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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 % homology for the clone No. 20, and 55 % homology for the clones No. 57 and 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., 2022) and understand microbial diversity (Shepherdson 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

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.

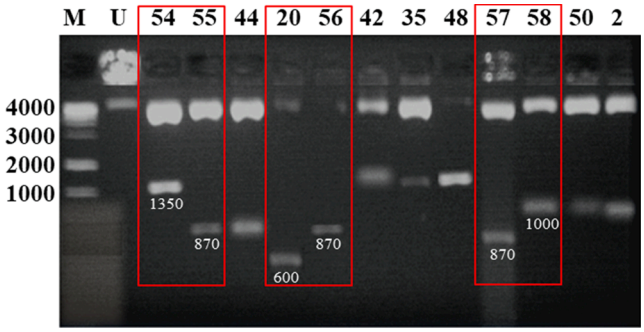


Fig. 1. 2 % agarose gel electrophoresis of purified plasmids digested with *Eco*RI restriction enzyme. Lane M: 1000 bp marker, lane U: uncut plasmid, No. from 54 to 2 represent the purified plasmids digested with *Eco*RI.

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 mini-columns, 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) T_4 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 µl of each ligation reaction was added to a sterile 1.5 ml micro-centrifuge tube on ice. Fifty µl 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 µl 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 µl 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 µl of plasmid prepared were incubated with 0.5 µl *Eco*RI, 1 µl buffer, 0.2 µl BSA, and nuclease free water to final volume of 10 µl for 2 h at 37 °C. Gel electrophoresis was then run, and bands visualized under U.V.

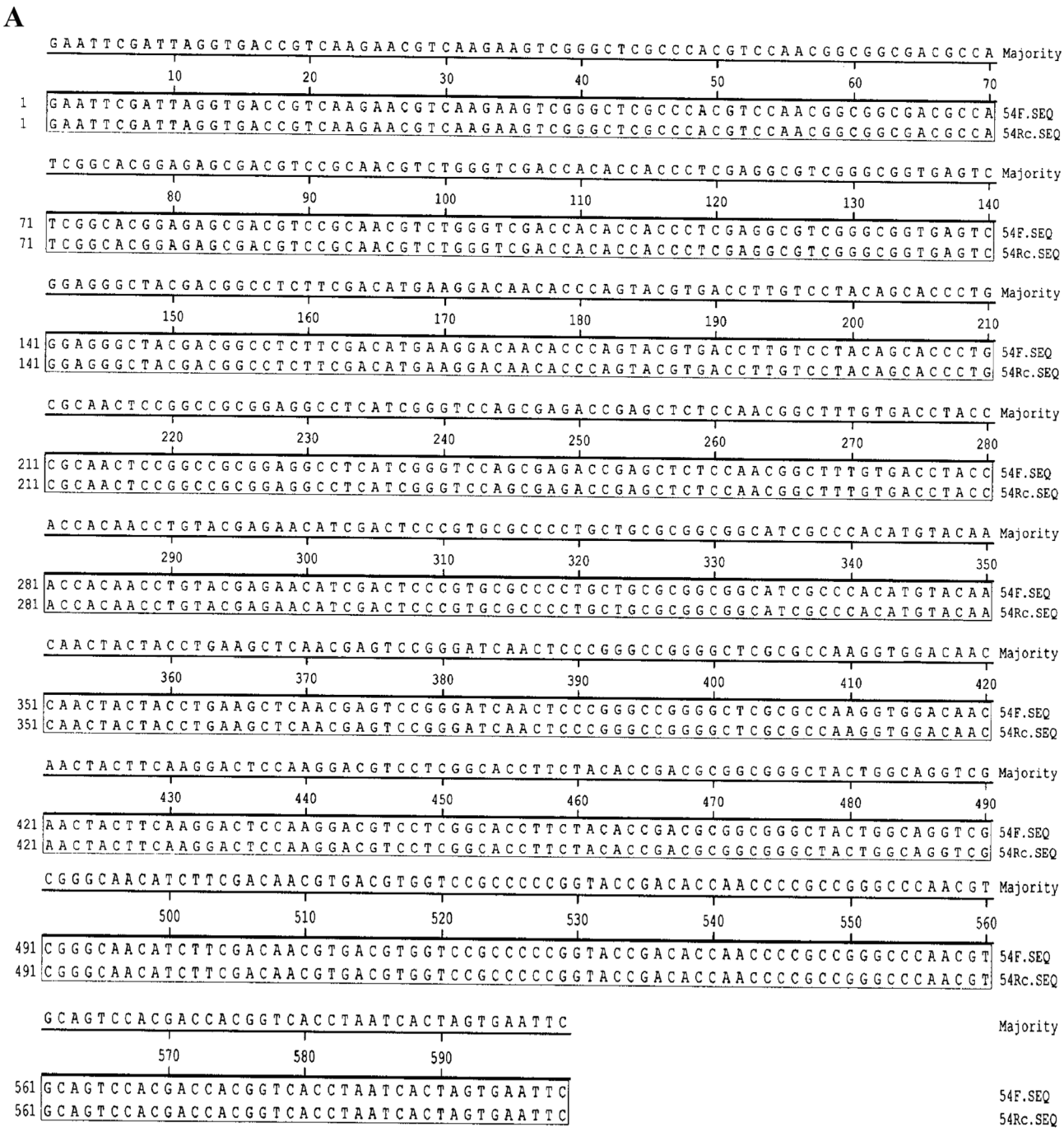


Fig. 2. Pairwise alignment of clone No. 54 (A); clone No. 55 (B); clone No. 20 (C); clone No. 56 (D); clone No. 57 (E); clone No. 58 (F) M13 forward and reverse complement sequences using DNA STAR software.

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 µl volume sequencing reaction tubes were set up as follows: 2 µl of the BigDye Terminator RR Mix (Applied Biosystems), 1 µl template DNA (plasmids), 2 µl of the pUC/M13 forward or reverse forward primer, and nuclease free water to the final volume of 10 µl. 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 µl 70 % ethanol. Samples were then dried in a vacuum concentrator (DNA mini,

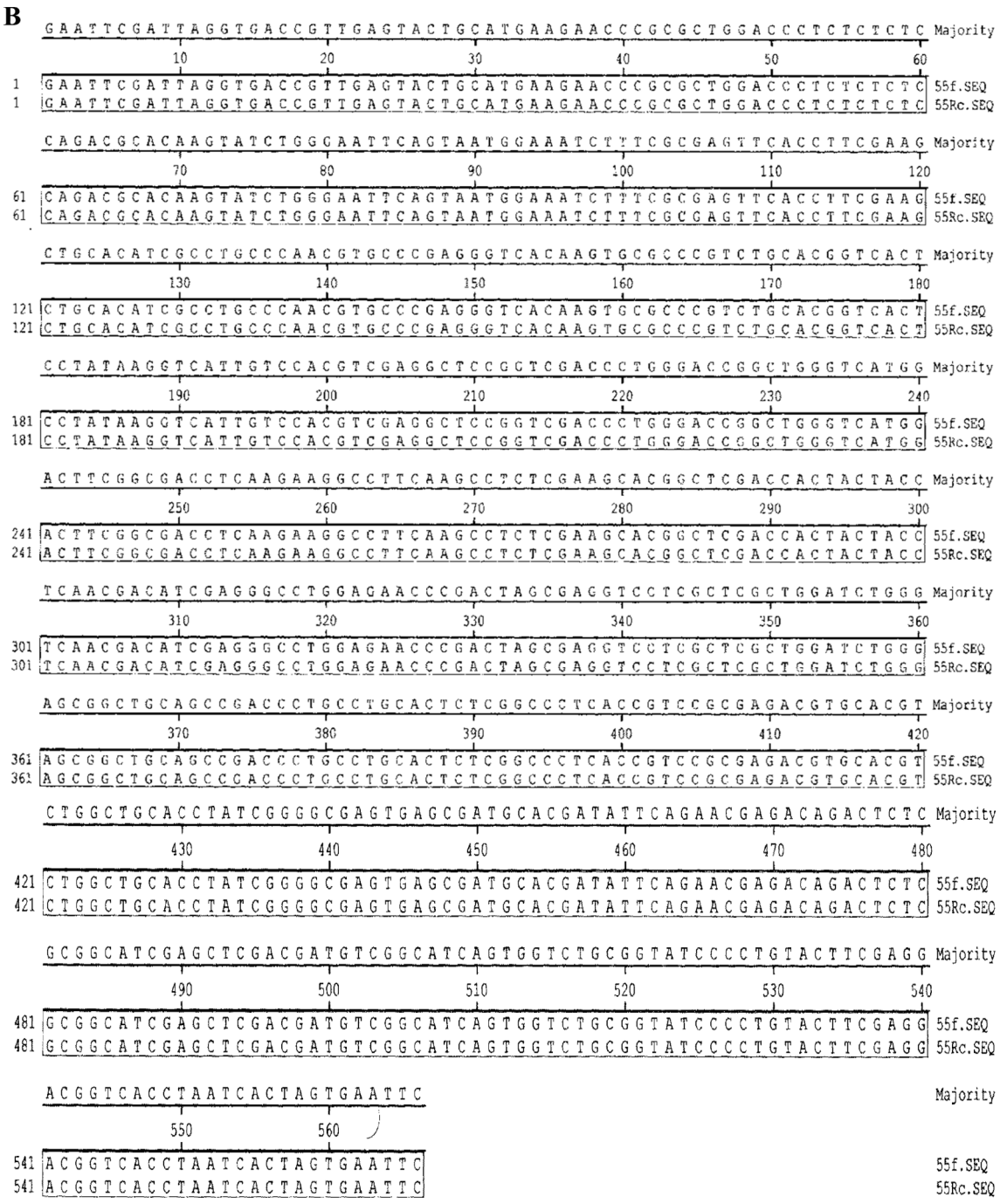


Fig. 2. (*continued*).

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 µl of Hi-Di ™ 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).

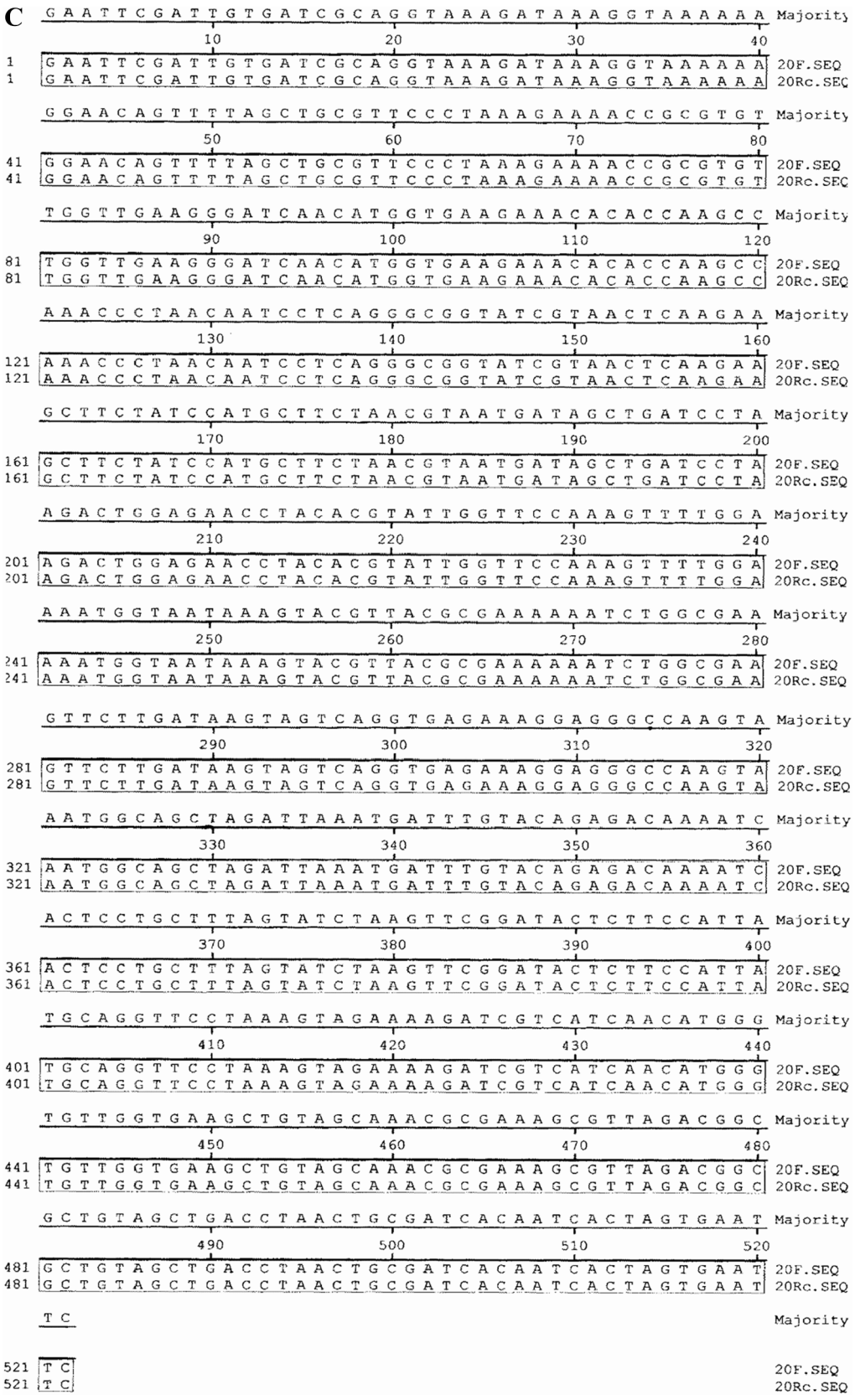
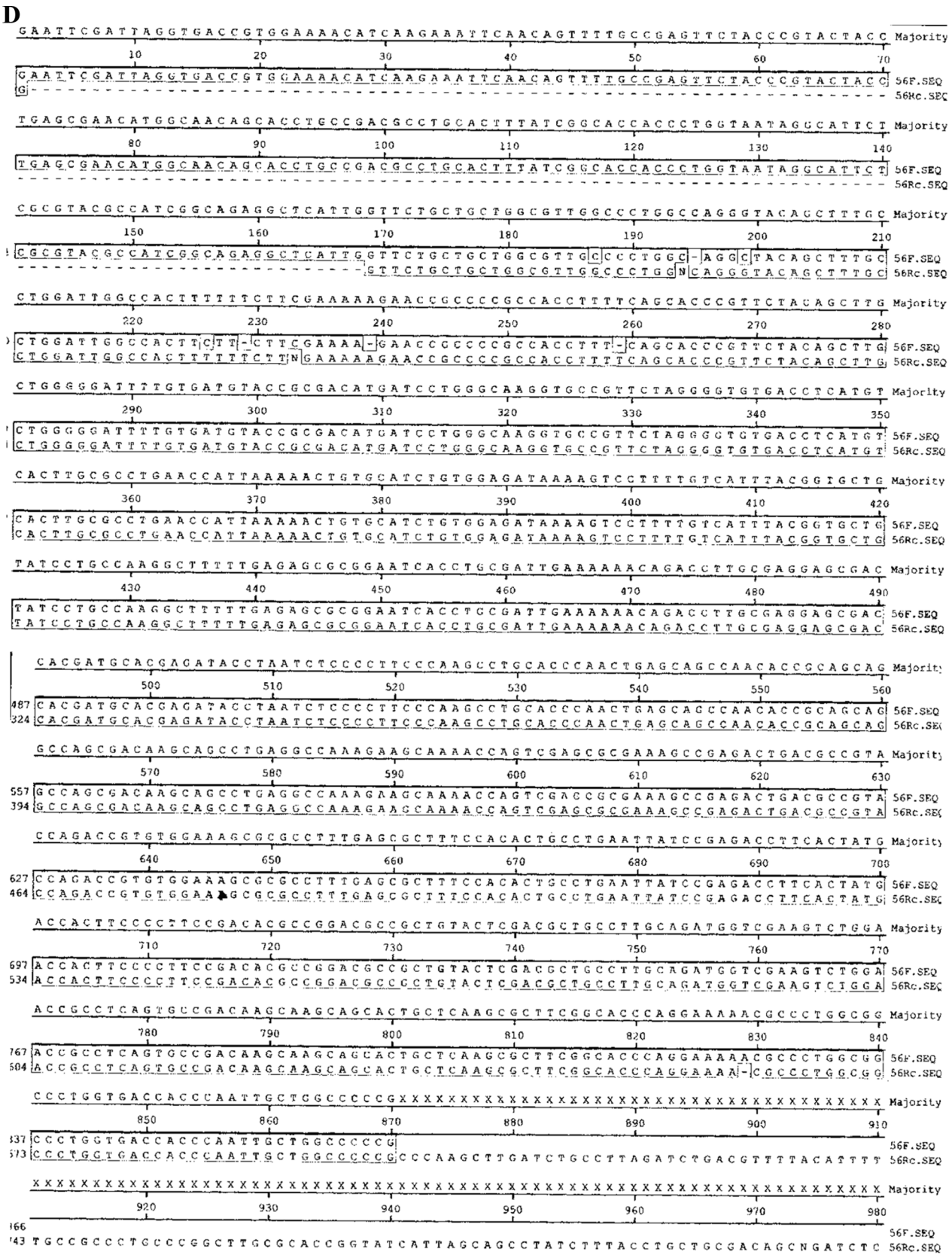


Fig. 2. (*continued*).



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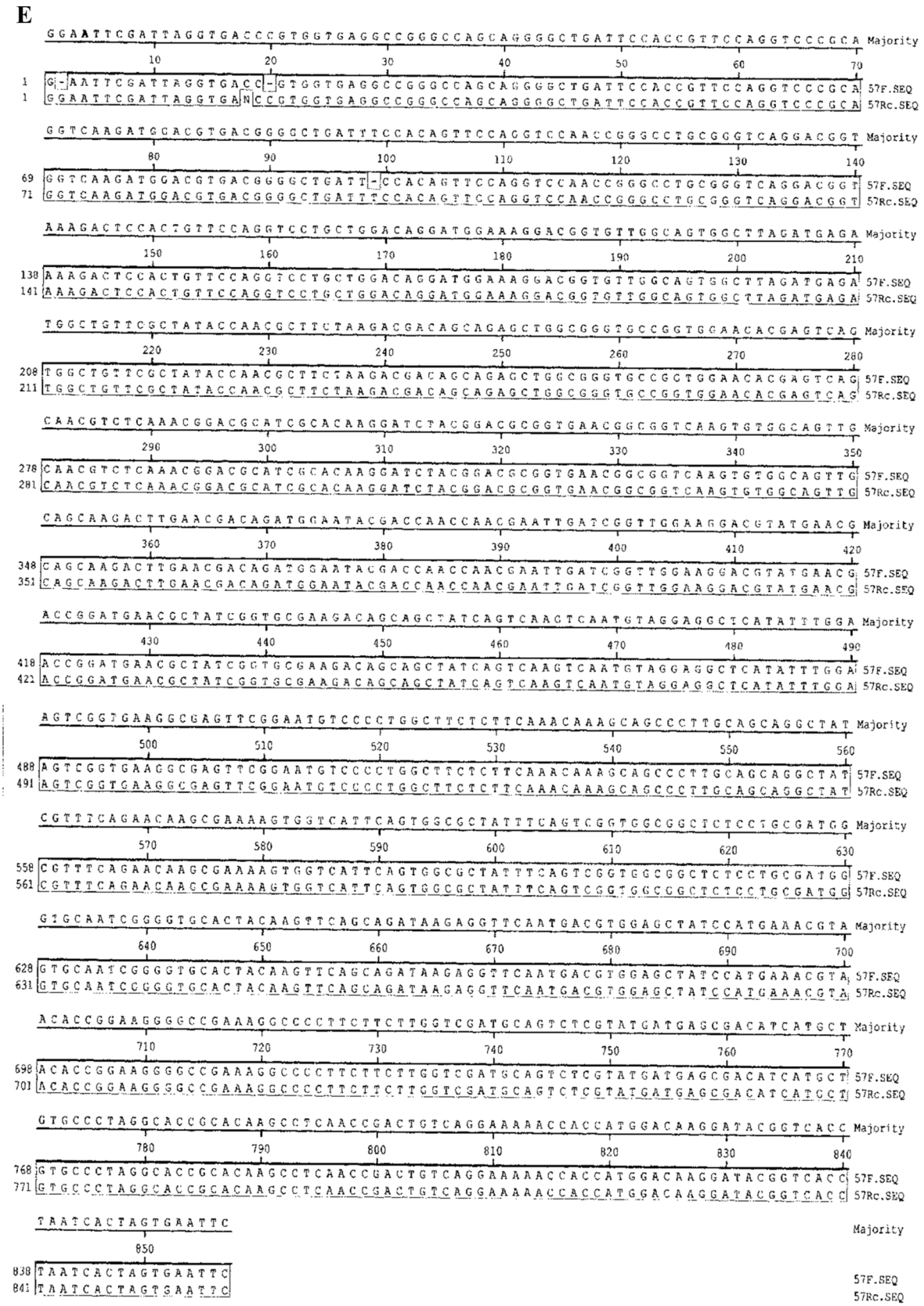


Fig. 2. (*continued*).

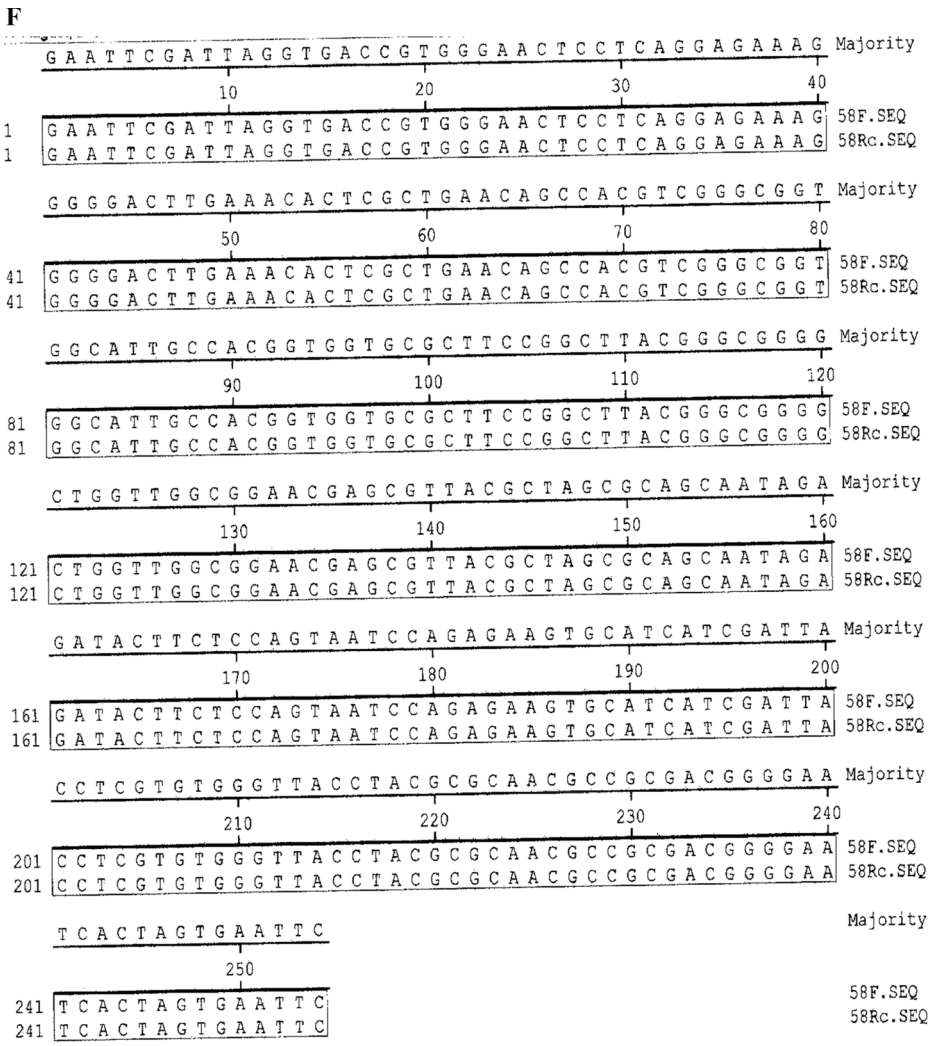


Fig. 2. (*continued*).

3.2. Digestion with EcoRI restriction enzyme

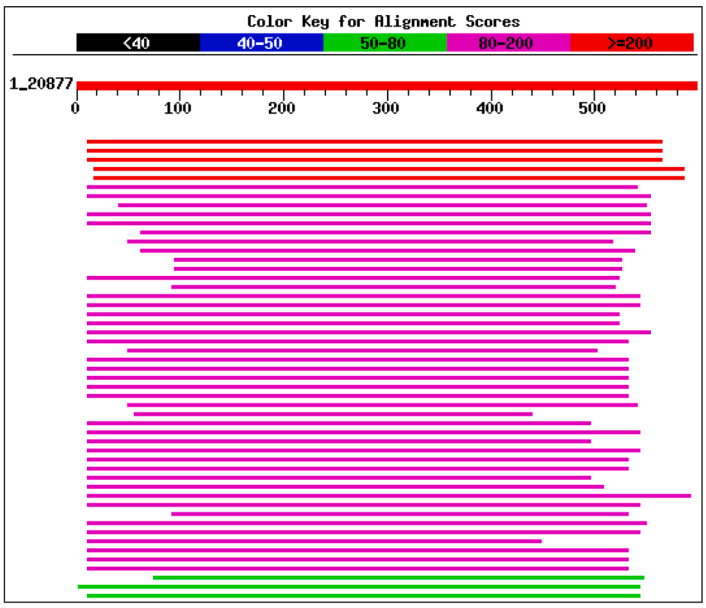
The success of the cloning process was confirmed by digesting purified plasmids with *Eco*RI restriction enzyme since the plasmid has two *Eco*RI sites flanking the inserted DNA. As shown in Fig. 1, two bands were observed for each tested sample (compared to the uncut plasmid); the first band represents 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 6-pyruvoyl 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*



A

Sequences producing significant alignments:	Score (bits)	E Value
gi|21221271|ref|NP_627050.1| putative secreted pectate lyas..	344	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 [*Bacillus* 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|1O8M|A Chain A, Pectate Lyase C From Erwini..	103	2e-21
gi|129757|sp|P11073|PELC_ERWCH Pectate lyase C precursor >g..	103	2e-21
gi|7437077|pir||S25262 pectate lyase (EC 4.2.2.2) B precurs..	101	9e-21

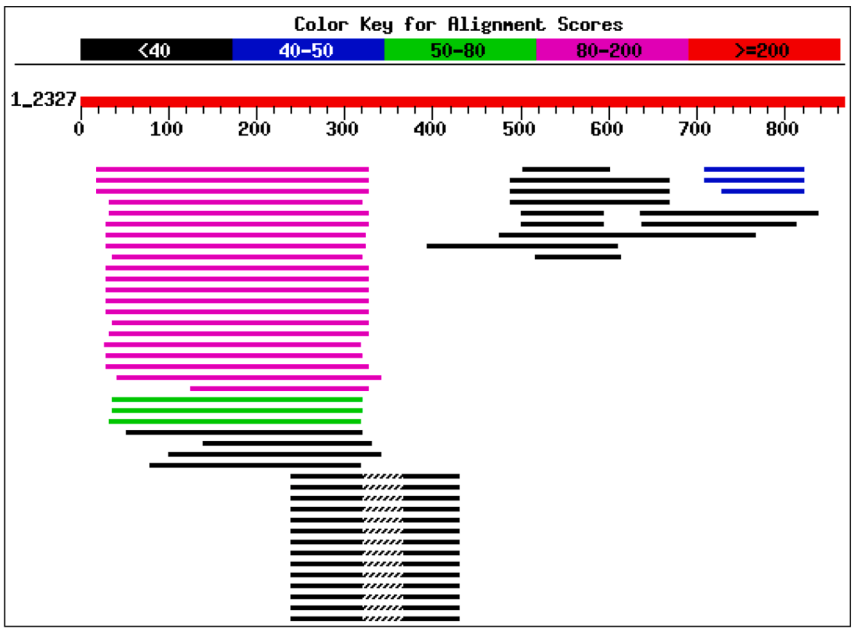
Fig. 3. Alignment results for clone (A) No. 54, (B) clone No. 55, (C) clone No. 20, (D) clone No. 56, (E) clone No. 57, (F) clone No. 58, forward sequence using DNA BLAST Software.

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).

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.



B

Sequences producing significant alignments:	Score (bits)	E Value
gi|48729636|ref|ZP_00263386.1| COG4323: Predicted membrane ..	172	7e-42
gi|28852246|gb|AAO55320.1| 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 ..	132	1e-29
gi|46320677|ref|ZP_00221062.1| COG4323: Predicted membrane ..	132	1e-29
gi|46311127|ref|ZP_00211738.1| COG4323: Predicted membrane ..	132	1e-29
gi|48788004|ref|ZP_00283983.1| COG4323: Predicted membrane ..	130	4e-29
gi|45519206|ref|ZP_00170757.1| COG4323: Predicted membrane ..	128	1e-28
gi|49079944|gb|AAT49952.1| PA3216 [synthetic construct]	121	2e-26
gi|15598412|ref|NP_251906.1| hypothetical protein [Pseudomo..	121	2e-26
gi|32038289|ref|ZP_00136561.1| COG4323: Predicted membrane ..	120	4e-26
gi|24373184|ref|NP_717227.1| conserved hypothetical protein..	115	1e-24
gi|28829682|gb|AAO52198.1| similar to Pseudomonas putida KT..	114	4e-24
gi|16127393|ref|NP_421957.1| hypothetical protein [Caulobac..	104	3e-21
gi|37912887|gb|AAR05223.1| conserved hypothetical protein ..	99	1e-19
gi|24196457|gb|AAN49847.1| conserved hypothetical protein ..	98	2e-19
gi|31195801|ref|XP_306848.1| ENSANGP00000000102 [Anopheles ..	97	6e-19
gi|50084387|ref|YP_045897.1| conserved hypothetical protein..	87	4e-16

Fig. 3. (*continued*).

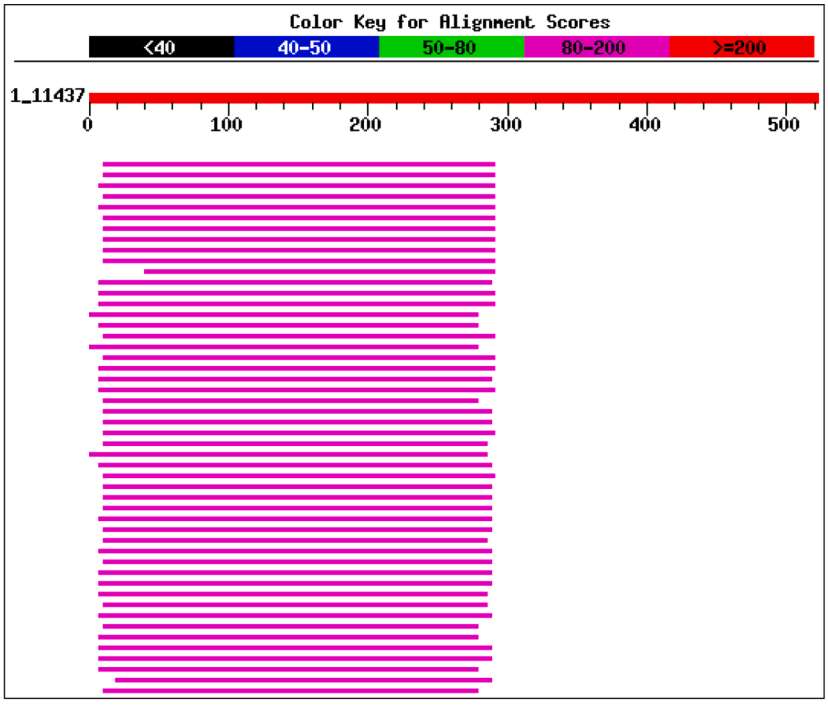
57 and 58, and highest for clone No. 54.

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.

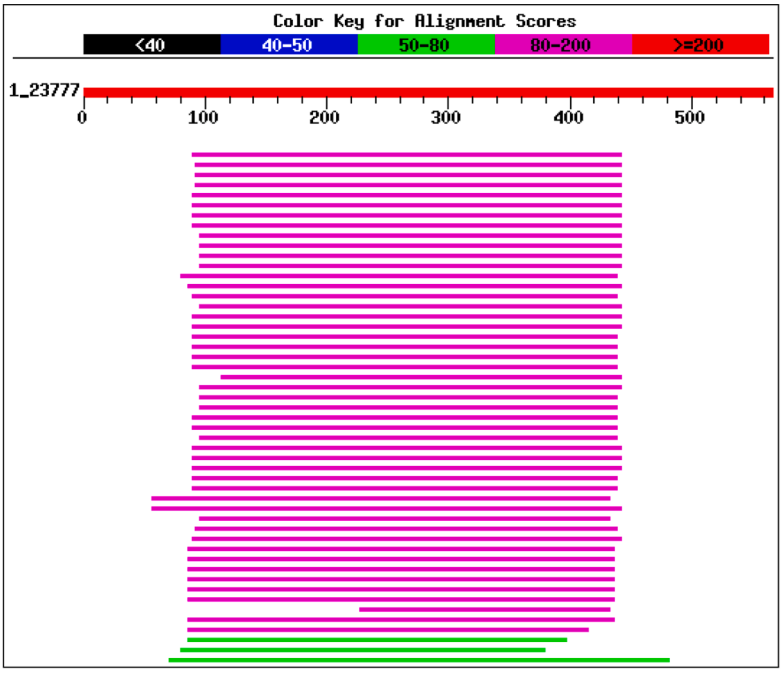
Using *Streptomyces* bacteria to produce pectinolytic enzymes that are important in the food and beverage industry for fruit juice extraction and in waste management to degrade organic matter (Haile and Ayele, 2022) will certainly increase the interest in eco-friendly industries to eradicate environmentally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



C

Sequences producing significant alignments:	Score (bits)	E value
gi|29893931|gb|AAP07223.1|LSU ribosomal protein L24P[Baci..	144	9e-34
gi|132812|sp|P04455|RL24_BACST 50S ribosomal protein L24	141	8e-33
gi|16077195|ref|NP_388008.1| ribosomal protein L24 (BL23)	140	2e-32
gi|15612708|ref|NP_241011.1| 50S ribosomal protein L24; rib	138	5e-32
gi|143448|gb|AAB59024.1| ribosomal protein L24	138	7e-32
gi|16801831|ref|NP_472099.1|ribosomal protein L24 [Lister..	136	2e-31
gi|16804659|ref|NP_466144.1|ribosomal protein L24 [Listeri..	136	2e-31
gi|47097192|ref|ZP_00234757.1|ribosomal protein L24 [Liste..	136	2e-31
gi|46113288|ref|ZP_00182610.2|COG0198: Ribosomal protein L..	131	6e-30
gi|23097585|ref|NP_691051.1|50S ribosomal protein L24 [Oce..	130	2e-29
gi|47570518|ref|ZP_00241145.1|ribosomal protein L24 [Bacil..	128	7e-29
gi|48765733|ref|ZP_00270283.1|COG0198: Ribosomal protein L..	127	9e-29
gi|15604494|ref|NP_221012.1|50S RIBOSOMAL PROTEIN L24 (rpl..	121	8e-27
gi|46202025|ref|ZP_00053914.2|COG0198: Ribosomal protein L..	120	1e-26
gi|34397847|gb|AAQ66908.1|ribosomal protein L24 [Porphyrom..	119	2e-26
gi|21283887|ref|NP_646975.1|50S ribosomal protein L24 [Sta..	119	2e-26
gi|29342320|gb|AAO80086.1|ribosomal protein L24 [Enterococ..	119	2e-26
gi|27316278|gb|AAO05453.1|50S ribosomal protein L24 [Staph..	119	3e-26
gi|48871245|ref|ZP_00323961.1|COG0198: Ribosomal protein L..	118	5e-26
gi|15892918|ref|NP_360632.1|50S ribosomal protein L24 [Ric..	114	8e-25

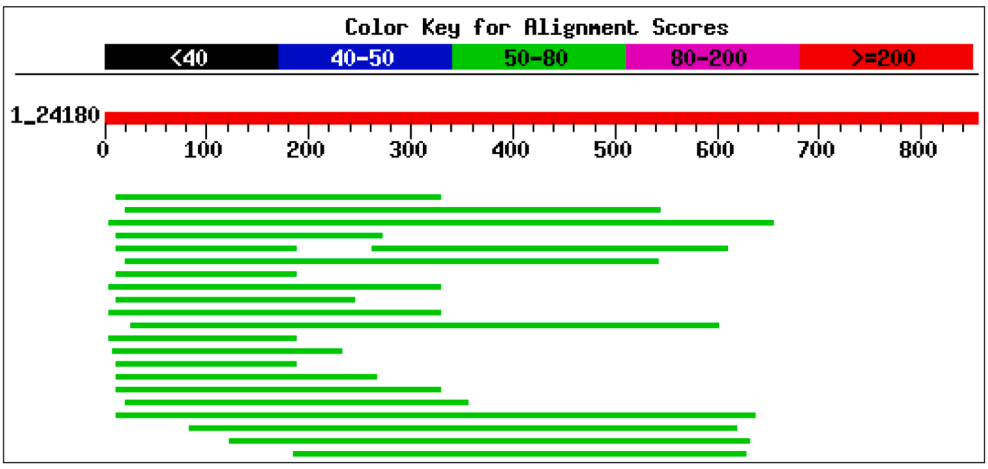
Fig. 3. (*continued*).



D

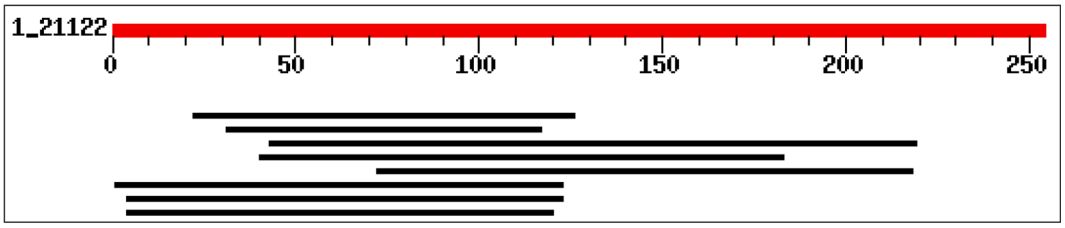
Sequences producing significant alignments:	Score (bits)	E Value
gi|46366239|ref|ZP_00228616.1| COG0720: 6-pyruvoyl-tetrahyd..	198	7e-50
gi|28898657|ref|NP_798262.1| putative 6-pyruvoyl tetrahydro..	188	7e-47
gi|37680272|ref|NP_934881.1| 6-pyruvoyl-tetrahydropterin sy..	187	1e-46
gi|9655787|gb|AAF94458.1| 6-pyruvoyl tetrahydrobiopterin sy..	186	2e-46
gi|48729945|ref|ZP_00263694.1| COG0720: 6-pyruvoyl-tetrahyd..	186	3e-46
gi|23105387|ref|ZP_00091843.1| COG0720: 6-pyruvoyl-tetrahyd..	186	3e-46
gi|32037704|ref|ZP_00135976.1| COG0720: 6-pyruvoyl-tetrahyd..	186	3e-46
gi|46187340|ref|ZP_00205296.1| COG0720: 6-pyruvoyl-tetrahyd..	185	4e-46
gi|16761721|ref|NP_457338.1| putative 6-pyruvoyl tetrahydro..	185	6e-46
gi|15832874|ref|NP_311647.1| putative 6-pyruvoyl tetrahydro..	183	2e-45
gi|30042370|gb|AAP18095.1| putative 6-pyruvoyl tetrahydrobi..	183	2e-45
gi|24053178|gb|AAN44270.1| putative 6-pyruvoyl tetrahydrobi..	183	2e-45
gi|15836798|ref|NP_297486.1| 6-pyruvoyl tetrahydrobiopterin..	182	4e-45
gi|21230064|ref|NP_635981.1| 6-pyruvoyl tetrahydrobiopterin..	182	5e-45
gi|26109570|gb|AAN81773.1| Putative 6-pyruvoyl tetrahydrobi..	182	5e-45
gi|50122474|ref|YP_051641.1| putative 6-pyruvoyl tetrahydro..	182	5e-45
gi|24983893|gb|AAN67954.1| 6-pyruvoyl tetrahydrobiopterin s..	181	6e-45
gi|28853842|gb|AAO56908.1| 6-pyruvoyl tetrahydrobiopterin s..	181	1e-44
gi|21109994|gb|AAM38457.1| 6-pyruvoyl tetrahydrobiopterin s..	180	1e-44
gi|22997319|ref|ZP_00041552.1| COG0720: 6-pyruvoyl-tetrahyd..	179	2e-44

Fig. 3. (*continued*).



E

Sequences producing significant alignments:	Score (bits)	E Value
gi|13560506|gb|AAK30079.1| collagen-like protein B [Strepto..	65	2e-09
gi|46313782|ref|ZP_00214370.1| COG5295: Autotransporter adh..	64	3e-09
gi|48785126|ref|ZP_00281431.1| COG5295: Autotransporter adh..	64	6e-09
gi|13235586|emb|CAC33776.1| SclB protein [Streptococcus pyo..	63	9e-09
gi|46322025|ref|ZP_00222398.1| COG5295: Autotransporter adh..	62	2e-08
gi|13235592|emb|CAC33779.1| SclB protein [Streptococcus pyo..	62	2e-08
gi|13560496|gb|AAK30077.1| collagen-like protein B [Strepto..	62	2e-08
gi|20178621|gb|AAL50184.1| collagen-like protein 2 [Strepto..	60	5e-08
gi|39593008|emb|CAE62622.1| Hypothetical protein CBG06744 ..	60	6e-08
gi|23115364|ref|ZP_00100473.1| COG5295: Autotransporter adh..	59	1e-07
gi|45516184|ref|ZP_00167737.1| COG5295: Autotransporter adh..	59	2e-07
gi|13235584|emb|CAC33775.1| SclB protein [Streptococcus pyo..	59	2e-07
gi|156286|gb|AAA28008.1| Collagen protein 91 [Caenorhabditi..	58	2e-07
gi|50914143|ref|YP_060115.1| Collagen-like surface protein ..	58	2e-07
gi|15675046|ref|NP_269220.1| putative collagen-like protein..	58	3e-07
gi|13235596|emb|CAC33780.1| SclB protein [Streptococcus pyo..	58	3e-07
gi|21904469|gb|AAM79345.1| collagen-like protein SclB [Stre..	57	5e-07
gi|25150119|ref|NP_505374.2| COLlagen structural gene (col-..	57	5e-07
gi|7494765|pir||T29837 hypothetical protein B0222.6 - Caeno..	57	5e-07
gi|17559056|ref|NP_505376.1| COLlagen structural gene (col-..	57	5e-07



F

Sequences producing significant alignments:	Score (bits)	E Value
gi|29829339|ref|NP_823973.1| hypothetical protein SAV2797	38	0.081
gi|23119687|ref|ZP_00102664.1| COG0175: 3'-phosphoadenosine..	35	0.69
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|50755581|ref|XP_429437.1| PREDICTED: hypothetical protei..	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 *Streptomyces coelicolor* 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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