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Growth Responses of Some Bacterial Isolates to Some Environmental Parameters

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Abstract

This study was carried out to investigate the antimicrobial activities of bacterial isolates of maize against plant pathogens as well as their growth responses to some environmental parameters. Twenty four bacterial isolates were obtained from maize plants collected from the Department of Biological Sciences, AfeBabalola University, Ado-Ekiti. The isolates were characterized by their biochemical and physiological characteristics and were identified as *Kurthiazopfu*, *Morganellamorganic*, *Rhodococcusequi*, *Bacillus subtilis*, *Catabaterhongkongensis*, *Brevibacteriumotitidis*, *Lactobacillus coleohominis*, *Staphylococcus aureus*, *Propionibacterium acnes* among others. Their responses to different NaCl concentrations, sugars, temperature as well as antibiotics were determined. Most of the isolates were able to withstand various environmental parameters in which they were subjected to. Also, eight isolates were able to ferment sucrose. The bacterial isolates showed a degree of resistance to the antibiotics tested. There was a high prevalence of multidrug resistant bacteria showing resistance to 3 - 8 drugs. The antagonistic effect of the bacterial isolates against selected fungi was determined. None of the isolates showed antagonistic potential against the fungal pathogens. However, the supposed antagonistic bacterial species can be genetically modified to produce secondary metabolites that will result in biocontrol.

Keywords

Maize Plants, Growth Responses, Bacterial Isolates, Secondary Metabolites, Biocontrol

1. Introduction

Plant diseases are threat to world agriculture and general food security of which

fungi cause about 75% of the diseases [1]. Significant yield losses due to pathogens' attack have been shown to occur in most agricultural and horticultural crops [2] [3]. About 25 million NGN was lost in Nigeria due to black pod disease which occurred in 1995 [4] [5].

Chemicals such as fertilizers and pesticides including fungicide have been used in the past in controlling plant diseases leading to increase productivity [3]. However, due to the negative effects of these chemicals on the environment and non target organisms, alternatives have been sought in controlling plant diseases and these alternatives include the use of microorganisms among others [3] [6]. Bacteria have been used as biological control agents for several decades [7]. Different plant associated bacteria often associated with and their metabolites have been identified as important contributors to the biological control of plant diseases [8]. Their mode of action has been linked to antibiotics such bacteria produce during their stationary phase. For example, biocontrol strains of *Pseudomonas* are known to synthesize phenazine carboxylic acid, pyrronitril and pyoluterin [9] [10] [11] [12]. Also, *Bacillus* species which are often associated with agricultural systems are known to be involved in biocontrol since they are capable of producing antibiotics [13] [14] [15] [16].

Growth of microorganisms is influenced by various environmental conditions and the effect may either favor their growth or retard their multiplication rate and the synthesis of different metabolites [17]. Environmental parameters such as temperature, aeration, nutrients or pH can become limiting factors for microorganism's survival. Biocontrol agents have been shown to be sensitive to varying environmental conditions such as temperature, pH and moisture content and these factors affect their usefulness. This current investigation therefore sought to determine the responses of maize associated bacteria to some environmental conditions as well as their antimicrobial properties.

2. Materials and Methods

2.1. Collection of Samples

Twenty four unidentified bacterial isolates were collected from the Microbiology unit of AfeBabalola University, Ado-Ekiti. The isolates were isolated from different parts of maize plants. *Ralstoniasolanacearum* strain Ogbomoso, *Ralstoniasolanacearum* strain Saki, *Ralstoniasolanacearum* strain Nihort, *Fusarium equiseti* strain Saki, *Trichoderma viride* from the Department of Biological Sciences, AfeBabalola University Ado-Ekiti, and *Pseudomonas* spp. were obtained from Ibadan. Also, a fungicide; Mancozeb was collected from AfeBabalola University Ado-Ekiti, Ekiti Farm.

2.2. Characterization of the Bacterial Isolates

The unidentified bacterial isolates were identified based on their morphological, cultural and biochemical characteristics after Gram staining according to standard

procedures [18].

2.3. Growth Characteristics of the Bacterial Isolates at Different Temperature

The test was done to identify organisms that can grow at different temperature (4˚C, 25˚C, 37˚C, 50˚C) for 24 h on nutrient agar plates. Presence of growth showed the organisms can tolerate the temperature [19].

2.4. Growth Characteristics of the Bacterial Isolates at Different pH

The test was done to identify organisms that can grow at different pH (2, 4, 6, 8, 10). Nutrient broth (Lab M) was prepared according to the manufacturer's procedure and their pH was adjusted to 2, 4, 6, 8 and 10 using Cacodylate and Succinic acid buffer solutions (brand name). The prepared broth were inoculated with the organisms and incubated at 37˚C for 24 h. The presence of growth was determined by the absorbance using the SP 600 Spectrophotometer (brand name) at wavelength of 600 nm.

2.5. Growth Characteristics of the Bacterial Isolates at Different Concentrations of NaCl

The test was done to identify organisms that can grow at different concentration of NaCl (1%, 2%, and 3%). Nutrient agar was prepared according to the manufacturer's procedure and the NaCl was added to the media. The prepared agar was inoculated with the organisms and incubated at 37˚C for 24 h. The presence of growth shows the organisms can utilize the salt and survive high salinity [19].

2.6. Antibiotic Susceptibility Test

The antimicrobial susceptibility testing was done using agar disk diffusion method. Fresh isolates were suspended in peptone broth in comparison to 0.5 McFarland standards. Each of the isolates was inoculated onto the surface of a sterile Mueller Hinton Agar plates using sterile swab in order to ensure even distribution while streaking. The plates were allowed to dry for 10 minutes and the antibiotic disc were placed on the surface of the agar plates using a sterile forceps. The plates were then inverted and incubated for 24 h at 37˚C. The antimicrobial disc includes the Gram negative disc which serves as a positive control for Gram negative organisms; Augmentin (30 µg), Ofloxacin (5 µg), Gentamicin (10 µg), Nalidixic acid (30 µg), Nitrofuratoin (200 µg), Cotrimoxazole (25 µg), Amoxycillin (25 µg), and Tetracyclines (25 µg). For the Gram positive organisms, Erythromycin (5 µg), Gentamicin (10 µg), Augmentin (30 µg), Streptomycin (10 µg), Tetracycline (10 µg), Chloramphenicol (10 µg), Cloxacillin (5 µg) and Cotrimoxazole (25 µg). The zone of inhibition was then measured and recorded according to the Clinical and Laboratory Standard Institute [20]. The tests were done in duplicate to ensure reliability [21].

2.7. Growth Characteristics of the Bacterial Isolates on Different Sugars

The test was done to identify organisms that can grow and utilize 2 sugars (glucose and sucrose) to determine if the organisms can ferment the sugars. Sugar solution was prepared according to the manufacturer's procedure and 5 ml was dispensed into test tube with durham tubes. The prepared solution was inoculated with the organisms and incubated at 37˚C for 24 - 72 h. Pale yellow indicated positive, negative no colour change was observed and gas production in the durham tubes also indicated a positive result.

2.8. Antagonistic Effect of Bacterial Isolates against the Test Fungi and *Pseudomonas* sp.

Each bacterial isolate was streaked on already prepared PDA plate and fungal plug was placed 23 mm away from the bacterial isolates in duplicates. The plates were then incubated at 25˚C for 1 - 7 days. Petri dishes inoculated with a fungus only were used as control treatment Antagonistic effect was determined according to Kucuk and Kivanc [22]. For *Pseudomonas* sp. a perpendicular streak was made.

2.9. Effect of Fungicide on the Selected Fungi

The test was done to identify organisms that can grow in the presence of the selected fungicide. The medium, PDA was prepared according to manufacturer's procedure and 0.01 g, 0.02 g and 0.03 g of the fungicide was added to the media. The poisoned medium was then inoculated with the fungal plugs and incubated at 25˚C respectively (Ogunmefun *et al*., 2015).

Growth on the media indicates that the organisms can survive in the presence of the fungicide.

3. Results

3.1. Growth Responses of the Bacterial Isolates to Different Environmental Parameters

The growth response of the bacterial isolates from maize plants to NaCl and temperature is represented in Table 1. Maximum growth was observed at 1%, followed by 2% and at 3% there was a reduction in growth. Growth response of the bacterial isolates from maize plants to different pH grown in nutrient broths is represented in Table 2. There was a progressive increase in the absorbance from pH 2 to 8. It was also observed that least growth was obtained from *Catabaterhongkongensis* 1 at pH 2 while the highest was *Rhodococcusequi* 1. Also there was reduction in the absorbance at pH 10 except *Vibrio fluvialis*.

3.2. Susceptibility Pattern of the Bacterial Isolates to Antibiotics

The antibacterial susceptibility of gram negative and gram positive bacterial are represented in Table 3 and Table 4 respectively. It was observed that all gram

Table 1. Qualitative growth response of bacterial isolates from maize plants to NaCl and temperature.

Bacterial isolates	Growth on NaCl			Temperature (˚C)			
	1	2	3	4	25	37	50
Catabaterhongkongensis 1	+	+	+	−	+	+	−
Anaerococcustetradius	+	+	+	+	+	+	−
Staphylococcus aureus 1	+	−	−	+	+	+	+
Corynebacterium argentoratense	+	+	+	−	+	+	−
Clostridium clostridiforme	+	+	+	+	+	+	−
Alistipeindistinctus	+	+	+	+	+	+	+
Proteus vulgaris	+	+	+	+	+	+	+
Pseudomonas aeruginosa	+	−	−	+	+	+	+
Leifsonia aquatic	+	+	+	+	+	+	−
Kurthiazopfu	+	+	+	−	+	+	−
Morganellamorganic	+	+	+	+	+	+	+
*Rhodococcusequi*1	+	−	−	−	+	+	−
Bacillus subtilis	+	−	−	+	+	+	+
Catabaterhongkongensis 2	+	+	+	+	+	+	+
Brevibacteriumotitidis	+	−	−	+	+	+	+
Rhodococcusequi 2	+	−	−	+	+	+	−
Lactobacillus coleohominis	+	+	+	+	+	+	+
Staphylococcus aureus 2	+	+	+	+	+	+	+
Rhodococcusequi 3	+	+	+	+	+	+	−
Propionibacterium acnes	+	+	+	+	+	+	−
Neisseria oralis	+	+	+	+	+	+	+
Catenibacteriummitsuokai	+	+	+	−	+	+	+
Legionella pnuemophila	+	+	+	+	+	+	−
Vibrio fluvialis	+	+	+	+	+	+	+

Keys: + = positive, − = negative.

positive organisms showed more than 50% resistance to the antibiotics used except Gentamycin (31.25%) and Streptomocin (50%) (Table 3). More than 50% of the Gram negative bacteria showed 100% resistance to the antibiotics tested. Generally. *Alistipesindistinctus*, *Proteus vulgaris* and *Vibrio fluvialis* were resistant to all the antibiotics (Table 4).

3.3. Antagonistic Activities of the Bacterial Isolates against the Selected Fungi

None of bacterial isolates had any antagonistic effect on fungi. Both organisms on the plates grew simultaneously without interfering with each other. However, there was reduction in the mycelia growth of the fungi in the presence of fungicide compared to when cultured on only PDA plate.

Table 2. Comparative growth response of bacterial isolates from maize plants to different pH grown in nutrient broths.

Bacterial isolates	pH range				
	2	4	6	8	10
Catabaterhongkongensis 1	0.42	0.58	1.51	1.51	0.70
Anaerococcustetradius	0.67	0.95	1.42	1.47	0.78
Staphylococcus aureus 1	0.52	0.97	1.37	1.49	0.75
Corynebacterium argentoratense	0.60	1.48	1.31	1.50	0.70
Clostridium clostridiforme	0.78	0.52	1.45	1.27	0.53
Alistipeindistinctus	0.62	1.42	1.51	1.50	1.41
Proteus vulgaris	0.67	1.45	1.51	1.51	1.33
Pseudomonas aeruginosa	0.57	0.90	1.29	1.49	0.65
Leifsonia aquatic	0.67	0.54	1.47	1.51	0.47
Kurthiazopfu	0.53	0.54	1.50	1.50	0.54
Morganellamorganic	0.69	1.13	1.51	1.52	0.55
Rhodococcusequi 1	0.93	0.52	1.36	1.46	0.54
Bacillus subtilis	0.57	0.55	1.51	1.33	0.70
Catabaterhongkongensis 2	0.57	0.61	1.51	1.50	0.47
Brevibacteriumotitidis	0.55	0.58	1.30	1.50	0.77
*Rhodococcusequi*2	0.58	0.53	1.28	1.00	0.54
Lactobacillus coleohominis	0.58	0.64	1.34	1.26	0.48
Staphylococcus aureus 2	0.58	0.56	1.48	1.45	0.98
*Rhodococcusequi*3	0.49	0.54	1.35	1.24	0.63
Propionibacterium acnes	0.57	0.73	1.21	1.25	0.86
Neisseria oralis	0.71	0.69	1.48	1.50	1.23
Catenibacteriummitsuokai	0.53	0.55	1.32	1.48	0.72
Legionella pnuemophila	0.52	0.60	1.49	1.52	0.47
Vibrio fluvialis	0.72	0.75	1.50	1.51	1.40

4. Discussion

Bacterial diversity is of particular importance in human sustenance since they comprise the majority of earth's species. It is also considered as one of the most useful resources with considerable significance in bioremediation and bio-prospecting [23].

In this study, different parameters were put into consideration such as responses to different pH, temperature and NaCl. Environmental factors may influence plant pathogens, biocontrol agents and the mechanisms of their interactions [24]. All the twenty four bacterial species were found to be mesophiles as they grew at a maximum temperature of 37˚C. Thirteen of the isolates grew at 50˚C which show that they were thermophiles. Similar observations were made by Javed *et al.* [25]. *Bacillus* spp. have been shown to live and survive in inhospitable environments including hot springs [26] [27].

Table 3. Antibiotic susceptibility patterns of selected Gram positive bacteria to antibiotics.

Organisms	Cotrimoxazole (25 µg)	Cloxacillin (5 µg)	Erythromycin (5 µg)	Gentamycin (10 µg)	Augmentin (30 µg)	Streptomycin (10 µg)	Tetracycline (10 µg)	Chloramphenicol (10 µg)
Catabaterhongkongensis 1	21(S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Anaerococcustetradius	15 (I)	0 (R)	0 (R)	20 (S)	0 (R)	20 (S)	13 (R)	16 (S)
Staphylococcus aureus 1	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Corynebacterium argentoratense	46 (S)	23 (S)	19 (S)	13 (I)	16 (S)	20 (S)	23 (S)	14 (I)
Leifsonia aquatic	0 (R)	0 (R)	0 (R)	19 (S)	0 (R)	0 (R)	0 (R)	0 (R)
Kurthiazopfu	0 (R)	0 (R)	0 (R)	19 (S)	0 (R)	18 (S)	0 (R)	15 (I)
Rhodococcus equi 1	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Bacillus subtilis	41 (S)	0 (R)	11 (R)	29 (S)	0 (R)	19 (S)	0 (R)	0 (R)
Catabacterhongkongensis 2	46 (S)	0 (R)	0 (R)	20 (S)	0 (R)	16 (S)	15 (I)	0 (R)
Brevibacteriumotitidis	0 (R)	0 (R)	0 (R)	22 (S)	0 (R)	14 (I)	0 (R)	33 (S)
Rhodococcusequi 2	0 (R)	0 (R)	0 (R)	18 (S)	0 (R)	21 (S)	0 (R)	29 (S)
Lactobacillus coleohominis	26 (S)	0 (R)	18 (S)	30 (S)	0 (R)	26 (S)	18 (S)	20 (S)
Staphylococcus aureus 2	20 (S)	0 (R)	0 (R)	24 (S)	0 (R)	11 (R)	14 (I)	18 (S)
Rhodococcusequi 3	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Propionibacterium acnes	16 (S)	18 (S)	25 (S)	28 (S)	32 (S)	15 (I)	18 (S)	13 (I)
Catenibacteriummitsuiokai	0 (R)	0 (R)	0 (R)	23 (S)	0 (R)	22 (S)	0 (R)	33 (S)
Susceptibility (%)	43.75	12.57	18.75	68.75	12.50	50	18.75	37.5
Resistance (%)	56.25	87.43	81.25	31.25	87.50	50	81.25	62.5

Key: I—Intermediate, S—Susceptible, R—Resistant.

All the isolates grew at pH 2, 4, 6, 7, 8 while seventeen isolates grew at pH 10, as opposed to the work of Javed *et al.* [25]. It was also observed that all the twenty four organisms grew 1% NaCl concentration, whereas only eighteen organisms grew at 2% NaCl concentration and seventeen organisms grew at 3% NaCl concentration. There was growth reduction at 2% and 3% concentration because they were halosensitive and couldn't tolerate high salinity. Jhala *et al.* [28] reported that endophytic bacteria can tolerate NaCl concentration up to 4%.

All the isolates showed varying degrees of resistance to commercial antibiotics. Although *C. equi* 1 and 3 were obtained from different sources both of them were resistant to antibiotics. The highest degree of resistance was observed among the Gram negative bacteria investigated in this study. Similar observation was made by Osibote *et al.* [29]. Iroha *et al.* [30] opined that the problem of antibiotic resistance in microorganisms may be due to the natural resistance of definite species to certain antibiotics, the transfer of antibiotic resistance among species and the use of sub-therapeutic doses of antibiotics.

Table 4. Antibiotic susceptibility patterns of selected Gram negative bacteria to antibiotics.

Bacterial isolates	Augmentine (30 µg)	Ofloxacin (5 µg)	Gentamincin (10 µg)	Nalidixic acid (30 µg)	Nitrofurantoin (300 µg)	Cotrimoxazole (25 µg)	Amoxycillin (25 µg)	Tetracycline (25 µg)
Clostridium clostridiforme	0 (R)	23 (S)	0 (R)	28 (S)	0 (R)	0 (R)	0 (R)	0 (R)
Alistipesindistinctus	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Proteus vulgaris	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Pseudomonas aeruginosa	0 (R)	31(S)	0 (R)	25 (S)	15 (I)	0 (R)	0 (R)	0 (R)
Morganellamorganic	0 (R)	14 (I)	0 (R)	16 (S)	0 (R)	0 (R)	0 (R)	0 (R)
Neisseria oralis	0 (R)	16 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Legionella pneumophila	0 (R)	22 (S)	0 (R)	16 (S)	11 (R)	0 (R)	0 (R)	0 (R)
Vibrio fluvialis	0 (R)	15 (I)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Susceptibility (%)	0	37.5	0	50	0	0	0	0
Resistance (%)	100	62.5	100	50	100	100	100	100

Key: I—Intermediate, S—Susceptible, R—Resistant.

The identification and characterization of microorganisms, useful as biocontrol agents or as producers of bioactive compounds, are of great relevance for the modern and ecocompatible agriculture [31]. Antibiosis often acts in concert with competition and/or parasitism [20]. The results of this investigation showed that none of the bacterial isolated were antagonistic to the fungal test pathogens tested at 25˚C on PDA.

5. Conclusion and Recommendation

Although none of the bacterial isolates used in this study exhibited antagonistic effect against the isolated fungal pathogens, the isolates can be genetically modified to produce secondary metabolites that will result in biocontrol. Also, since some of the isolates were thermophilic, further work is therefore needed to test their antagonistic properties against the fungal pathogens at high temperature.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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