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Determination of the genetic dimension of *Pseudomonas stutzeri* which of nitrogen-fixation bacteria in a random PCR method

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Abstract

Bacteria play an important role in fixing atmospheric nitrogen and converting it into compounds that can be used by plants. Due to the importance of this topic, this type of bacteria was chosen, and its genetic dimension was studied. The nitrogen-fixing strain of *Pseudomonas stutzeri*, or those that have already been positively identified, should be collected from the wild or from laboratory cultures. *Pseudomonas stutzeri* strain was collected and their genomic DNA was extracted. This can be done using regular methods for extracting DNA. The purpose of this research is to generate a diverse panel of PCR amplification primers. Primers chosen at random are short sequences of nucleotides that can bind to various locations in the bacterial genome. These primers can be used to initiate polymerase chain reaction (RPCR) amplification at a high number of random positions within the DNA, allowing researchers to probe a wide range of genes. Fifteen different bundles, ranging in size from 200 to 3000 base pairs, were recorded. Thirteen differential bundles of 350, 425, 600, 650, 700, 800, 950, 1000, 1300, 1400, 1500, 1700, and 3000 base pairs are featured by the inclusion of two public bundles of 200 and 2000. Fifteen different bundles, ranging in size from 200 to 3000 base pairs, were recorded. Thirteen differential bundles of 350, 425, 600, 650, 700, 800, 950, 1000, 1300, 1400, 1500, 1700, and 3000 base pairs are featured by the inclusion of two public bundles of 200 and 2000.

Keywords: *pseudomonas*, nitrogen fixation, and RPCR.

1. Introduction

Nitrogen fixation is a chemical process to produce ammonium from nitrogen that is already present in the atmosphere. Nitrogen, with the chemical symbol N_2, is considered as an inert compound because of undergone a

chemical reaction [1, 2]. When nitrogen gas combined with other elements it can produce new compounds [3, 4]. Upon fixing, nitrogen undergoes a transformation from its binary form N_2. Nitrogen gas amenable to a wider range of approaches [5,6]. Algae and microorganisms that repair atmospheric nitrogen produce ammonia. A portion of the ammonia evaporates into the air, while the majority is absorbed by plants [7]. Denitrification is the inverse process of nitrogen fixation and returns about the same amount of nitrogen to the atmosphere [8]. As a result of photosynthesis, the bacteria generate energy and, in exchange, fix nitrogen into a form that plants can use [9]. Moreover, to facilitate the plant's growth, the fixed nitrogen is subsequently transferred to various sections of the plant and utilized to build tissue [10]. A symbiotic interaction exists between legumes and nitrogen-fixing bacteria called "rhizobia," which live in unique nodules found in legume roots [2]. The plant gives the bacteria glucose and proteins, and the bacteria give the plant a form of nitrogen that the plant can use [4]. Because of its high concentration of nitrogenous bases (guanine and cytosine) in its DNA, its ability to use multiple sources of nitrogen, its location where mutations can occur frequently, and the ease with which genetic factors can be transferred. This microbe is utilized to perform genetic studies on nitrogen fixation.

2. Methodology

1.1 Samples Collection

The nitrogen-fixing strains of *Pseudomonas stutzeri*, or those that have already been positively identified, were collected from the wild or from laboratory cultures.

2.2. DNA Extraction

Pseudomonas stutzeri strains were collected and their genomic DNA was extracted. This can be done using regular methods for extracting DNA [11].

2.3. Random PCR Primer Design

The purpose of this research is to generate a diverse panel of PCR amplification primers. Primers chosen at random are short sequences of nucleotides that can bind to various locations in the bacterial genome. These primers can be used to initiate polymerase chain reaction (PCR) amplification at a high number of random positions within the DNA, allowing researchers to probe a wide range of genes [12].

2.4. Random PCR Amplification

Each DNA sample's PCR reaction mix was produced with the random primers already included. Perform multiple rounds of PCR using different sets of random primers. The procedure outlined here will yield a collection of DNA snippets from various parts of the bacterial genome. The term "gel" used to describe a semi-solid substance with a jelly-like consistency [13]. The subject of biochemistry makes frequent use of electrophoresis, a standard laboratory method. After obtaining amplified DNA fragments from a PCR reaction, run them through an agarose gel electrophoresis to separate them depending on size. This procedure will cause the gel to display a distinct banding pattern. The act of isolating segments of DNA. Excluding the corresponding DNA bands from the gel will reveal the amplified fragment [13].

Table 1: Sequences of 10-mer RAPD primers producing reproducible polymorphisms with *Pseudomonas stutzeri*.

Primer	Sequence (59 to 39)
270	TGCGCGCGGG
275	CCGGGCAAGC

3. Result and discussion

The procedures used to harvest DNA from 25 unique nitrogen-fixing isolates are depicted in figure 1.

Figure 1: Gel electrophoresis of genomic DNA extraction from Bacteria, 1% agarose gel at 5 vol /cm for 30 minutes.

Pseudomonas stutzeri is a multidrug-resistant opportunistic pathogen that can infect anyone. However, particularly individuals with weak immune systems or other pre-existing disorders. When people hear about this phenomenon, they often associate it with the production of infections

rather than with nitrogen fixation. Bacteria like *Rhizobium*, *Azotobacter*, and *Clostridium* are commonly thought to be responsible for nitrogen fixation because of their specialized enzymes and metabolic processes. These bacteria play a critical role in improving soil fertility by converting atmospheric nitrogen into forms that may be easily absorbed by plants. Some strains of *Pseudomonas stutzeri*, found naturally in soil, are known as nitrogen-fixing bacteria. Bacteria that fix nitrogen from the air are essential to the process of making atmospheric nitrogen usable for plants and other ecosystems. The nitrogen-fixing bacteria *Pseudomonas stutzeri* is well-known for the crucial roles it plays in a variety of habitats. However, its contagiousness and pathogenicity are more widely acknowledged. Discovering and using nitrogen-fixing bacteria in agriculture and environmental conservation is crucial to the spread of sustainable practices and the planet's health.

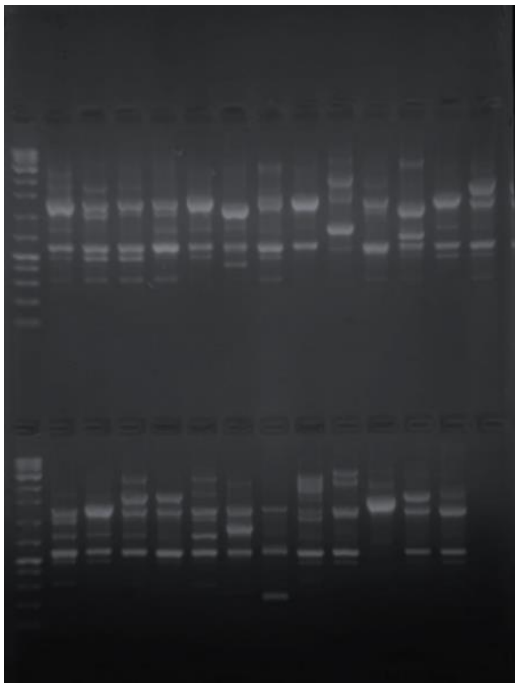

Figure 2: PCR product of primer 270 The product was electrophoresis on 2 % agarose at 5 volt/cm^2. 1x TBE buffer for 90 minutes. N: DNA ladder1000 plus bp.

Fifteen different bundles, ranging in size from 200 to 3000 base pairs, were recorded. Thirteen differential bundles of 350, 425, 600, 650, 700, 800, 950, 1000, 1300,1400, 1500, 1700, and 3000 base pairs are featured by the inclusion of two public bundles of 200 and 2000.

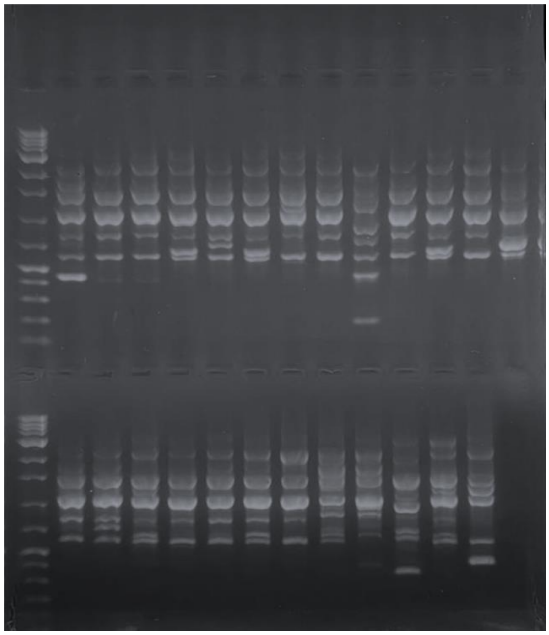

Figure 3: PCR product of primer 275 the product was electrophoresis on 2 % agarose at 5 volt/cm^2. 1x TBE buffer for 90 minutes. N: DNA ladder1000 plus bp.

There was a total of 14 different bundles found, and their sizes ranged from 250 to 3000 bp. A total of 1200 public bundles ranging in size from 250 to 3000 base pairs are represented in the dataset, along with 13 unique differential bundles. Differential bundles can be created with sizes of 250, 300, 400, 500, 600, 700, 750, 800, 1000, 1200, 1500, 1700, 2500, and 3000 base pairs. Primer 275 used the RBC approach to identify 18 genetic segments, according to the researcher [3]. The current study's findings are not entirely consistent with this outcome. However, the identical primer was claimed to identify a different number of genomic segments by a separate researcher [4].

Table 2: The prefixes outputs from the total and differentiated bundles with their efficiency ratios and discriminatory ability for the samples studied.

Primer	Number of binds	Differentiated bundles	The formal plurality of each prefix	Efficiency ratios	Discriminatory ability
270	15	13	86.66667	51.72	50
275	14	13	92.85714	48.27	50
Total	29	26			

Furthermore, (table 2) shows the number of main and different packages for each primer, as well as the efficiency and discriminatory ability of each primer. The number of main packages for the primer reached 270 (15 packages), while it's different packages (13 packages). As for its efficiency rate and discriminatory ability, it was swallowed (51.72, 50 on the number of main packages for the primer was 275) (14 packages), while the different packages for it were (13 packages). As for the rate of efficiency and discriminatory ability, it was swallowed (48.27, 50, respectively. The current study agreed with K. Thilo [5], in revealing the efficiency and discriminatory ability of the primer and did not agree with

Bingbing [8] that results related to the efficiency and discriminatory ability of the primer.

4. Conclusion

The nitrogen-fixing strain of *Pseudomonas stutzeri*, or those that have already been positively identified, were collected from the wild or from laboratory cultures. *Pseudomonas stutzeri* strain was collected and their genomic DNA was extracted to generate a diverse panel of PCR amplification primers. Primers chosen at random are short sequences of nucleotides that can bind to various locations in the bacterial genome. These primers can be used to initiate polymerase chain reaction (RPCR) amplification at a high number of random positions within the DNA, allowing researchers to probe a wide range of genes. Fifteen different bundles, ranging in size from 200 to 3000 base pairs, were recorded. Thirteen differential bundles of 350, 425, 600, 650, 700, 800, 950, 1000, 1300, 1400, 1500, 1700, and 3000 base pairs are featured by the inclusion of two public bundles of 200 and 2000. Fifteen different bundles, ranging in size from 200 to 3000 base pairs, were recorded. Thirteen differential bundles of 350, 425, 600, 650, 700, 800, 950, 1000, 1300, 1400, 1500, 1700, and 3000 base pairs are featured by the inclusion of two public bundles of 200 and 2000.

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