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Molecular Detection of Biosynthetic Genes for Anti-fungal Metabolite Production by Yam (*Dioscorea* sp.) Rhizobacteria

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Abstract: Biosynthetic genes are responsible for antibiotic production by rhizobacterial antagonists. Detection of antibiotic biosynthetic genes of an antagonist is therefore important for the identification of genes and antibiotics responsible for disease suppression. The study was carried out to detect the antibiotic producing genes of eight rhizobacteria responsible for fungal disease suppression. Eight yam (*Dioscorea* sp.) rhizobacterial isolates which were found to possess antifungal properties against several plant pathogenic fungi and identified as *Bacillus* species were tested for the presence of genes for biosynthesis of antifungal lipopeptides; bacillomycin D, iturin A, surfactin, fengycin, and aminopolyols, zwettemycin A as possible antibiotic tools for biocontrol using specific primers. The detection of bacillomycin D gene by PCR amplification, gene sequencing, and BLAST analysis, was achieved through the use of the primer pair, BACC1-F/BACC1-R, capable of detecting 875-bp region, iturin A through the use of ITUD1-F/ITUD1-R primer pair, capable of detecting 647-bp region while primer pair SUR3-F/SUR3-R, capable of detecting 441-bp region was also used for the detection of surfactin. Three separate primer pairs were used for fengycin *viz*. FEND1-F/FEND1-R, FENA1-F/FENA1-R, and FENB2-F/FENB2-R, capable of detecting 964-bp region corresponding to fengycin D, fengycin A and fengycin B respectively. Zwettermycin A was detected through the use of ZWET-F2/ZWET-R1 primer pair, capable of detecting 1-kb region. The outcome of the study shows that all the eight rhizobacteria possessed biosynthetic genes for the production of bacillomycin D, iturin A, and surfactin, however, neither the three types of fengycin nor the zwettermycin A were detected. Sequenced data of these antibiotics have been deposited with GenBank and the following accession numbers assigned to bacillomycin D (MW263002-MW263009), iturin A (MW263010-MW263017), and surfactin (MW263018-MW263025). All the eight rhizobacteria tested were found to possess three out of the five biosynthetic genes namely bacillomycin D, iturin A and surfactin. The detection of these biosynthetic genes confirms and justify why these rhizobacteria are potential biocontrol agents of plant pathogens.

Keywords: Antifungal, Lipidopeptides, Iturin A, Bacillomycin D, Surfactin, Fengycin and Zwettermycin A

1. Introduction

Antibiotic production by rhizobacteria as a mechanism of biocontrol for the suppression of plant diseases has been widely studied [1-3]. Biocontrol using bacteria antagonists has therefore been the main focus of research for pathologists as a potential substitute of chemical pesticides for the management of fungal diseases [4]. Particularly, Gram-positive and endospore producing *Bacillus* spp. have the ability to be easily formulated into stable products [5]. *Bacillus* species produce a wide range of peptides and lipopeptides in culture media as well as in the field that possess antifungal properties against plant pathogens, among the lipopeptides are iturin A, bacillomycin D, surfactin, and fengycin [6], and aminopolyols such as zwettermycin [7]. Iturin and fengycins are noted for their powerful antifungal activity and have been reported as growth inhibitors against several plant pathogens [4]. Surfactin is also reported not to be toxic to fungal pathogens by themselves but exhibits synergic effect on Iturin A antifungal activity [8]. Detection of antibiotic ability by an antagonist is an important factor in determining the capability of the antagonist as a potential biocontrol agent against plant diseases [9]. Direct detection of an antibiotic profile of a particular antagonist through advanced techniques such as PCR will also provide a rapid approach in confirming the antibiotic potential of the antagonist with the traditional methods of screening [10]. PCR-based detection of antibiotic biosynthetic genes is a very appropriate approach for the identification of genes responsible for disease suppression.

A previous study with eight yam (*Dioscorea* sp.) rhizobacterial isolates suggests that they possessed strong antifungal properties not only against the black pod and damping-off diseases of cocoa caused by *Phytophthora palmivora* and *Phytophthora megakarya in vitro* and in the field [11] but also several fungi of different phyla [12]. The *in vitro* and *in situ* studies suggested that the mechanism of biocontrol was antibiotic production. Therefore, this study sought to detect the presence of antibiotic biosynthetic genes responsible for fungal diseases suppression.

2. Materials and Methods

2.1. Bacterial Isolates and Cultural Conditions

Eight rhizobacterial isolates from the yam rhizosphere were isolated according to the method of Akrasi [12] and were maintained at 4ºC under refrigerated conditions before used in this study. Pure cultures were maintained on nutrient agar (Oxoid, Unpath Ltd, Basingstoke, Hampshire, UK). New cultures of each bacterium were started by streaking them onto fresh nutrient agar and incubated at 28 ± 2ºC for 24 hrs. before used.

2.2. DNA Extraction and PCR Analysis

The DNA extraction was carried out at the Biotechnology Laboratory of the Council for Scientific and Industrial Research-Crop Research Institute (CSIR-CRI), Kumasi-Ghana and sent to Functional Biosciences for analysis. The genomic DNA of each rhizobacterium was isolated as follows; Bacterial cells on 24 h nutrient agar (Oxoid, Unipath Ltd, Basingstoke, Hampshire, UK) were harvested with a sterile plastic loop and suspended in 2 ml Eppendorf tubes containing 200 µl Nuclease free sterile water (NFSW). DNA was then extracted from bacterial cells, using Zymo fungal/bacterial DNA miniprep kits according to the manufacturer's instructions (Zymo Research Corp. USA). The extracted DNA was eluted in 100 µl elution buffer and sent to the Functional Biosciences Laboratories in Madison, Wisconsin, USA where they were stored at -80^{0}C in a freezer till needed. PCR reactions were carried out with a methodology established by [13] with negative control of water and antibiotic specific primers (Table 1).

Table 1. *Specific primer sequences used for the detection gene for the production of iturin A, bacilomycin D, surfactin, zwettermicin A and fengycin in this study.*

Antibiotics	Primer name	Primer sequence	Expected band sizes (bp)
Zwettermicin A	ZWETF2	TTGGGAGAATATACAGCTCT	1000 (1kb)
	ZWETR1	GACCTTTTGAAATGGGCGTA	
Iturin A	ITUD1F	GATGCGATCTCCTTGGATGT	647
	ITUD1R	ATCGTCATGTGCTGCTTGAG	
Bacillomycin D	BACC1F	GAAGGACACGGCAGAGAGTC	875
	BACC1R	CGCTGATGACTGTTCATGCT	
Surfactin	SUR3F	ACAGTATGGAGGCATGGTC	441
	SUR3R	TTCCGCCACTTTTTCAGTTT	
Fengycin	FEND1F	TTTGGCAGCAGGAGAAGTTT	964
	FEND1R	GCTGTCCGTTCTGCTTTTTC	
	FENA1F	GACAGTGCTGCCTGATGAAA	964
	FENA1R	GTCGGTGCATGAAATGTACG	
	FENB2F	CAAGATATGCTGGACGCTGA	964
	FENB2R	ACACGACATTGCGATTGGTA	

Source: [13, 38]

2.3. Gene Sequencing and BLAST Analysis

DNA products were also sequenced at the Functional Biosciences Inc. (Madison-Wisconsin, USA) and specific homologies for sequenced data were searched for in the GenBank database through the NCBI n-BLAST search tool for the Basic Local Alignment Search Tool (BLAST) [14] for the detection of biosynthetic genes of the eight rhizobacteria. Sequencing was done, using Big Dye Terminator v3 following purification of PCR product using standard cycling conditions. Sequencing output data were produced in a FSTAT format.

2.4. Submission of Sequence Data to GenBank

Sequenced data for the bacillomycin D, iturin A, and surfactin were submitted and deposited at the GenBank for accession numbers for the eight rhizobacteria respectively. Before the submission, the data were presented in the fstat format. Data were processed and accession numbers were assigned to each of the antibiotics of the eight rhizobacteria based on the sequence data of the three antibiotics detected.

3. Results

3.1. PCR Analysis, Gene Sequencing and BLAST Search

Primers specific for bacillomycin D, iturin A, surfactin, fengycin and zwettermycin A as used in the amplification of biosynthetic genes from the eight *Bacillus* isolates, using PCR (Figure 1).

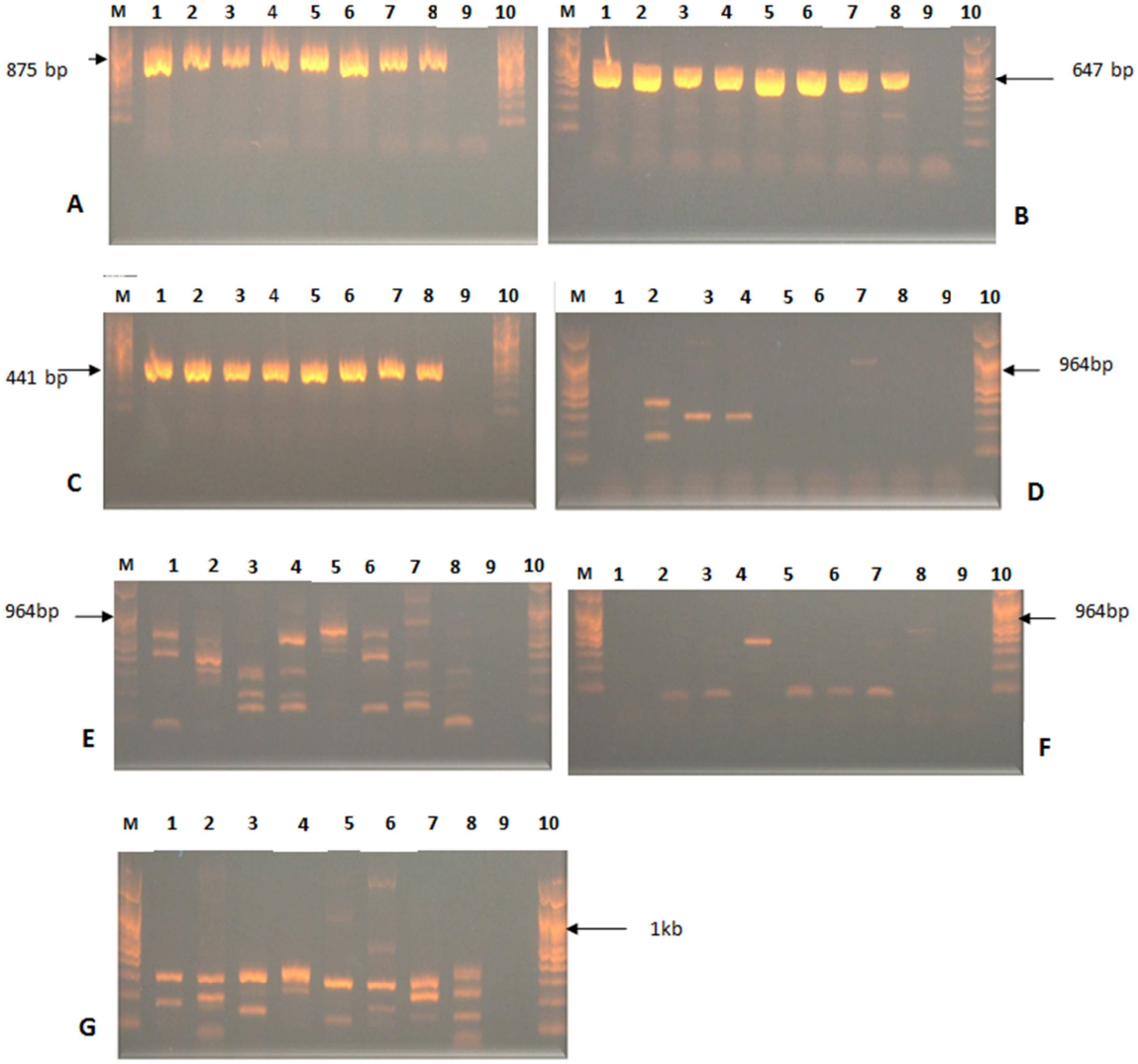

Figure 1. *PCR gel product of amplification for the presence of various antifungal metabolites biosynthetic genes of the eight Bacillus isolates (A-G). A: Isolates producing bacillomycin D; B: Isolates producing iturin A: C: Isolates producing surfactin; D-F (D=fengycin D; E=fengycin A; F=fengycin B); None of the isolates produced fengycin and G: None of the isolates produced zwittermicin. From Lanes of each gel picture (L-R) are, Lane M, 1kb marker; lane 1 ESI; Lane 2, E7B8; Lane 3, E7B1; Lane 4, M7; Lane 5, M8; Lane 6, M32; Lane 7, K4; Lane 8, M78; Lane 9, negative control (water); Lane 10, 1kb marker. Each figure represents the product size for detection of the antifungal metabolite.*

All isolates were positive for bacillomycin D, iturin A and surfactin biosynthetic gene. Fengycin and zwittermycin produced various amplifications with various product sizes, however, none of the sizes corresponded to the expected product sizes. Even though some isolates (ESI and K4) produced bands around the expected product size of fengycin, they produced bad (noisy) sequences at these product sizes. Therefore, none of the eight isolates was positive for fengycin and zwittermycin A biosynthetic genes (Figure 1). PCR gel product amplification for the presence of various metabolites of one of the most promising *Bacillus* isolates, *Bacillus amyloliquefaciens,* ESI (Figure 2).

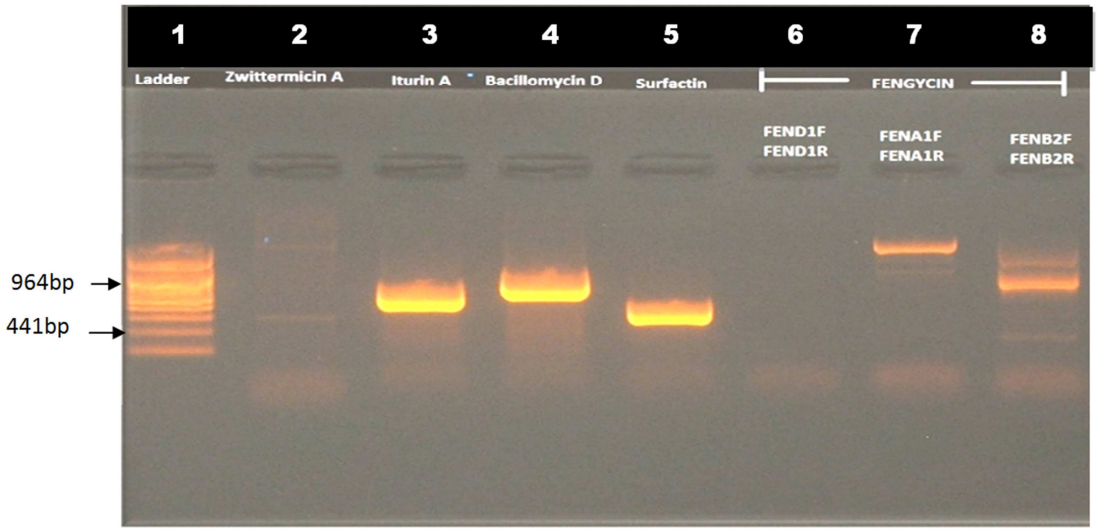

Figure 2. *PCR gel product of amplification for the presence of various antifungal metabolites biosynthetic gene of one of the Bacillus isolates (ESI). Lane 1, 100bp marker; lane 2, zwittermicin A; lane 3, iturin A; lane 4, bacillomycin D; lane 5, surfactin and lane 6, fengycin D; lane 7, fengicin A and lane 8, fengycin B.*

Primer pair BACC1F/BACC1R, showed an 875 base pairs (bp) band corresponding to the bacillomycin antibiotic biosynthetic gene (Figure 1A) of bacillomycin D operon of *Bacillus subtilis* (GenBank acc. No. AY137375.1) (Table 2).

Table 2. *BLAST results of the sequenced products obtained from Polymerase Chain Reaction amplification, using gene-specific primers biosynthetic gene of bacillomycin D synthetase.*

Isolate	Primer	Product size(bp)	GenBank acc. No.	Obtained GenBank match	S-score	E-value	% identity
Bacillus amyloliquefaciens (ESI)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of Bacillus subtilis*	1397	0	98
B. methylotrophicus (E7B8)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1380	0	98
B. subtilis (E7B1)	BACC1F/BACC1R	875	AY137375	Bacillomycin D operon of *B. subtilis*	1373	0	98
B. subtilis subsp. (M7)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1367	0	98
B. amyloliquefaciens (M8)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1393	0	98
B. amyloliquefaciens (M32)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1376	0	98
B. subtilis (K4)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1391	0	98
B. subtilis (M78)	BACC1F/BACC1R	875	AY137375	*Bacillomycin D operon of B. subtilis*	1404	0	98

S-score= Similarity score; E-value= Expected value

Note: S-score is a measure of the similarity of the query to the sequence shown. E-value is a measure of the reliability of the S-score. E.g. E-value= 0-1; 0= high reliability/significance, 1= low reliability/significance. Higher S-score, lower the E-value.

All isolates also showed 647 bp bands, corresponding to the iturin A antibiotic biosynthetic gene (Figure 1B), and the sequenced PCR products, using primer pair ITUD1F/ITUD1R, were highly homologous to iturin A synthetase gene cluster of *Bacillus* sp. CY22 (GenBank acc. No. AF534617.1) (Table 3).

Table 3. *BLAST results of the sequenced products obtained from Polymerase Chain Reaction amplification, using gene-specific primers biosynthetic gene of iturin A synthetase.*

Isolate	Primer	Product size(bp)	GenBank acc. No.	Obtained GenBank match	S-score	E-value	% identity
Bacillus amyloliquefaciens (ESI)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	977	0	97
B. methylotrophicus (E7B8)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	965	0	97
B. subtilis (E7B1)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	970	0	97
B. subtilis (M7)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	965	0	97
B. amyloliquefaciens (M8)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	970	0	97
B. amyloliquefaciens (M32)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	970	0	97
B. subtilis (K4)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	959	0	97
B. subtilis (M78)	ITUD1F/ ITUD1R	647	AF534617	Iturin A synthetase cluster gene of *Bacillus* sp. CY22	959	0	97

S-score= Similarity score; E-value= Expected value

Note: S-score is a measure of the similarity of the query to the sequence shown. E-value is a measure of the reliability of the S-score. E.g. E-value= 0-1; 0= high reliability/significance, 1= low reliability/significance. Higher S-score, lower the E-value.

The PCR products from the eight *Bacillus* isolates, using primer pair SUR3F/SUR3R showed a 441 bp band, corresponding to the surfactin antibiotic biosynthetic gene (Figure 1C). Sequenced products showed a high similarity to GenBank accession No AF534916.1 (surfactin synthetase gene cluster of *Bacillus* sp. CY22) also, confirming that gene with the GenBank match (Table 4). Even though some isolates (ESI and K4) had bands for fengycin at an expected size of 964 bp, those bands did not correspond to the fengycin biosynthetic gene when sequenced, using primer pair FENA1F/FENA1R (Figure 1D), confirming that the product size was not of the correct size. However, primer pairs of FEND1F/FEND1R and FENB2F/FENB2R either did not amplify at all or amplified at wrong product sizes (Figure 1E and F). None of the eight isolates was able to show amplification for zwittermycin A, using primer pair ZWETF2/ZWETR1 at an expected product size of 1 kb corresponding to the detection of zwittermycin A biosynthetic gene (Figure 1G).

Table 4. *BLAST results of the sequenced products obtained from Polymerase Chain Reaction amplification, using gene-specific primers biosynthetic gene of surfactin synthetase.*

Isolate	Primer	Product size(bp)	GenBank acc. No.	Obtained GenBank match	S-score	E-value	% identity
Bacillus amyloliquefaciens (ESI)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	651	0	98
B. methylotrophicus (E7B8)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	634	0	98
B. subtilis (E7B1)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	632	0	98
B. subtilis (M7)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	632	0	98
B. amyloliquefaciens (M8)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	634	0	98
B. amyloliquefaciens (M32)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	634	0	98
B. subtilis (K4)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	665	0	98
B. subtilis (M78)	SUR3F/ SUR3R	441	AF534916	Surfactin synthetase cluster gene of *Bacillus* sp. CY22	665	0	98

S-score= Similarity score; E-value= Expected value

Note: S-score is a measure of the similarity of the query to the sequence shown. E-value is a measure of the reliability of the S-score. E.g. E-value= 0-1; 0= high reliability/significance, 1= low reliability/significance. Higher S-score, lower the E-value.

3.2. Accession Numbers Obtained from GenBank for the Three Biosynthetic Sequenced Genes

For each of the eight rhizobacteria namely; *Bacillus amyloliquefaciens* (ESI), *B. velezensis* (E7B8), *B. subtilis* (E7B1), *B. subtilis* (M7), *B. amyloliquefaciens* (M8), *B. amyloliquefaciens* (M32), *B. subtilis* (K4), *B. subtilis* (M78), the following GenBank accession numbers of their

biosynthetic genes for bacillomycin D (MW263002-MW263009), iturin A (MW263010-MW263017), and surfactin (MW263018-MW263025) were assigned respectively.

4. Discussion

Using a PCR-based approach, this study found all eight rhizobacterial isolates bearing gene clusters responsible for the production of three antibiotics *viz.* Iturin A, Bacillomycin D, and Surfactin. These antibiotics have been associated with *Bacillus* spp. notably *B. subtilis* [15-17, 13], *B. amyloliquefaciens* [18, 13, 19] and *B. methylotrophicus* [20]. The three antibiotics are efficacious against several plant diseases. Iturin A, for example, has been used effectively against *Botrytis cinerea* and *Rhizoctonia solani* causing damping-off of tomato [16, 17]. Phae *et al*. [21] reported that more than 23 types of plant pathogens were suppressed *in vitro* by iturin A producing *B. subtilis*. Akrasi & Awuah [22] reported that, 22 fungi from four different classes were also suppressed by culture filtrate of the rhizobacterium isolate ESI which is now shown to be *B. amyloliquefaciens* and bears gene cluster for the production of iturin A. The finding that ESI bears gene cluster for the production of iturin A may explain why its culture filtrate also showed broadspectrum antifungal activity as reported by Phae *et al*. [21] and [22]. Bacillomycin D identified in this study, suppressed *Aspergillus flavus* causing aflatoxin accumulation in groundnut [15] and *Fusarium oxysporum*, a wilt pathogen of corn [18], whereas surfactin also identified in the current study is effective against several plant pathogens [23, 24].

Spore-forming ability of *Bacillus* spp., [25] as well as their antagonistic effect which is ascribed to antibiotics such as bacillomycin, bacilysin, iturin, surfactin, fengycin, and siderophore production [26] makes them ideal to be used as biopesticides. *Bacillus amyloliquefaciens* produces various antibacterial and antifungal antibiotics such as bacillomycin D, surfactin, iturin A and fengycin [27]. The *Bacillus* spp. are also capable of inducing growth and defense responses in their host [28]. *Bacillus* spp. produce spores resistant to UV light and heat which allows them to resist adverse environmental conditions, enabling easy formulation for commercial purposes [2].

The rhizobacterium ESI (which is now known to be *B. amyloliquefaciens*) suppressed black pod lesion development on detached cocoa pods [29] which the current study has confirmed with the kind of antibiotics it produces. Control of damping-off of cocoa seedling has also been achieved with ESI. Field control of black pod with ESI has also been attempted with some measure of success [11]. Hence the inhibition of *P. palmivora* in culture plates and on pods observed with ESI could be attributed to antibiotics such as Iturin A, Bacillomycin and Surfactin or related kinds suspected to be produced by ESI.

Biological control of plant pathogens through antibiosis is ascribed to either one or the combined effect of several antibiotics produced by the biocontrol agent [24, 30, 31]. This is likely to be the case with the biocontrol of *P. palmivora* with ESI. This needs to be confirmed by isolating each antibiotic type produced by each bacterial filtrate and examining individual and combined effects of the constituents on *P. palmivora.*

In studies with the culture filtrate of ESI (identified in this study as *B. amyloliquefaciens*), it was reported that the filtrate, maintained activity after autoclaving at 121°C for 30 min. [22]. In the current study, the culture filtrate of ESI was routinely autoclaved at 121°C for 30 min without losing its antibiotic potency. This thermostability of the antibiotic properties of the ESI filtrate could be attributed to the presence of iturin A, bacillomycin D, or surfactin, or related product. While [21], demonstrated that iturin A is thermostable, [6] also reported that bacillomycin D and surfactin have a high degree of thermostability as they resisted autoclaving at 100°C for 30 min. Thermostability of the three antibiotics i.e. iturin A, bacillomycin D and surfactin is desirable as it would enhance the usefulness of the antibiotics producing bacteria as a biocontrol agent [2, 32] as they would resist heat and desiccation in nature and facilitate formulation for commercial purposes. The thermostable ability of ESI is also advantageous from a research point of view in the sense that the filtrate can be sterilized by autoclaving, thus obviating the need to use Millipore filters which is often difficult to do without proper facilities.

B. amyloliquefaciens produces a wide array of antibiotics *viz*. iturin A. bacillomycin D, surfactin, macrolactin, difficidine, bacillacine, bacilysin, fengycin and bacillibactin [33]. This study targeted only four of these antibiotics *viz*. iturin A, bacillomycin D, surfactin and fengycin. It is very likely the *B. amyloliquefaciens* used in the present study could have produced more antibiotics than just the four if other antibiotics had been targeted as done by Arguelles-Arias *et al*. [33].

In general, further characterization of the rhizobacterial isolates may also be inferred from the antibiotics which they produce. From this research, gene clusters for the production of three antibiotics (iturin A, bacillomycin D, and surfactin) have been identified in all eight isolates. The high percent similarities to the best sequences in the GenBank and the E-value of zero (0) show high reliability in their identification. While bacillomycin D synthetase PCR products from all isolates match best to *B. subtilis*, iturin A synthetase and surfactin synthetase PCR products from all isolates match best to *Bacillus* sp. CY22. The antibiotics produced from the gene cluster belong to the family of cyclic lipopeptide (LPs) found among *Bacillus* spp. [34-37]. Hence, it is not surprising that all isolates are showing antifungal properties. Since these are mostly peptide antibiotics and likely undergo point mutations, a change in even single amino acids in their coding region will be sufficiently enough to produce a different antibiotic. The isolates are likely to be pure, therefore, there is the likelihood that each isolate potentially produces multiple antibiotics. A hint of variability in the type of antibiotics produced may, therefore, be obtained from the

patterns of inhibition (differential potencies against different organisms and also the extent of their inhibition spectrum). For example, ESI that is mentioned in this study as being the most promising, and K4 show some product from fengycin, hence, they may produce more types of antibiotics than the other isolates.

So far, only genes for known antibiotics were identified. There may be other genes for other antibiotics not yet identified. Identification and characterization of any novel antibiotics may provide the rationale for identifying their gene cluster. These antibiotics and possibly others should be studied further to determine the range of antibiotics produced by all eight rhizobacteria. The current study has therefore demonstrated that the eight yam rhizobacteria possess three antibiotic biosynthetic genes viz. bacillomycin D, iturin A, and surfactin out of the five targeted antibiotics. It has also been demonstrated that those antibiotics were responsible for fungal diseases suppression. Successful attempt was also made to deposit the sequenced data of all the three antibiotics of the eight rhizobacteria at the GenBank and the following accession numbers were assigned to Bacillomycin D (MW263002-MW263009), Iturin A (MW263010-MW263017), and Surfactin (MW263018-MW263025). The study has therefore confirmed and justified why the rhizobacteria have potential as biocontrol agents of plant pathogens from previous studies.

5. Conclusion

The current study has therefore demonstrated that all the eight yam rhizobacteria possess three out of the five antibiotic biosynthetic genes namely. bacillomycin D, iturin A, and surfactin out of the five targeted antibiotics. However, niether fengycin nor the zwettermycin A were detected with none of the rhizobacteria. It has also been demonstrated that those antibiotics were possibly responsible for fungal diseases suppression. Successful attempts were also made to deposit the sequenced data of all the three antibiotics of the eight rhizobacteria at the GenBank and the following accession numbers were assigned to Bacillomycin D (MW263002-MW263009), Iturin A (MW263010-MW263017), and Surfactin (MW263018-MW263025). The detection of these biosynthetic genes confirms and justify why these rhizobacteria are potential biocontrol agents of plant pathogens from previous studies.

Conflicts of Interest

The authors declare no conflicts of interest.

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