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THE EFFECT OF NUCLEOTIDE TRANSFER FROM SOME MICROBES TO IMPROVE PLANTS FOR BIOTECHNOLOGICAL ADVANCEMENT

TABOWEI ONUOSA CHURCHILL, JAJA T. Emylia & ADELEKE V.T. Martina

(123) Rivers State University Department of Plant Science and Biotechnology Port Harcourt.

Corresponding author: *Tabowei Onuosa Churchill
Email : Churchill.tabowei@ust.edu.ng

ABSTRACT

The advance in plant biotechnology has some challenges with the evolutionary trend and methods adopted to resolve some of these problems: to improve the host morphological and genotypic features by nucleotide alteration leading to changes in mitochondrial molecular structure in the eukaryotic and prokaryotic plants. However, some biotechnological designs used in this research are DGGE, Phoretix 1D, and the Shannon-wiener index (H). While the microbial DNA concentration, virulent qualities coupled with the adaptative features of both the microbes and host plant and bioactive compounds reduction effects on the transformed host plant were the findings from this research.

KEYWORDS

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) denature gradient, gel electrophoresis DGGE, 16srRNA (16S ribosomal RNA), this is a component of the ribosome of the prokaryotic cell with 30S subunit also 'S' found in 16s is the sedimentation coefficient, Clustered Regularly Interspaced Short Palindromic Repeats(CRISPR-Cas9) and virus (short repeat Cas9), *Thermus aquaticus* polymerase chain reaction (Taq PCR).

Introduction

Epigenetic of Plant MicrobialGene Transfer

There are different types of plant microbial interaction: Endosymbionts, parasitic, competition, commensalism, and mutualism. However, the most beneficial to biotechnological approach which could be used in the modulation of bioactive compounds and bioremediation of industrial pollutant is endosymbionts and (commensalism and mutualism)(Zhao *et al*., 2022).Moreover, humans could influence the metabolism of microbes negatively or positively depending on

The activity and the conditions in the ecosystem. Thus, in the plant ecosystem most microbes could respond to stress due to pollution, availability of nutrient supply (carbon: nitrogen), pH, temperature, molecular oxygen availability (aerobic or anaerobic), salinity, and other trace nutrients. However, when the anthropogenic product increases in the ecosystem, some microbes in the ecosystem could survive while some may not survive the environmental disturbance. Thus, the adverse restructuring of the microbial community could lead to microbial community adaptation(Zhao *et al*., 2022). The bacterial horizontalgene transfer could be greater where the cell density appears high due to cell to cell close interaction. The identification of bacteria due to 16srRNA could pose a limitation because it represents a single genomes. Hence, the horizontal gene transfer bacterial community existing in a close ecosystem interaction due to disturbance in the ecosystem would display very close features. In the face of disturbance, some microbes could remain unchanged while some microbes would easily adjust back to the original structure after been disturbed(Altieri and Biroli, 2022). However, in the face of disturbance a new microbial community could arise, although it would still have similar functions and composition like the ancestral origin. Size of microbial genomes varies from one specie to the other. Hence, the importance of gene editing to get specific size of gene needed to be transferred to specific plant host to produce a desired physiological and genetic effect. Thus, the advantage of invention of clustered regularly interspaced short palindromic repeats **CRISP** which are family of DNA sequence first found in a bacteria *Escherichia coli,archaea* andvirus (short repeat Cas9)(El-Mounadi *et al., 2020).* Although, the size of the concentration of microbial DNA also affects the horizontal gene transfer; a short DNA could transfer faster and easier than an elongated gene to a genome(IbarraCaballero *et al*., 2022)both in traditional and modern gene transfer methods.

The identification of microbial gene transfer in plant host due to interaction.

The breakthrough on the microbial ecology could have not come to the mainstream of molecular studies without the effort of Professor Thomas D. Brock and his student Freeze Hudson at Mushroom Spring Geyser Yellowstone National Park in 1966, where they discovered a pink, non-spore forming, Gram-negative, filamentous bacteria called *Caldobactertrichrogenes. Thus,* DNA isolation, bacteria growth conditions and enzymatic activity at a specific optimal temperature were carried out, protein metabolism was determined, and the taxonomy identified. Restriction endonuclease enzyme Taq1 was named after the initials of *Thermus aquaticus (*hence the technique Taq PCR polymerase chain reaction)(Gurunathan *et al*., 2021 ;Goswami, 2021).

Analysis of DNA Band and Band Detection.

The analyses of bands size, position of band and amount of band present in a denatured gradient gel electrophoresis could be estimated using Phoretix 1D software(Honglei et al., 2018). Thus, Phoretix 1D software could measure the number of band present in the gel and retardation factor (RF), hence RF could give the specific position of each bands on the gel. The RF value normally backs up the melting point theory and covers the error margin of ± 0.01(Phoretix 1D software manufacturer's

instruction). (Honglei et al., 2018)In addition, the peak height and number of band present in a gel could be extrapolated into the Shannon-Wiener index to calculate the species abundance and the species relative abundance in a gel, as a representative of microbial communities. However, the number of species in a sample equals one when (H=0),where the species pictures and the total species present would be represented in the sample H= maximum while Shannon-wiener index (H), represent an information model used to estimate the mean rate of uncertainty when the degree of uncertainty rises, the species number increases with even distribution(Castro *et al*., 2022). However, the importance of horizontal gene transfer from some microbes to plants for biotechnological advancement triggered this research work.

Materials and Methods

Rivers State University Biology Laboratory and Research farm where use for field trial, Phoretix 1D and Shannier Wannier index while PCR and DGGE were carried out in University of Port Harcourt Regional Centre for Bioresearch (RCBBR).

Microorganism and Plant Extraction

The microorganisms that were used in this research include three strains: *Bacillus licheniformis, Bacillus substili, and Pseudomonas putida* while eight leaves of *Cucurbita maxima* were plucked in triplicate from each bed of transgenic plants and immediately put into an icebag labeled properly with modification from (Ali *et al*., 2019)and were transported to (RCBBR) for processing. Also, the Subculturing was done after gram staining, DNA isolation, PCR, Agarose gel electrophoresis and DGGE.

DGGE

The DGGE was performed by use of Ingeny System with little modification from (Sabu *et al*., 2018) using the following standard: 1-milimeter gel, denaturant gradient of 45% to 75% Acrylamide, urea and formamide at temperature of 70^{o}C with a constant voltage supply of 110V for 12hours.
Gel Band and Statistical Analysis

Phoretix 1D software was used to analysis the DNA band size while the Shannon-wiener index (H)with modification from (Rabae et al., 2020)was used to study microbial relative abundance in the host genomes observed in each gel lane.

RESULT

The gel lanes as observed using phoretix 1D extrapolated from the DGGE showing microbial relative abundance depicting level of invasiveness between each microbes from the peak height.

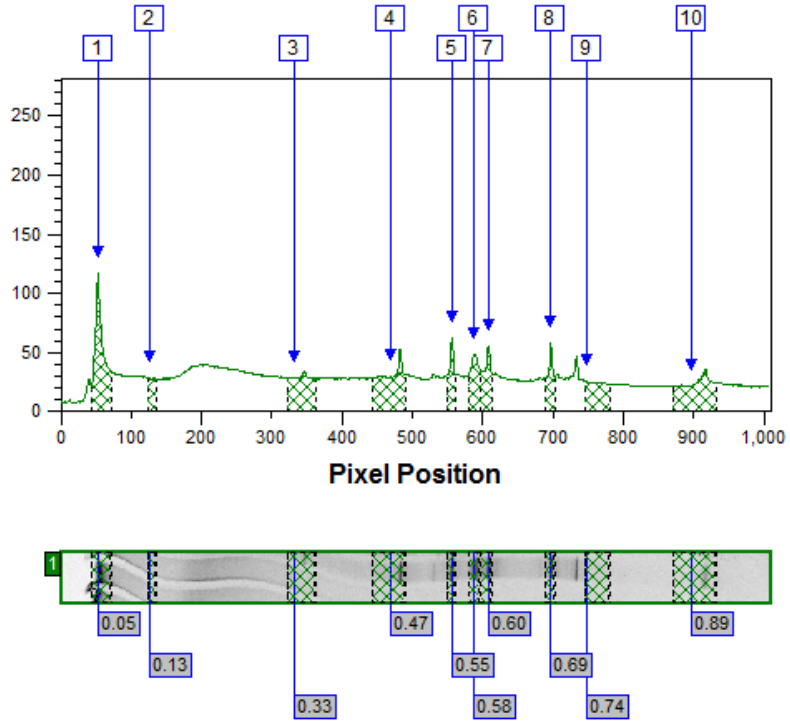

Figure 1. showing gel 1 lane 23 depicting the relative abundance of microbial band in each lane

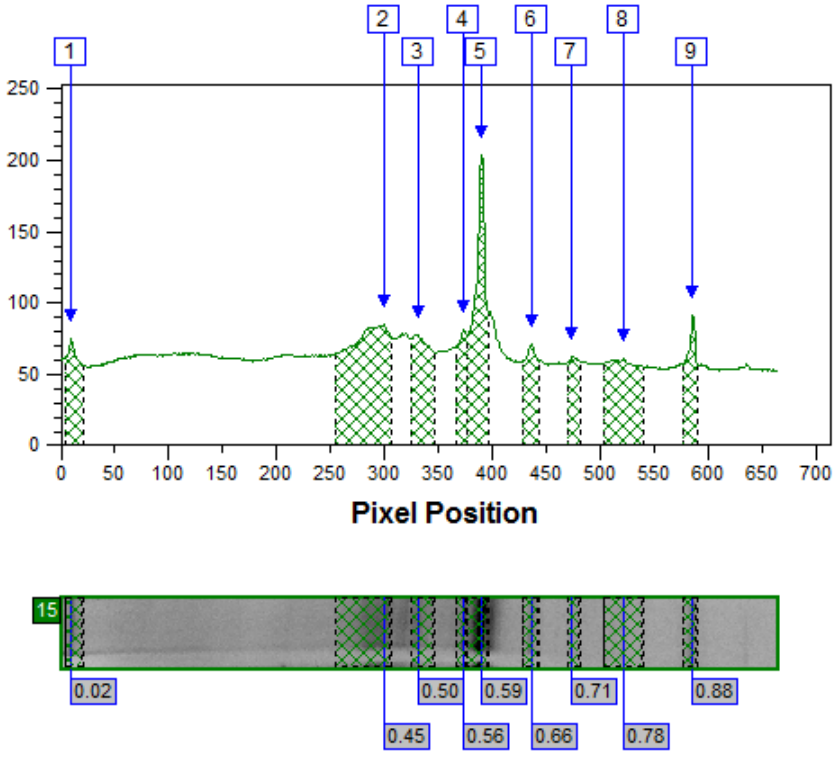

Figure 2. showing gel 2 lane 20 depicting the relative abundance of microbial band in each lane

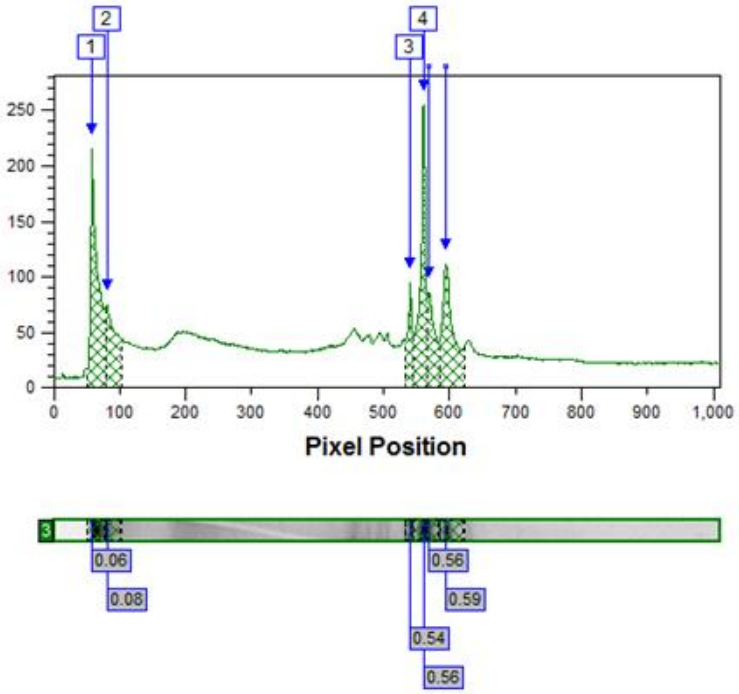

Figure 3. showing gel 3 lane 18 depicting the relative abundance of microbial band in each lane

Discussion

From the study conducted it was observed that microbial relative abundance identified during the research of samples in plant microbial genomes having fewer DNA peak migrated faster in the plant host. This could be due to the concentration or quantity of DNA isolated from microbial gene edited, delivered to the plant host, DNA isolated and observed using DGGE in line with the previous researcher (Na etal., 2016). Although, the broader the relative abundance of DNA band and size the slower the flow of the DNA in the gel lanes loaded, this would also influence their mobility and virulent in any ecosystem they are found in line with the earlier researcher (Quispe-Huamanquispe *et al*., 2017).

The transformation and transfer of inherit gene to the host plant was determined by the concentration of the microbial DNA edited and delivered to cause knockin or knock out of specific DNA sequenced. However, the bioactive compounds in plant could be managed by the direct insertion of microbial genomes into the plant host serving as a modern plant biotechnology model for controlling the rate of bioactive compounds in plant host as reported from the previous research (Tabowei et al., 2021) depicting that some vital gene could be inherited from microbial genomes when transformed directly using modern method (CRISPR-Cas9) or any traditional system. This is one of the limitation following the traditional method. Thus, microbial probiotic features are determinant factors to be used before an organism DNA is transferred directly into a plant genomes as researched by (Tabowei *et al*., 2021). The clustering of microbes in the gel lanes could also depict that the invasiveness of some microbes in certain suitable substrates would enhance their virulence probably due to the susceptibility of the host genomes possessing some open ligands to accept the molecular traits from the microbes in the case of horizontal transfer gene researched by(Quispe-Huamanquispe *et al*., 2017). Also, a research observed *Cucurbita maxima* have the ability to accept genomes directly from

Bacilluslicheniformis with *phy*L gene altering nucleotide molecular structure resulting to frame shift mutation cited by (Tabowei *et al*., 2021).

Conclusion

Nonetheless, the effect of Nucleotide transfer from some microbes to improve plants for advancement of biotechnological produce, could trigger more horizontal gene transfer from microbial sources to plant host genomes. Since the research aid and objectives would also be achieved using both traditional and modern technique gene editing and transformation, hence, the desired traits of interest could be achieved using gold standard of biotechnological protocol adherence. Thus, the relative abundance of each microbial genomes identified in transgenic plant could determine the type of nucleotide altered, affecting the final protein translated due to the concentration of the microbial genomes transferred thereby influencing various bioactive compounds, mitochondrial molecular restructuring and inducting the plant for a viable bioremediation toolbox.

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