PERIODICUM BIOLOGORUM
VOL. 124, No 1–2, 21–27, 2022
DOI: 10.18054/pb.v124i1-2.21463

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original Research Article

Development of strain-specific SCAR primers to beneficial rhizobacteria *Bacillus subtilis* OSU-142

SECKIN EROGLU*

Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

***Correspondence:**
Seckin Eroglu
erogluseckin@gmail.com

Keywords: RAPD; SCAR; PGPR; *Bacillus subtilis*; OSU-142; biofertilizer; yield

Abbreviations

ARC – ARC Seibersdorf Collection
AUC – Ataturk University Collection
EUC – Erciyes University Collection
OSU-142 – *Bacillus subtilis* OSU-142
PCR – Polymerase chain reaction
PGPR – Plant Growth Promoting Rhizobacteria
RAPD – Randomly Amplified Polymorphic DNA
SCAR – Sequence-Characterized Amplified Region
YUC – Yeditepe University Collection

Received March 19, 2022
Revised June 13, 2022
Accepted June 14, 2022

Abstract

Background and purpose: *Synthetic fertilizers damage the environment. Biofertilizers that consist of microorganisms emerge as an environmentally friendly alternative. Biofertilizers improve plant growth by mobilizing soil nutrients, triggering plant hormone synthesis, or competing with pathogenic bacteria. However, biofertilizers often fail due to insufficient colonization of the plant roots.*

Materials and methods: *To explore the colonization dynamics of a bacterial strain commonly used in biofertilizers,* Bacillus subtilis *OSU-142 (OSU-142), developing a set of primers specific to OSU-142 was aimed. Since its genome is unknown, to identify genomic regions unique to OSU-142 strain, DNAs of more than 40 bacterial strains were fingerprinted, most of which belong to* Bacillus subtilis *using the Randomly Amplified Polymorphic DNA (RAPD) method.*

Results: *This approach identified a polymorphic band at 880 bp, which was then cloned and sequenced. The sequence showed no perfect match to any known sequences in the tested genomic databases indicating that the region of OSU-142 DNA is highly unique. The primer was converted to Sequence-Characterized Amplified Region (SCAR) primers, and its functionality in detecting OSU-142 genome was confirmed. Newly designed primer set can specifically detect OSU-142.*

Conclusions: *These primers can be useful for basic science or commercial applications on tracking OSU-142 in various environments, thus contributing to biofertilizers' adoption in the long run.*

INTRODUCTION

Fertilizers are inevitable in agriculture yet damage the environment. Phosphate fertilizers infiltrate groundwater, leading to cyanobacterial overgrowth *(1)*. Nitrogen fertilizers are produced by fixed atmospheric nitrogen at extremely high temperatures and pressure, requiring fossil fuels *(2,3)*. Fertilizer use can be lessened by supplementing Plant Growth Promoting Rhizobacteria (PGPR) to the rhizosphere of the plants.

PGPR are the bacteria that live near plant root cells and promote plant growth by forming a symbiotic relationship with the plant. PGPR help plants by solubilizing phosphates, fixing atmospheric nitrogen, and producing phytohormones such as Indole-3-acetic acid *(4)*, while benefit from them by receiving carbon source as sugars, organic acids, and other secretions *(5)*. Furthermore, some PGPR, including *Bacillus subtilis,* mitigate biotic and abiotic stresses *(6,7)*. For over three decades, people

have isolated PGPR from its natural environment, grown in large amounts in synthetic mediums, and delivered them back to plants to promote agricultural production.

One of the most significant drawbacks of PGPR fertilization is the inconsistencies in its effect due to insufficient colonization of the bacteria in the rhizosphere. The fertilization efficiency varies as the season, the agricultural site, or the applied crop changes *(8)*. Non-optimal conditions may favor other microorganisms to outcompete PGPR in the rhizosphere *(9,10)*. Tracking the bacterial faith showed PGPR cell numbers could decrease rapidly following the fertilization reaching below a certain threshold, where the beneficial effect is completely lost *(11,12)*. The factors affecting colonization are not well understood but vaguely suggested to be climate, pH of the soil, the nature of the plant, and soil management practices. To exploit the PGPR potential, we must investigate the biotic/abiotic factors determining PGPR colonization; however, tracking bacteria after inoculation is complicated when the bacterial genome is unknown.

Various techniques can detect PGPR. These include reporter genes, immuno-based or nucleic acid techniques *(13–15)*. Nucleic acid-based methods take advantage of unique regions in the DNA sequence of the bacteria of interest to differentiate them from other bacteria abundant in soil. Among the nucleic acid techniques, the Randomly Amplified Polymorphic DNA technique (RAPD) has advantages, as it does not require prior knowledge about the genomic sequence and does not lead to the production of genetically modified organisms; unlike reporter-gene-based techniques. RAPD takes advantage of natural polymorphic DNA regions in differentiating bacteria of interest from the others *(16)*. RAPD compares DNA patterns of microorganisms based on PCR amplification where random short primers are used *(17)*. However, RAPD bands may offer poor reproducibility in PCR amplifications leading to inconsistent results *(18)*.

Furthermore, in addition to polymorphic regions of the DNA, they also amplify non-polymorphic ones, which are unnecessary. Therefore, once a polymorphic RAPD band is obtained, the RAPD primer that amplifies the band is usually converted into a set of SCAR (Sequence-Characterized Amplified Region) primers. These are typically elongated versions of the RAPD primers *(19)*. Tracking bacteria with SCAR primers provides more specificity, simplicity, and reproducibility over RAPD primers.

Bacillus subtilis OSU-142 (OSU-142) is a renowned PGPR. It promoted growth in several crops; i.e., potato, sweet cherry, and barley *(4,20,21)*, in several different settings, such as cold or hot climates with varied soil types across Turkey *(22)*. Furthermore, OSU-142 acts as a biocontrol agent, at least in apricot trees, against shot-hole disease *(23)*. It is commercially available for agricultural use (BACTOGEN, Istanbul, Turkey). Despite being isolated more than twenty years ago, methods to track these bacteria are still missing. We aimed to develop a nucleic acid-based strategy to track OSU-142 in the soil. To this end, RAPD method was used to take advantage of the unique regions in the DNA of OSU-142, compared to that of other *Bacillus subtilis* strains.

MATERIALS AND METHODS

Bacterial strains

All of the bacterial strains used in this study were listed in Table 1. This table included 52 bacterial isolates, 40 of which belonged to different strains of *B. subtilis.* Strains were obtained from -80 °C stocks and plated on Luria-Bertani (LB) agar medium *(24)*. To work with fresh bacterial cultures, strains were plated on LB agar plates. All culture plates were incubated at 30 °C for a week. Freshly collected bacteria were used in DNA extraction.

DNA extraction

Bacterial DNA was isolated with a bacterial genomic DNA kit (Product No: NA2110, Merck, Darmstadt, Germany) according to the manufacturer's manual for the strains obtained from Ataturk University Collection (Erzurum Atatürk University, Erzurum, Turkey) and ARC Seibersdorf Collection (Seibersdorf Research Center, Wien, Austria). Bacterial DNA from Erciyes University Collection (Erciyes University, Kayseri, Turkey) and Yeditepe University Collection (Yeditepe University, Istanbul, Turkey) were isolated manually *(25)*. Genomic DNAs were eluted in 50 µl DNAse-free water. The presence of genomic DNA and its purity was confirmed by NanoDrop (Nanodrop Technologies, Wilmington, DE, USA).

RAPD analysis and optimization

To amplify random parts of the DNA, we used ready-to-use 10mer primer sets from Operon Technologies (Eurofins, Luxembourg, Belgium). These included the following primers: OPG1, OPG-5, OPB-5, OPG16, OPB-1, OPB-7, OPC-2, OPC-3, OPC-4, OPC-5 (Table 2). For each primer, unique bands that represent polymorphic regions of OSU-142 DNA were sought. The number and intensity of the PCR products changed immensely; therefore, not to miss any polymorphic band, PCR was conducted in different conditions. Dependent on the PCR results obtained with each primer, these diverse conditions included four different concentrations of MgSO4 (1.2-1.6-2-2.4-2.8 mM), two different annealing times (45-60 s), two different primer concentrations (0.2-0.6 uM), and four different annealing temperatures (50 °C to 32 °C) *(26)*. To conduct a faster screening, at first, only a subset of the available strains was initially compared with OSU-142. If a polymorphic band was detected in the initial screen, the screening continued with the same parameter extended to the other strains. PCR cycles were; initial denaturation at 92 °C for 2 min, denaturation at

Table 1. *Bacterial strains used in this study.*

Genus Species	Strain/ID	Reference/source
Bacillus subtilis	OSU142	YUC
Bacillus subtilis	305	ARC
Bacillus subtilis	295	ARC
Bacillus subtilis	383	ARC
Bacillus subtilis	753	ARC
Bacillus subtilis	460	ARC
Bacillus subtilis	181	ARC
Bacillus subtilis	RK342	AUC
Bacillus subtilis	TV13B	AUC
Bacillus subtilis	RK339	AUC
Bacillus subtilis	TV12H	AUC
Bacillus subtilis	TV17C	AUC
Bacillus subtilis	TV89E	AUC
Bacillus subtilis	TV6F	AUC
Bacillus subtilis	RK341	AUC
Bacillus subtilis	TV77B	AUC
Bacillus subtilis	TV130B	AUC
Bacillus subtilis	TV90D	AUC
Bacillus subtilis	TV93B	AUC
Bacillus subtilis	49.3	EUC
Bacillus subtilis	1.1.1	EUC
Bacillus subtilis	73.4	EUC
Bacillus subtilis	10.5	EUC
Bacillus subtilis	35.3	EUC
Bacillus subtilis	39.24	EUC
Bacillus subtilis	54.3	EUC
Bacillus subtilis	8.2	EUC
Bacillus subtilis	59.5	EUC
Bacillus subtilis	65.11	EUC
Bacillus subtilis	36.4	EUC
Bacillus sp.	Dil4	YUC
Bacillus subtilis /amyloliquefaciens	745	ARC
Bacillus subtilis /amyloliquefaciens	749	ARC
Bacillus subtilis /amyloliquefaciens	748	ARC
Bacillus subtilis /amyloliquefaciens	747	ARC
Bacillus subtilis /amyloliquefaciens	744	ARC
Bacillus subtilis /amyloliquefaciens	746	ARC
Bacillus subtilis /amyloliquefaciens	742	ARC
Bacillus subtilis /amyloliquefaciens	685	ARC
Bacillus subtilis /amyloliquefaciens	743	ARC
Bacillus subtilis /licheniformis	122	ARC
Bacillus cereus	DY2	YUC
Bacillus cereus	DA1	YUC
Bacillus cereus	DA3	YUC
Bacillus cereus	DY4	YUC
Bacillus cereus	DA2	YUC
Pantoea agglomeran	Said1	YUC
Pseudomonas putid	Said2	YUC
Erwinia amylovora	Said3	YUC
Pseudomonas tolaasii	Said4	YUC
Bacillus megaterium	TV6D	AUC
Bacillus megaterium	182	ARC

ARC – ARC Seibersdorf Collection; AUC – Ataturk University Collection; YUC – Yeditepe University Collection; EUC – Erciyes University Collection

Table 2. *The sequences of primers used in this study.*

Name	Sequence
OPB-01	GTTTCGCTCC
OPB-05	TGCGCCCTTC
OPB-07	GGTGACGCAG
OPC-02	GTGAGGCGTC
OPC-03	GGGGGTCTTT
OPC-04	CCGCATCTAC
OPC-05	GATGACCGCC
OPG-01	CTACGGAGGA
OPG-05	CTGAGACGGA
OPG-16	AGCGTCCTCC
SEC1	TGCCCGTCGTCTCTACGTC
SEC2	TGCCCGTCGTGGATACAGT

92 °C for 30 sec, primer annealing for 45 sec, primer extension at 72 °C for 1 min for 40 cycles, final extension at 72 °C for 10 min. Reaction mixture consisted of dNTP's (0.2 mM), TSG polymerase (1U) and buffer (10x) from Biobasic (Toronto, Canada), primers (0.2 uM) and template DNA (0.1-10 ng for pure, 400 ng for unpure DNA) at 25 µl total volume. After the amplification, agarose gel electrophoresis was conducted. 5 µl of PCR products and 3 µl of DNA marker (Sigma D-7058) were loaded in 2% Agarose gel and operated at 80 V for 60 min. The gel image was obtained in Bio-Rad (Berkeley, California) GelDoc imaging system.

Cloning and sequencing

The polymorphic band was excised from the gel and purified by Wizard® SV Gel and PCR Clean-Up System. To clone the DNA belonging to the polymorphic band, Qiagen PCR cloning kit was used according to the manufacturer's recommendations (Qiagen, Hilden, Germany). The transformation step was carried out using chemically competent cells (C8620-03, Invitrogen, Waltham, USA) according to the manufacturer's suggestions. After the transformation and selection, for the plasmid isolation step, single colonies were inoculated into 100 uM ampicillin-containing liquid LB medium. The medium was incubated for 4 hours at 37 °C with horizontal shaking. Plasmids were isolated by using the NucleoSpin plasmid isolation kit (Macherey-Nagel, Düren, Germany). To validate the correct size of the insert, 1 ng plasmid was used in PCR amplification as a template with M13 universal primers. The plasmids were then sequenced from both directions. The sequence of the OSU-142 polymorphic band was deposited to GenBank under accession number FR773525.1.

SCAR primer design

The sequence of the polymorphic band was analyzed on NCBI BLASTN through nr/nt database using the default parameters (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers were designed by extending the OPG1 primer annealing sites from the flanking regions of the sequence, using Primer3 software *(27)* (http://bioinfo.ut.ee/primer3-0.4.0/).

The name and sequence of the primers generated from extending the flanking region of the polymorphic band are as follows:

sec1: 5'-TGCCCGTCGTCTCTACGTC-3'

sec2: 5'-TGCCCGTCGTGGATACAGT-3'

Designed primers were confirmed for their specificity on Primer Specificity Check Software (PSC) (http:// biocompute.bmi.ac.cn/PSC). Newly designed primers were tested with all available bacterial strains (Table 1). For this test, stringent conditions were met by increasing annealing temperature to 65 °C and decreasing annealing time to 30 seconds.

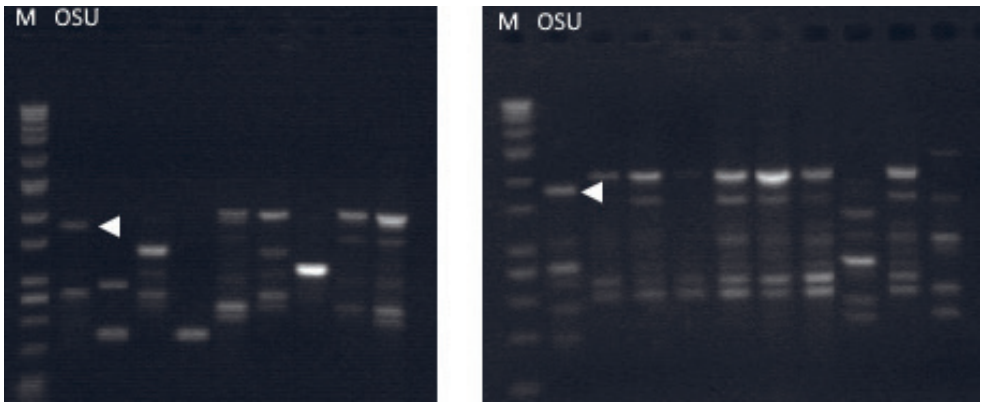

Figure 1. *Representative agarose gel images of PCR products amplified with OPG1 primer. The first lanes of both gels: DNA marker (100bp); second wells, PCR products when OSU-142 DNA was used; other wells, PCR products when DNA of other bacterial strains was used as templates. All the bacterial stocks in Table 1 have been tested but only two of the gels were shown. Arrow heads show polymorphic bands.*

RESULTS

Since genomic data lacks for OSU-142, we used RAPD method for fingerprinting OSU-142 specific bands compared with other *Bacillus* strains. PCR amplification of OSU-142 genome with the random primer set OPG-1 resulted in amplification of two bands (Figure 1). The first band was approximately 900 bp, while the second band was found to be approximately 600 bp. Other weaker bands were also produced, ranging between 300 to 700 bp. PCR amplification with genomes of other bacteria produced bands that have sizes between 300 to 900 bp. Many of these bands were shared between OSU142 and the rest. However, the 900 bp band produced with OSU-142 DNA as a template appeared to be unique.

Table 3. *The most similar sequences to OSU-142 polymorphic band sequence in the NCBI database.*

Description	Max Score	Total Score	Query Cover	Identity	Accession
Bacillus subtilis RAPD fragment OPG1, strain OSU-142	1626	1626	100%	100.00%	FR773525.1
Bacillus velezensis strain 10075 chromosome, complete genome	1613	3226	99%	99.89%	CP025939.1
Bacillus velezensis strain SCGB 1 chromosome, complete genome	1408	1408	99%	95.66%	CP023320.1
Bacillus velezensis strain SCDB 291 chromosome, complete genome	1408	2308	99%	95.66%	CP022654.2
Bacillus velezensis strain L-1 chromosome, complete genome	1303	1303	94%	95.05%	CP023859.1
Bacillus amyloliquefaciens strain LM2303, complete genome	1230	2461	94%	93.48%	CP018152.1
Bacillus velezensis strain MH25 chromosome, complete genome	1064	1064	94%	90.05%	CP034176.1
Bacillus velezensis strain SRCM103788 chromosome, complete genome	911	911	84%	88.95%	CP035399.1
Bacillus velezensis strain Lzh-a42 chromosome, complete genome	911	911	83%	89.13%	CP025308.1
Bacillus amyloliquefaciens strain ZJU1 chromosome, complete genome	752	752	55%	94.47%	CP041691.1

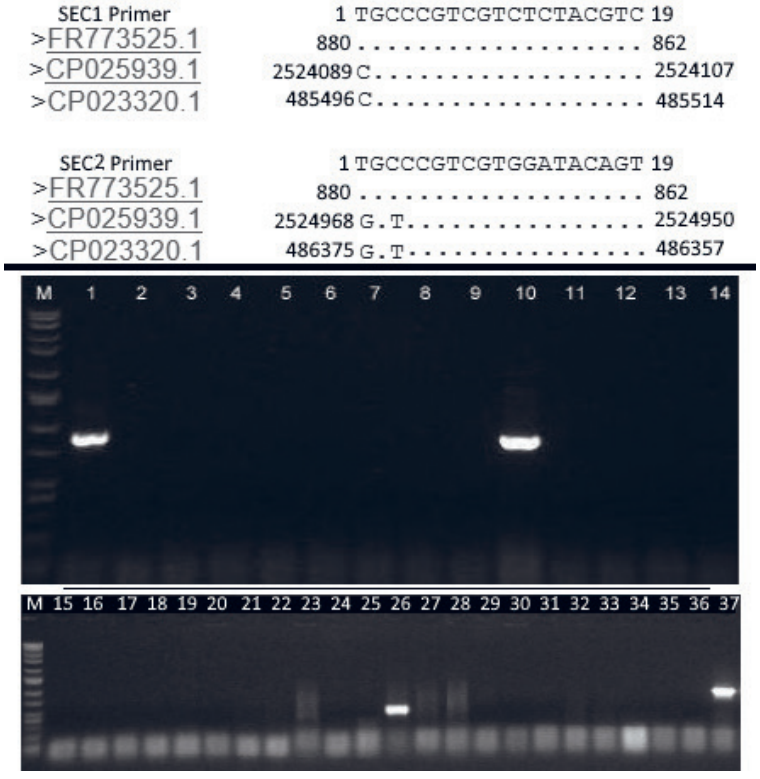

Figure 2. *Specificity of SEC1 and SEC2 primers for OSU-142. Upper panel, SEC1 and SEC2 primers were subjected to primer blast. FR773525.1 is the Genebank accession number for OSU-142 polymorphic band. CP025939.1 and CP023320.1 represent sequences that have highest match with SEC1 and SEC2 primers in the Genebank database. SEC1 has one and SEC2 has 2 mismatches with them. Below panel, M:DNA marker; lane 1and 37: OSU-142; lane 10: Bacillus subtilis TV77B strain; Lane 26: Bacillus subtilis TV6F strain. All other lanes include PCR products from various other bacterial DNAs that belong to Table 1. Bands in OSU-142-related wells are at 880 bp. Note that the band on the lane 26 is far below than that of the lane 37.*

To obtain the DNA sequence of this band, DNA piece was extracted from gel and sequenced. BLAST result of the sequence indicated the sequence is distinct from the known genomic sequences (Table 3).

SCAR primers were designed by extending the OPG-1 primers. These primers were examined for their specificity to known sequences. Designed primers showed no match to the existing sequences (Figure 2). PCR amplification using the newly designed primers amplified the intended band from the OSU-142 genome, but not with the genomes of other bacteria tested, except the DNA of *Bacillus subtilis* TV77 strain, which produced a similar size band.

DISCUSSION

Scientists must monitor the number of PGPR to investigate what factors help or inhibit their successful establishment in the rhizosphere. A primer set was designed to detect and monitor a renowned PGPR, OSU-142 and validate its specificity using blast and PCR-based methods. Genomic DNA of OSU-142 was randomly identified in comparison with other bacterial DNAs and screened for a unique band. Obtained band was cloned and sequenced. SCAR primer set was shown to detect OSU-142 reproducibly.

Since OSU-142's DNA sequence is unknown, designing a primer using bioinformatic methods was impossible. Instead, a sequence that is unique for OSU142 was sought and primers were designed to detect the bacteria, a technique called RAPD-SCAR *(28)*. It can quantify the bacteria and does not require genetic engineering *(19,29)*, thus advantageous over other techniques such as molecular probes and hybridization-based techniques. The release of genetically engineered organisms to the environment is strictly forbidden in many countries, including Turkey *(30)*.

To obtain unique DNA regions to differentiate OSU-142 from other bacteria, bacterial genomic DNAs were amplified by using 10-mer oligos. These primers have fingerprinted OSU-142 and other bacterial genomes tested. Among these primers, OPG1 primer amplified a band corresponding to 880 bp only when OSU-142 DNA was used as a template (Figure 1). OPG1 primer amplified additional bands as well, but these found to be shared between genomic DNAs of other bacteria. Among the amplified bands, 880 bp one was brighter than the others, indicating the primer perfectly matched the genomic DNA. The sequence of the polymorphic band was most similar to the sequences from *Bacillus* sp. (Table 3), confirming the origin of the amplified band.

The OPG1 primer successfully amplified a DNA region that can differentiate OSU-142 from others but using OPG1 to detect bacteria in the rhizosphere had practical disadvantages in applications. First, the primer was short, 10-mers, likely producing non-specific bands *(31)*. Second, it produced additional bands that complicate the assessment. Therefore, next, SCAR primers were designed by extending the OPG1 primer sequence (Figure 2, top panel).

Newly designed primers were evaluated for their specificity using bioinformatics and wet lab (Figure 2). Regarding bioinformatics, PSC software analysis showed none of the primers perfectly match any sequence in existing databases (Figure 2). This indicated that the designed primers bind specifically to the polymorphic region but not to other tested microorganisms' DNA. Since the newly designed primers amplified an unwanted band from only one strain out of 52, 40 of which belonged to different *B. subtilis* strains, we concluded that newly designed primers specifically bind to OSU-142 DNA; thus, can differentiate the bacteria in strain-level specificity. Since it was out of the study's focus, the unwanted band from the strain TV77 was left unexplored. A simple sequencing would determine how similar the unwanted band is to OSU-142 DNA.

In this study, we developed strain-specific primers for PCR detection of *B. subtilis* OSU-142. *B. subtilis* OSU-142 strain has been used in numerous studies *(23,32)*, but molecular tools to confirm its existence after application has been missing. These primers can be used individually or as a complementary tool to cultivation-based techniques to monitor the bacteria in any medium. Several primers have been successfully developed for other beneficial bacteria, including *Pseudomonas fluorescens*, *Pantoea agglomerans*, *Bacillus subtilis, Pseudomonas brassicacearum* , *Azospirillum brasilense* in strain-specific levels *(19,33–36)*. These primers can detect and quantify the bacteria of interest in complex matrices such as rhizosphere soil. The primers we developed here can detect the bacteria successfully, yet, whether they can be used in the rhizosphere should be further investigated in the future.

REFERENCES

1. MULLINS G L 2009 Phosphorus, agriculture & the environment. https://vtechworks.lib.vt.edu/handle/10919/55777 Last accessed 20-07-2022
2. XING G X, ZHU Z L 2000 An assessment of N loss from agricultural fields to the environment in China. Nutrient Cycling in Agroecosystems 57(1): 67–73. https://doi.org/10.1023/A:1009717603427
3. BICER Y, DINCER I, VEZINA G, RASO F 2017 Impact Assessment and Environmental Evaluation of Various Ammonia Production Processes. Environmental Management 59(5): 842–855. https://doi.org/10.1007/s00267-017-0831-6
4. EKIN Z, OĞUZ F, ERMAN M, ÖĞÜN E 2009 The effect of Bacillus sp. OSU-142 inoculation at various levels of nitrogen fertilization on growth, tuber distribution and yield of potato (Solanum tuberosum L.). African Journal of Biotechnology 8(18): 4418–4424. https://doi.org/10.4314/ajb.v8i18.62394
5. STRIGUL N S, KRAVCHENKO L V. 2006 Mathematical modeling of PGPR inoculation into the rhizosphere. Environmental Modelling and Software 21(8): 1158–1171. https://doi.org/10.1016/j.envsoft.2005.06.003
6. YANG J, KLOEPPER J W, RYU C M 2009 Rhizosphere bacteria help plants tolerate abiotic stress. Trends in plant science 14(1): 1-4. https://doi.org/10.1016/j.tplants.2008.10.004
7. WEI LIU, DIANKUI CHEN, HANWEN YIN, MIN SONG, SHIRONG GUO 2010 Bacillus Subtilis for Salt Stress Relief in Vegetable Cultivation. Acta Horticulturae (856): 237–242. https://doi.org/10.17660/actahortic.2010.856.33

8. VESSEY J K 2003 Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255(2): 571–586. https://doi.org/10.1023/A:1026037216893
9. KRAGELUND L, HOSBOND C, NYBROE O 1997 Distribution of metabolic activity and phosphate starvation response of lux-tagged Pseudomonas fluorescens reporter bacteria in the barley rhizosphere. Applied and Environmental Microbiology 63(12): 4920–4928. https://doi.org/10.1128/aem.63.12.4920-4928.1997
10. LUGTENBERG B J J, DEKKERS L C 1999 What makes Pseudomonas bacteria rhizosphere competent? Environmental microbiology 1(1): 9–13. https://doi.org/10.1046/j.1462-2920.1999.00005.x
11. BULL C T 1991 Relationship Between Root Colonization and Suppression of Gaeumannomyces graminis var. tritici by Pseudomonas fluorescens Strain 2-79 . Phytopathology 81(9): 954. https://doi.org/10.1094/phyto-81-954
12. SCHIPPERS B, BAKKER A W, BAKKER P A H M 1987 Interactions of Deleterious and Beneficial Rhizosphere Microorganisms and the Effect of Cropping Practices. Annual Review of Phytopathology 25(1): 339–358. https://doi.org/10.1146/annurev.py.25.090187.002011
13. GYANESHWAR P, JAMES E K, REDDY P M, LADHA J K 2002 Herbaspirillum colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytologist 154(1): 131–145. https://doi.org/10.1046/j.1469-8137.2002.00371.x
14. PODILE A R, KISHORE G K 2006 Plant growth-promoting rhizobacteria, In: Plant-Associated Bacteria, Springer Netherlands, p 195
15. MOURYA S, JAUHRI K S 2002 LacZ Tagging of Phosphate Solubilizing Pseudomonas striata for Rhizosphere Colonization. Indian Journal of Biotechnology 1(3): 275–279.
16. AKKAYA M S, BHAGWAT A A, CREGAN P B 1992 Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132(4): 1131–1139. https://doi.org/10.1093/genetics/132.4.1131
17. WILLIAMS J G K, KUBELIK A R, LIVAK K J, RAFALSKI J A, TINGEY S V. 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18(22): 6531–6535. https://doi.org/10.1093/nar/18.22.6531
18. PENNER G A, BUSH A, WISE R, KIM W, DOMIER L, KASHA K, LAROCHE A, SCOLES G, MOLNAR S J, FEDAK G 1993 Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. Genome Research 2(4): 341–345. https://doi.org/10.1101/gr.2.4.341
19. CHAPON A, BOUTIN M, RIMÉ D, DELALANDE L, GUILLERM A Y, SARNIGUET A 2003 Direct and specific assessment of colonisation of wheat rhizoplane by Ps eudomonas fluorescens Pf29A. European Journal of Plant Pathology 109(1): 61–70. https://doi.org/10.1023/A:1022084728087
20. ESITKEN A, PIRLAK L, TURAN M, SAHIN F 2006 Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Scientia Horticulturae 110(4): 324–327. https://doi.org/10.1016/j.scienta.2006.07.023
21. ŞAHIN F, ÇAKMAKÇI R, KANTAR F 2004 Sugar beet and barley yields in relation to inoculation with N 2-fixing and phosphate solubilizing bacteria. Plant and Soil 265(1–2): 123–129. https://doi.org/10.1007/s11104-005-0334-8
22. ACIKGOZ E, BILGILI U, SAHIN F, GUILLARD K 2016 Effect of plant growth-promoting Bacillus sp. on color and clipping yield of three turfgrass species. Journal of Plant Nutrition 39(10): 1404–1411. https://doi.org/10.1080/01904167.2016.1143501
23. EŞITKEN A, KARLIDAĞ H, ERCIŞLI S, ŞAHIN F 2002 Effects of foliar application of Bacillus subtilis OSU-142 on the yield, growth and control of shot-hole disease (Coryneum blight) of apricot. Gartenbauwissenschaft 67(4): 139–142.
24. BERTANI G 1951 Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli. J Bacteriol 62(3): 293–300. https://doi.org/10.1128/JB.62.3.293-300.1951
25. LAU S K P, WOO P C Y, FUNG A M Y, CHAN K M, WOO G K S, YUEN K Y 2004 Anaerobic, non-sporulating, Gram-positive bacilli bacteraemia characterized by 16S rRNA gene sequencing. Journal of Medical Microbiology 53(12): 1247–1253. https://doi.org/10.1099/jmm.0.45803-0
26. HARINI S S, LEELAMBIKA M, SHIVA KAMESHWARI M N, SATHYANARAYANA N 2008 Optimization of DNA isolation and PCR - RAPD methods for molecular analysis of urginea indica kunth. International Journal of Integrative Biology 2(2): 138–144
27. ROZEN S, SKALETSKY H 2000 Primer3 on the WWW for general users and for biologist programmers., In: Methods in molecular biology (Clifton, N.J.), Springer, p 365
28. FELICI C, VETTORI L, TOFFANIN A, NUTI M 2008 Development of a strain-specific genomic marker for monitoring a Bacillus subtilis biocontrol strain in the rhizosphere of tomato. FEMS Microbiology Ecology 65(2): 289–298. https://doi.org/10.1111/j.1574-6941.2008.00489.x
29. GLANDORF D C M, VERHEGGEN P, JANSEN T, JORRITSMA J W, SMIT E, LEEFLANG P, WERNARS K, THOMASHOW L S, LAUREIJS E, THOMAS-OATES J E, BAKKER P A H M, LOON L C VAN 2001 Effect of Genetically Modified Pseudomonas putida WCS358r on the Fungal Rhizosphere Microflora of Field-Grown Wheat. Applied and Environmental Microbiology 67(8): 3371–3378. https://doi.org/10.1128/AEM.67.8.3371-3378.2001
30. ABBOTT A 2010 Turkish law could cripple bioscience. Nature 464(7288): 478. https://doi.org/10.1038/464478a
31. YE J, COULOURIS G, ZARETSKAYA I, CUTCUTACHE I, ROZEN S, MADDEN T L 2012 Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13(134): 134. https://doi.org/10.1186/1471-2105-13-134.
32. EKIN Z, OĞUZ F, ERMAN M, ÖĞÜN E 2009 The effect of Bacillus sp. OSU-142 inoculation at various levels of nitrogen fertilization on growth, tuber distribution and yield of potato (Solanum tuberosum L.). African Journal of Biotechnology 8(18): 4418–4424. https://doi.org/10.4314/ajb.v8i18.62394
33. NUNES C, BAJJI M, STEPIEN V, MANSO T, TORRES R, USALL J, JIJAKLI M H 2008 Development and application of a SCAR marker to monitor and quantify populations of the postharvest biocontrol agent Pantoea agglomerans CPA-2. Postharvest Biology and Technology 47(3): 422–428. https://doi.org/10.1016/j.postharvbio.2007.07.016
34. HOLMBERG A I J, MELIN P, LEVENFORS J P, SUNDH I 2009 Development and evaluation of SCAR markers for a Pseudomonas brassicacearum strain used in biological control of snow mould. Biological Control 48(2): 181–187. https://doi.org/10.1016/j.biocontrol.2008.10.016
35. REDDY PRIYA P, SELASTIN ANTONY R, GOPALASWAMY G, BALACHANDAR D 2016 Development of sequence-characterized amplified region (SCAR) markers as a quality standard of inoculants based on Azospirillum. Archives of Microbiology 198(3): 257–267. https://doi.org/10.1007/s00203-016-1187-7
36. COUILLEROT O, POIRIER M A, PRIGENT-COMBARET C, MAVINGUI P, CABALLERO-MELLADO J, MOËNNE-LOCCOZ Y 2010 Assessment of SCAR markers to design real-time PCR primers for rhizosphere quantification of Azospirillum brasilense phytostimulatory inoculants of maize. Journal of Applied Microbiology 109(2): 528–538. https://doi.org/10.1111/j.1365-2672.2010.04673.x