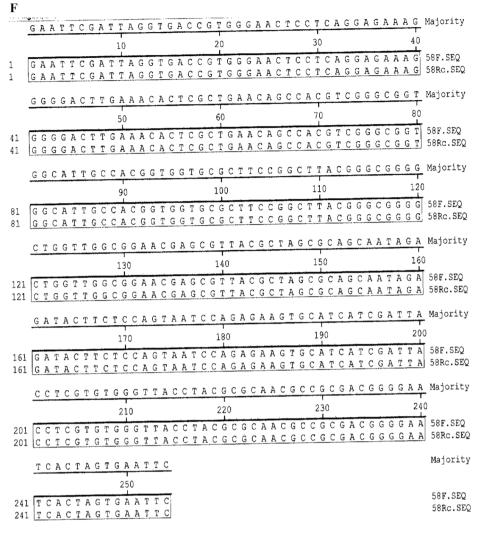


Fig. 2. (continued).

3.2. Digestion with EcoRI restriction enzyme

The success of the cloning process was confirmed by digesting purified plasmids with *Eco*RI restriction enzyme since the plasmid has two *Eco*RI sites flanking the inserted DNA. As shown in Fig. 1, two bands

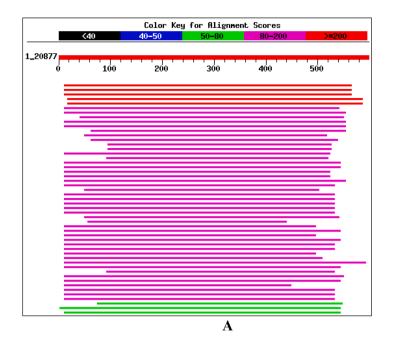
were observed for each tested sample (compared to the uncut plasmid); the first band represents the linear vector plasmid after being digested with *Eco*RI and the second band represents the DNA fragment of interest with expected size of 600, 870, 1000, and 1350 bp. All the six tested clones have verified the presence of the DNA fragment of interest after digestion.

3.3. Sequencing of the DNA clones

The six clones (No. 54, 55, 20, 56, 57, and 58) were sequenced using the DNA BigDye Terminator Sequencing System utilizing the M13 forward or reverse primer found in the pGEM- T Easy vector. Fig. 2A shows the sequence of clone No. 54 with a size of 599 bp. Fig. 2B shows the sequence of clone No. 55 with the size of 566 bp. The size of clone No. 20 is 522 bp as indicated in Fig. 2C. The sequence of clone 56 is 870 bp using the forward primer, the same when using the reverse primer (Fig. 2D). Clone No. 57 (Fig. 2E) shows a sequence of 857 bp. Finally, clone No. 58 with a size of 254 bp is represented in Fig. 2F.

3.4. Identification of homologous proteins

Using the DNA BLAST software, the alignment, and homologous protein results of clones No. 54, 55, 20, 56, 57, and 58 are shown in Fig. 3. A summary of the most homologous protein to the six cloned sequences and their homology percentages is shown in Table 2. The highest score of protein homology was scored for clone No. 54 with 87 %homology to putative secreted pectate lyase [Streptomyces coelicolor A3 (2)], 81 % to pectate lyase [Thermobifida fusca], 68 % to pectate lyase B [Pseudoalteromonas haloplanktis], and 61 % to pectate lyase [Microbulbifer degradans] (Fig. 3A). The other clones showed less homology as follows: clone No. 55 scored 77 % homology to 6-pyruvoyl-tetrahydropterin synthase [Kineococcus radiotolerans SRS30216], 69 % to putative 6pyruvoyl tetrahydrobiopterin synthase [Vibrio parahaemolyticus RIMD 2210633], 68 % to 6-pyruvoyl-tetrahydropterin synthase [Vibrio vulnificus YJ016], and 68 % to 6-pyruvoyl tetrahydrobiopterin synthase, putative [Vibrio cholera] (Fig. 3B); clone No. 20 scored 73 % homology to LSU ribosomal protein L24P [Bacillus cereus ATCC 14579], 71 % homology to ribosomal protein L24 - Bacillus stearothermophilus, 73 % homology to ribosomal protein L24 (BL23) (histone-like protein HPB12) [Bacillus subtilis], and 71 % 50S ribosomal protein L24 [Bacillus halodurans C-125] (Fig. 3C); clone No. 56 scored 77 % homology to predicted membrane protein [Pseudomonas fluorescens PfO-1], 68 % to conserved hypothetical protein [Pseudomonas syringae pv. Tomato str. DC3000], 66 % to predicted membrane protein [Pseudomonas syringae



	Score	Ε
Sequences producing significant alignments:	(bits)	Value
gi 21221271 ref NP 627050.1 putative secreted pectate lyas.	. <u>344</u>	6e-94
gi 48835481 ref ZP 00292481.1 COG3866: Pectate lyase [Ther.	.327	1e-88
gi 9255879 gb AAF86343.1 pectate lyase B [Pseudoalteromona.	.270	1e-71
gi 48860626 ref ZP 00314538.1 COG3866: Pectate lyase [Micr.	.243	3e-63
gi 48860627 ref ZP 00314539.1 COG3866: Pectate lyase [Micr.	.240	1e-62
gi 37222165 gb AAP70368.1 PelA [uncultured bacterium]	165	5e-40
gi 27552303 emb CAD56882.1 pectate lyase [Bacillus licheni.	.153	2e-36
gi 48864071 ref ZP 00317964.1 COG3866: Pectate lyase [Micr.	.151	8e-36
gi 4589751 dbj BAA76884.1 pectate lyase [<i>Bacillus</i> sp.]	139	4e-32
gi 4589753 dbj BAA76885.1 pectate lyase [Bacillus sp.]	135	6e-31
gi 15616381 ref NP 244686.1 high-alkaline pectate lyase [B.	.133	2e-30
gi 31652279 gb AAF86344.2 pectin methylesterase/pectate ly.	.131	9e-30
gi 48859774 ref ZP 00313704.1 COG3866: Pectate lyase [Clos.	.130	2e-29
gi 21232246 ref NP 638163.1 pectate lyase II [Xanthomonas .	.119	4e-26
gi 21109296 gb AAM37831.1 pectate lyase II [Xanthomonas ax.	.119	4e-26
gi 4191260 emb CAA10642.1 pectate lyase [Erwinia chrysanth.	.108	6e-23
gi 129755 sp P04959 PELB ERWCH Pectate lyase B precursor >g.	.105	9e-22
gi 28374120 pdb 108M A Chain A, Pectate Lyase C From Erwini.	.103	2e-21

Fig. 3. Alignment results for clone (A) No. 54, (B) clone No. 55, (C) clone No. 20, (D) clone No. 56, (E) clone No. 57, (F) clone No. 58, forward sequence using DNA BLAST Software.

gi|129757|sp|P11073|PELC ERWCH Pectate lyase C precursor >g..103

gi|7437077|pir||S25262 pectate lyase (EC 4.2.2.2) B precurs..101

pv. syringae B728a], and 66 % to conserved hypothetical protein [*Pseudomonas putida* KT2440] (Fig. 3D); clone No. 57 scored 55 % homology to collagen-like protein B [*Streptococcus pyogenes*] and 37 % to autotransporter adhesin [*Burkholderia cepacia* R18194] (Fig. 3E); clone No. 58 scored 55 % homology to hypothetical protein SAV2797 [*Streptomyces avermitilis* MA-4680], 51 % to 3'-phosphoadenosine 5'-phosphosulfate sulfotransferase (PAPS reductase)/FAD synthetase and related enzymes [*Desulfitobacterium hafniense*], 35 % to hypothetical protein [*Oryza sativa* (japonica cultivar-group)], and 33 % to predicted: like thyroid hormone receptor associated protein 2; protein like TRAP240 [*Gallus gallus*] (Fig. 3F).

4. Discussion

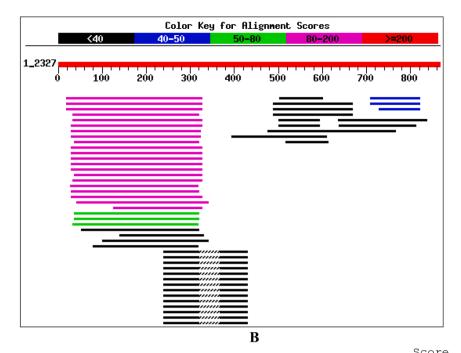
The most common bands of the 6 clones were purified out of the RAPD profile gels and as shown in Table 1, they vary in size. This could be related to the primer being used in that reaction; however, the presence of a shared common band might suggest the existence of certain sequences that are commonly shared among the isolates under investigation (Van Rossum et al., 2020).

2e-21

9e-21

The 6 clones No. 54, 55, 20, 56, 57, and 58 were successfully cloned as confirmed by the *Eco*RI digestion of the purified plasmids. Sequencing of these cloned bands indicated size range between 254 and 870 bp, and the pairwise alignment of the clones showed almost exact match. The homologous proteins to the six cloned sequences indicated percentage of homology ranged between 55 % and 87 % with the lowest for clones No.

F



	Score	E
Sequences producing significant alignments:	(bits)	Value
gi 48729636 ref ZP 00263386.1 COG4323: Predicted membrane .	172	7e-42
gi 28852246 gb AA055320.1 conserved hypothetical protein .	158	2e-37
gi 46187561 ref ZP 00127411.2 COG4323: Predicted membrane .	.154	2e-36
gi 24986242 gb AAN70083.1 conserved hypothetical protein .	.145	1e-33
gi 17546279 ref NP 519681.1 PUTATIVE TRANSMEMBRANE PROTEIN.	136	5e-31
gi 48772282 ref ZP_00276624.1 COG4323: Predicted membrane .	<u>132</u>	1e-29
<u>gi 46320677 ref ZP_00221062.1 </u> COG4323: Predicted membrane .		1e-29
<u>gi 46311127 ref ZP 00211738.1 </u> COG4323: Predicted membrane .		1e-29
<u>gi 48788004 ref ZP_00283983.1 </u> COG4323: Predicted membrane .	<u>130</u>	4e-29
<u>gi 45519206 ref ZP_00170757.1 </u> COG4323: Predicted membrane .	<u>128</u>	1e-28
gi 49079944 gb AAT49952.1 PA3216 [synthetic construct]	<u>121</u>	2e-26
gi 15598412 ref NP 251906.1 hypothetical protein [Pseudomo.	<u>121</u>	2e-26
<u>gi 32038289 ref ZP 00136561.1 </u> COG4323: Predicted membrane .	<u>120</u>	4e-26
gi 24373184 ref NP_717227.1 conserved hypothetical protein.	<u>115</u>	1e-24
gi 28829682 gb AA052198.1 similar to Pseudomonas putida KT.	<u>114</u>	4e-24
gi 16127393 ref NP 421957.1 hypothetical protein [Caulobac.	<u>104</u>	3e-21
gi 37912887 gb AAR05223.1 conserved hypothetical protein .	••• <u>99</u>	1e-19
gi 24196457 gb AAN49847.1 conserved hypothetical protein .	98	2e-19
gi 31195801 ref XP_306848.1 ENSANGP00000000102 [Anopheles .	97	6e-19
<u>gi 50084387 ref YP 045897.1 </u> conserved hypothetical protein	1 <u>87</u>	4e-16

Fig. 3. (continued).

57 and 58, and highest for clone No. 54.

Interestingly, the clone No. 54 exhibited a homology of 87 % with the putative secreted pectate lyase [*Streptomyces coelicolor* A3(2)]. Pectate lyase represents an important member of pectinolytic enzymes that hydrolyze pectins and responsible for the pathogenesis and softening of plant tissues (Hassan et al, 2013). It also has a role in fruit juice clarification and in retting of natural fibers (Amit et al., 2016). Thus, Pectinolytic enzymes derived from natural microorganisms have gained growing interest as potential bio-catalysts suitable for a wide range of applications in industrial processes (Alkorta et al., 1998; Ahlawat et al., 2008). In fact, pectinases have been isolated, purified and characterized from different Streptomyces spp. (Ladjama et al., 1991; Saoudi and Ladjama, 2006; Saoudi et al. 2007; Tonouchi et al., 2010; Yuan et al., 2012).

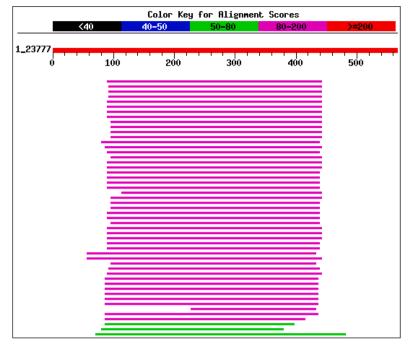
The other homologous protein identified in the same *Streptomyces* spp., *Streptomyces avermitilis* MA-4680, we found another homologous protein located in clone No. 58. However, it's worth noting that this

protein, known as SAV2797, is hypothetical and exhibits a relatively low homology score (55 %). This emphasizes the distinctiveness of clone No. 54 and its vital role in pectate lyase production, as well as its promising potential for future applications. Moreover, the recognition of homologous proteins in species other than *Streptomyces* highlights the uniqueness of clone No. 54 and its promise for future research endeavors.

Identification of homologous proteins arising from DNA cloned fragments deserves deeper molecular investigation through gene expression systems. Additionally, employing Southern blot technique with these cloned DNA fragments as probes holds promise for the precise identification of *Streptomyces* within pure cultures and soil samples.

Using *Streptomyces* bacteria to produce pectinolytic enzymes that are important in the food and beverage industry for fruit juice extraction and in waste management to degrade organic matter (Haile and Ayele, 2022) will certainly increase the interest in eco-friendly industries to eradicate environmentally harmful substances and advance the adoption of cleaner, more environmentally friendly technologies and

Color Key for Alignment Scores		
√40 40−50 50−80 80−200 >=2	200	
1_11437		
0 100 200 300 400	500	
C		
С		
	Score	
${f C}$ quences producing significant alignments:	Score (bits)	
quences producing significant alignments:	(bits)	val
quences producing significant alignments: <u> 29893931 gb AAP07223.1 </u> LSU ribosomal protein L24P[Baci	(bits)	val 9e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24</pre>	(bits) <u>144</u> <u>141</u>	val 9e-3 8e-3
<pre>quences producing significant alignments: <u> 29893931 gb AAP07223.1 LSU</u> ribosomal protein L24P[Baci <u> 132812 sp P04455 RL24 BACST</u> 50S ribosomal protein L24 <u> 16077195 ref NP_388008.1 </u> ribosomal protein L24 (BL23</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$	val 9e-3 8e-3 2e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP_388008.1 ribosomal protein L24 (BL23 15612708 ref NP_241011.1 50S ribosomal protein L24; r.</pre>	(bits) <u>144</u> <u>141</u>) <u>140</u> ib <u>138</u>	val 9e-3 8e-3 2e-3 5e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ 138 138	val 9e-3 8e-3 2e-3 5e-3 7e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{138}{138}$ $\frac{136}{136}$	val 9e-3 8e-3 2e-3 5e-3 7e-3 2e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister]</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{138}{136}$ i $\frac{136}{136}$	val 9e-3 8e-3 2e-3 5e-3 7e-3 2e-3 2e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister]</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{138}{138}$ $\frac{136}{136}$ e $\frac{136}{136}$	val 9e-3 8e-3 2e-3 5e-3 7e-3 2e-3 2e-3 2e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 46113288 ref ZP 00182610.2 COG0198: Ribosomal protein I</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{138}{138}$ $\frac{136}{136}$ e $\frac{136}{136}$ L $\frac{131}{131}$	val 9e-3 8e-3 2e-3 5e-3 7e-3 2e-3 2e-3 6e-3
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 46113288 ref ZP 00182610.2 COG0198: Ribosomal protein L24 [Ocd 23097585 ref NP 691051.1 50S ribosomal protein L24 [Ocd 1505 ribosomal protein L24 [Ocd </pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{138}{138}$ $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{131}{130}$ e. $\frac{130}{130}$	val 9e-3 8e-3 2e-3 5e-3 2e-3 2e-3 2e-3 6e-3 2e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister. 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister. 46113288 ref ZP 00182610.2 COG0198: Ribosomal protein L24 [Ocd 23097585 ref NP 691051.1 50S ribosomal protein L24 [Data 47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci.</pre>	(bits) $\frac{144}{141}$) $\frac{140}{138}$ $\frac{136}{138}$ $\frac{136}{136}$ $\frac{136}{136}$ L $\frac{131}{130}$ e $\frac{130}{130}$ l $\frac{128}{128}$	val 9e-3 8e-3 2e-3 7e-3 2e-3 2e-3 2e-3 2e-2 2e-2 7e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 23097585 ref NP 691051.1 50S ribosomal protein L24 [Oct 47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP 00270283.1 COG0198: Ribosomal protein 124</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i $\frac{136}{136}$ E $\frac{131}{130}$ l $\frac{130}{128}$ L $\frac{127}{127}$	val 9e-3 8e-3 2e-3 5e-3 2e-3 2e-3 2e-3 6e-3 2e-2 7e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 23097585 ref NP 691051.1 50S ribosomal protein L24 [Oct 47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP 00270283.1 COG0198: Ribosomal protein 124 [Baci 15604494 ref NP 221012.1 50S RIBOSOMAL PROTEIN L24 (rp.)</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ e $\frac{136}{136}$ L $\frac{131}{128}$ L $\frac{127}{121}$	val 9e-3 2e-3 5e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 23097585 ref NP 691051.1 50S ribosomal protein L24 [Oct 47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP 00270283.1 COG0198: Ribosomal protein 124</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ e $\frac{136}{136}$ L $\frac{131}{128}$ L $\frac{127}{121}$	val 9e-3 2e-3 5e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2 8e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP 388008.1 ribosomal protein L24 (BL23 15612708 ref NP 241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 16801831 ref NP 472099.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 16804659 ref NP 466144.1 ribosomal protein L24 [Lister 47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister 23097585 ref NP 691051.1 50S ribosomal protein L24 [Oct 47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP 00270283.1 COG0198: Ribosomal protein 124 [Baci 15604494 ref NP 221012.1 50S RIBOSOMAL PROTEIN L24 (rp.)</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i. $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{131}{130}$ l. $\frac{128}{127}$ L. $\frac{127}{121}$ L. $\frac{121}{120}$	val 9e-3 2e-3 5e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2 8e-2 1e-2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP_388008.1 ribosomal protein L24 (BL23 15612708 ref NP_241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 [Lister 16801831 ref NP_472099.1 ribosomal protein L24 [Lister 16804659 ref NP_466144.1 ribosomal protein L24 [Lister 16804659 ref NP_466144.1 ribosomal protein L24 [Lister 46113288 ref ZP_00234757.1 ribosomal protein L24 [Lister 23097585 ref NP_691051.1 50S ribosomal protein L24 [Occ 47570518 ref ZP_00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP_00270283.1 COG0198: Ribosomal protein 1 15604494 ref NP_221012.1 50S RIBOSOMAL PROTEIN L24 (rp. 46202025 ref ZP_00053914.2 COG0198: Ribosomal protein 1 24 (rp.) </pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i. $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{131}{128}$ L. $\frac{127}{121}$ L. $\frac{121}{120}$ m. $\frac{119}{119}$	val 9e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2 8e-2 1e-2 2e-2
<pre>quences producing significant alignments: [29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci [132812 sp P04455 RL24 BACST 50S ribosomal protein L24 [16077195 ref NP 388008.1] ribosomal protein L24 (BL23 [15612708 ref NP 241011.1] 50S ribosomal protein L24; r. [143448 gb AAB59024.1] ribosomal protein L24 [16801831 ref NP 472099.1 ribosomal protein L24 [Lister [16804659 ref NP 466144.1 ribosomal protein L24 [Lister [16804659 ref NP 466144.1 ribosomal protein L24 [Lister] [47097192 ref ZP 00234757.1 ribosomal protein L24 [Lister] [23097585 ref NP 691051.1 50S ribosomal protein L24 [Occ [47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci] [48765733 ref ZP 00270283.1 COG0198: Ribosomal protein] [15604494 ref NP 221012.1 50S RIBOSOMAL PROTEIN L24 (rp. [46202025 ref ZP 00053914.2 COG0198: Ribosomal protein] [34397847 gb AAQ66908.1 ribosomal protein L24 [Porphyron] [240705000]</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{136}{131}$ e. $\frac{130}{130}$ L. $\frac{128}{127}$ L. $\frac{127}{121}$ L. $\frac{121}{121}$ L. $\frac{120}{119}$ a. $\frac{119}{119}$	val 9e-3 2e-3 5e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2 8e-2 1e-2 2e-2 2e-2
<pre>quences producing significant alignments: [29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci [132812 sp P04455 RL24 BACST 50S ribosomal protein L24 [16077195 ref NP 388008.1] ribosomal protein L24 (BL23 [15612708 ref NP 241011.1] 50S ribosomal protein L24; r. [143448 gb AAB59024.1] ribosomal protein L24 [16801831 ref NP 472099.1 ribosomal protein L24 [Lister [16804659 ref NP 466144.1 ribosomal protein L24 [Lister] [4601328 ref ZP 00234757.1 ribosomal protein L24 [Lister] [46113288 ref ZP 00182610.2 COG0198: Ribosomal protein 124 [Occ [47570518 ref ZP 00241145.1 ribosomal protein L24 [Baci] [48765733 ref ZP 00270283.1 COG0198: Ribosomal protein 124 [48765733 ref ZP 00053914.2 COG0198: Ribosomal protein 124 (rp] [46202025 ref ZP 00053914.2 COG0198: Ribosomal protein 124 (rp] [46202025 ref ZP 00053914.2 COG0198: Ribosomal protein 124 [States] [34397847 gb AAQ66908.1 ribosomal protein L24 [Porphyron] [21283887 ref NP 646975.1 50S ribosomal protein L24 [States] [50]</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i. $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{131}{130}$ l. $\frac{128}{127}$ L. $\frac{127}{121}$ L. $\frac{127}{121}$ L. $\frac{129}{120}$ m. $\frac{119}{20}$ c. $\frac{119}{119}$	E val 9e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-2 7e-2 9e-2 2e-2 2e-2 2e-2 2e-2 2e-2 2e-2 2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP_388008.1 ribosomal protein L24 (BL23 15612708 ref NP_241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 [Lister 16801831 ref NP_472099.1 ribosomal protein L24 [Lister 16804659 ref NP_466144.1 ribosomal protein L24 [Lister 16804659 ref NP_466144.1 ribosomal protein L24 [Lister 46113288 ref ZP_00182610.2 COG0198: Ribosomal protein 124 23097585 ref NP_691051.1 50S ribosomal protein L24 [Cod 47570518 ref ZP_00270283.1 COG0198: Ribosomal protein 124 48765733 ref ZP_00270283.1 COG0198: Ribosomal protein 124 46202025 ref ZP_00053914.2 COG0198: Ribosomal protein 13 15604494 ref NP_221012.1 50S RIBOSOMAL PROTEIN L24 (rp. 46202025 ref ZP_00053914.2 COG0198: Ribosomal protein 13 34397847 gb AAQ66908.1 ribosomal protein L24 [Porphyron 21283887 ref NP_646975.1 50S ribosomal protein L24 [States] 23042320 gb AAO80086.1 ribosomal protein L24 [Enterocod</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i. $\frac{136}{136}$ e. $\frac{136}{136}$ L. $\frac{131}{130}$ l. $\frac{128}{127}$ L. $\frac{127}{121}$ L. $\frac{127}{121}$ L. $\frac{129}{120}$ m. $\frac{119}{119}$ c. $\frac{119}{119}$	val 9e-3 2e-3 5e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-2 2e-2 2
<pre>quences producing significant alignments: 29893931 gb AAP07223.1 LSU ribosomal protein L24P[Baci 132812 sp P04455 RL24 BACST 50S ribosomal protein L24 16077195 ref NP_388008.1 ribosomal protein L24 (BL23 15612708 ref NP_241011.1 50S ribosomal protein L24; r. 143448 gb AAB59024.1 ribosomal protein L24 [Lister 16801831 ref NP_472099.1 ribosomal protein L24 [Lister 16804659 ref NP_466144.1 ribosomal protein L24 [Lister 16804659 ref ZP_00234757.1 ribosomal protein L24 [Lister 46113288 ref ZP_00182610.2 COG0198: Ribosomal protein 124 [Occ 47570518 ref ZP_00241145.1 ribosomal protein L24 [Baci 48765733 ref ZP_00270283.1 COG0198: Ribosomal protein 124 [Baci 48765733 ref ZP_00270283.1 COG0198: Ribosomal protein 124 [Coc 47570518 ref ZP_0025914.2 COG0198: Ribosomal protein 124 [Star 15604494 ref NP_221012.1 50S_RIBOSOMAL_PROTEIN_L24 (rp 46202025 ref ZP_00053914.2 COG0198: Ribosomal protein 124 [Star 23937847 gb AAQ66908.1 ribosomal protein_L24 [Porphyron 21283887 ref NP_646975.1 50S_ribosomal_protein_L24 [Star 23942320 gb AAO80086.1 ribosomal_protein_L24 [Enterocod 27316278 gb AAO5453.1 50S_ribosomal_protein_L24 [Star]</pre>	(bits) $\frac{144}{141}$) $\frac{140}{140}$ ib $\frac{138}{138}$ $\frac{136}{136}$ i. $\frac{136}{136}$ i. $\frac{136}{136}$ i. $\frac{136}{131}$ e. $\frac{130}{131}$ l. $\frac{128}{127}$ l. $\frac{127}{121}$ L. $\frac{127}{121}$ L. $\frac{129}{19}$ a. $\frac{119}{119}$ c. $\frac{119}{119}$ L. $\frac{118}{118}$	val 9e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2e-3 2



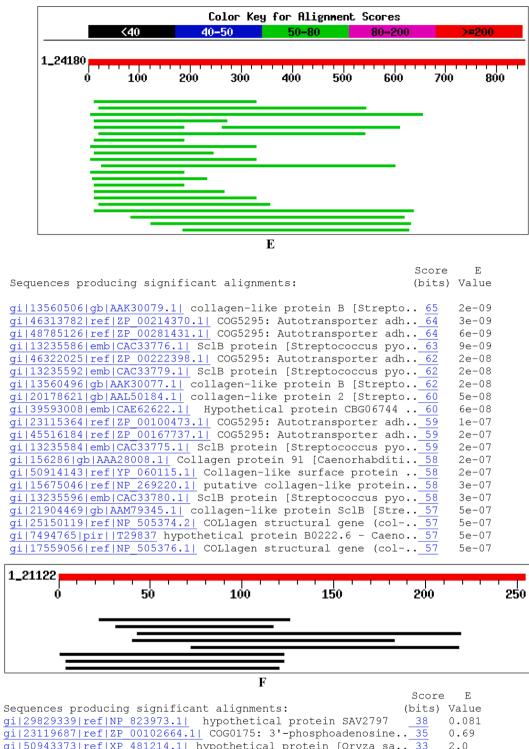
D

Score E (bits) Value

Sequences producing significant alignments:

gi 46366239 ref ZP_00228616.1 COG0720: 6-pyruvoyl-tetrahyd198	7e-50
gi 28898657 ref NP 798262.1 putative 6-pyruvoyl tetrahydro188	7e-47
gi 37680272 ref NP 934881.1 6-pyruvoyl-tetrahydropterin sy187	1e-46
gi 9655787 gb AAF94458.1 6-pyruvoyl tetrahydrobiopterin sy186	2e-46
gi 48729945 ref ZP_00263694.1 COG0720: 6-pyruvoyl-tetrahyd186	3e-46
gi 23105387 ref ZP 00091843.1 COG0720: 6-pyruvoyl-tetrahyd186	3e-46
gi 32037704 ref ZP 00135976.1 COG0720: 6-pyruvoyl-tetrahyd186	3e-46
gi 46187340 ref ZP 00205296.1 COG0720: 6-pyruvoyl-tetrahyd185	4e-46
gi 16761721 ref NP 457338.1 putative 6-pyruvoyl tetrahydro185	6e-46
gi 15832874 ref NP 311647.1 putative 6-pyruvoyl tetrahydro183	2e-45
gi 30042370 gb AAP18095.1 putative 6-pyruvoyl tetrahydrobi183	2e-45
gi 24053178 gb AAN44270.1 putative 6-pyruvoyl tetrahydrobi183	2e-45
gi 15836798 ref NP 297486.1 6-pyruvoyl tetrahydrobiopterin182	4e-45
gi 21230064 ref NP 635981.1 6-pyruvoyl tetrahydrobiopterin182	5e-45
gi 26109570 gb AAN81773.1 Putative 6-pyruvoyl tetrahydrobi182	5e-45
gi 50122474 ref YP 051641.1 putative 6-pyruvoyl tetrahydro182	5e-45
gi 24983893 gb AAN67954.1 6-pyruvoyl tetrahydrobiopterin s181	6e-45
gi 28853842 gb AAO56908.1 6-pyruvoyl tetrahydrobiopterin s181	1e-44
gi 21109994 gb AAM38457.1 6-pyruvoyl tetrahydrobiopterin s180	1e-44
gi 22997319 ref ZP 00041552.1 COG0720: 6-pyruvoyl-tetrahyd179	2e-44

Fig. 3. (continued).



 gi | 50943373 | ref | XP 481214.1 | hypothetical protein [Oryza sa.. 33
 2.0

 gi | 50756777 | ref | XP 415317.1 | PREDICTED: similar to thyroid .. 32
 3.4

 gi | 21219580 | ref | NP 625359.1 | putative sugar transport sugar.. 31
 7.6

 gi | 23129915 | ref | ZP 00111736.1 | COG3409: Putative peptidogly.. 31
 10.0

 gi | 32415868 | ref | XP 328412.1 | predicted protein [Neurospora .. 31
 10.0

Fig. 3. (continued).

Table 2

Summary of the most homologous proteins to the six cloned sequences and their homology percentages.

Clone No.	Homologous Protein	Homology (%)
54	Putative secreted pectate lyase <i>Streptomyces coelicolor</i> A3	87
55	COG0720: 6-pyruvoyl-tetrahydropterin synthase Kineococcus radiotolerans SRS30216	77
20	50S ribosomal protein L24 Bacillus stearothermophilus	73
56	Predicted membrane protein Pseudomonas fluorescens PfO-1	77
57	Collagen-like protein B Streptococcus pyogenes	55
58	Hypothetical protein SAV2797 Streptomyces avermitilis MA-4680	55

procedures.

5. Conclusion

The association of homologous proteins (pectate lyase) to RAPD analysis of the dominant white and gray aerial mycelium-bearing *Streptomyces* isolates is reported for the first time. This breakthrough underscores the potential for advancing our understanding of *Streptomyces* diversity and ecology.

Ethical disclosures

The authors confirm that this research adheres to the ethical standards relevant to the field of study, with no animal experimentation or patient data collection, demonstrating their commitment to maintaining research integrity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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