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Isolation and Antibiotic Susceptibility of Aerobic Bacterial Food Pathogens Associated with Cassava Products

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Abstract: Indicator bacteria of pathogenic and food spoilage capabilities were isolated from grated cassava, starch and flour using MacConkey agar, Nutrient agar, KF agar and Mann Rogosa Sharpe medium. The most frequently encountered bacterial isolates were *Bacillus* sp. (42.86%). Plasmids DNA were isolated from *B. pumilus, B. sphaericus, B. subtilis* and *Pseudomonas flourescens*. The sensitivity patterns of the isolates showed common resistance to Erythromycin, Gentamycin, Cotrimozadole, Amoxycillin and Nalixidic acid among all isolates while *P. flourescens* and *B. subtilis* were resistant to Ampiclox. *P. flourescens* was most resistant to both discs followed by *B. pumilus* and *B. sphaericus*. *B. substilis* was found to be most sensitive to Cotrimidazole (14.0 mm) followed by Ofloxacin (13.0 mm), Tetracycline (12.0 mm) and Amoxycillin and Augmentin (10.0 mm). *P. flourescens* was found to be most sensitive to Ofloxacin (12.0 mm), Gentamycin and Chloramphenicol (3.0 mm) among the Gram-negative isolates. The resistance of the isolates to most antibiotics probably resulted from the acquisition of resistance plasmids DNA. These plasmids confer both toxigenicity and resistance factor characteristics on the bacteria.

Key words: Bacterial strains, antibiotic sensitivity, antibiotic resistance plasmid

INTRODUCTION

Microbes present in food may cause food spoilage or disease when consumed. For foods to be of good sanitary quality, they must be shown to be free of hazardous micro-organisms or those present should be at a safe low level. The quality of food is therefore determined by their content of indicator organisms; their presence or number serve to indicate the condition or quality of materials. Most foods are regarded as unwholesome when they have large population of micro-organisms even if the organisms have not altered the characteristics of the food[1]. Abba-Kareem and Okagbue[2] reported that cassava flour samples are highly contaminated with various groups of micro-organisms including bacteria, lactose and non-lactose fermenting *Enterobacteriaceae*. The aerobic spore-forming bacteria have been enumerated and used to investigate quality of cassava flour. These organisms have been implicated in spoilage of bread and starch-based foods and in food intoxication[3].

Antibiotics are widely used in combating bacterial infections. The molecular basis of action is well understood[4-6]. The key to antibiotic action is a selective toxicity for the infecting organism but not for the patient[7]. Bacterial resistance to antibiotics is a major therapeutic problem[8]. The transfer of plasmid-mediated resistance is the most important in the emergence of resistant organisms. The extra-chromosonal resistance factors in Gram-negative bacteria are called R-factors. With recent scientific and biotechnological discoveries, various strains of micro-organisms with unique physiological and biochemical characteristics have emerged from the environments. Thus, much attention is focused on assessing the impacts such organisms have on the ecological environments. Their clinical effects on human health, industrial values and other socio-economic importance are also considered. Similarly, some normal flora of foods may be pathogenic which invariably may lead to infection. Branca *et al*.[9] reported a link between diet and disease by ascertaining their biological outcome. The study of the antibiotic sensitivity profile of the organisms therefore becomes necessary to effectuate treatment or control. This present study was undertaken to determine the antimicrobial sensitivity patterns of indicator and other food bacterial pathogens present in cassava products with a view to proffer treatment or control and detect the presence of genetic plasmids containing antibiotic resistant determinants.

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MATERIALS AND METHODS

Bacterial strains: *Bacillus subtilis, B. pumilus, B. sphaericus, Pseudomonas flourescens, P. aeruginosa, Lactobacillus brevis, Escherichia coli, Staphylococcus aureus, Proteus* sp. were isolated from three cassava products viz: Grated cassava, starch and flour[10].

Plasmid isolation: Plasmids DNA were isolated from 5 bacterial strains both by alkali lysis and glucose mediated detergent lysis methods. Agarose gel electrophoresis was done including a DNA molecular weight marker as molecular size marker. Plasmid DNA was electrophoresed in 1.4 percent w/v agarose gel (Sigma, USA) in TB-EDTA buffer (Sigma, USA). Gels were visualised by staining with ethidium bromide and banding patterns photographed over uv light using a red filter. The investigations using these strains were carried out at the Nigerian Institute of Molecular Research (NIMR) Yaba, Lagos, Nigeria.

Antibiotic susceptibility pattern: Determination of susceptibility of the bacterial isolates to 12 different antibiotics was determined by the disc agar diffusion technique[11] using antibiotics discs (Oxoid). For the Gram-positive isolates: Amplicox, Chloramphenicol, Erythromycin and Cloxacillin. For the Gram-negative isolates: Nitrofuratoin, Nalicidic acid and Oflaxocin. For both types of isolates single discs of Gentamycin, Augmentin, Tetracycline, Amoxycillin and Cotrimozadole were applied.

RESULTS AND DISCUSSION

Microbial contamination occurs as a result of the presence of micro-organisms found in food. The introduction of antibiotics for the chemotherapy of bacterial infections has been one of the most important achievements of the past years. The sensitivity patterns of each bacterial isolate to the antibiotics is shown in (Table 1). The organisms were generally resistant to

achievements of the past years. The sensitivity patterns of each bacterial isolate to the antibiotics is shown in (Table 1). The organisms were generally resistant to Erythromycin, Ampiclox, Cotrimodazole, Amoxycillin and Nalixidic acid. Among the Gram positive isolates, *B. pumilus* was found to be most resistant to eight antibiotics. *B. subtilis* was most sensitive to all antibiotics except Nalixidic acid and Ampiclox. *Bacillus* species are widely distributed in nature. Their ability to develop such a wide range may be associated with its reduction in the antibiotic uptake (impermeability or efflux), ability to degrade the drug (enzymatic attack), modification of specific target sites, overproduction of the target or bypass of the antibiotics sensitive step by duplication of the target site[3,8]. Among the Gram negative bacterial pathogens, *P. flourescens* was found to be most sensitive to Ofloxacin (12.0 mm), Chloramphenicol and Gentamycin (3.0 mm) and resistant to other antibiotics screened. The broad spectrum antibiotics were more effective on Gram positive bacteria than the Gram negative organisms. This may be due to differences in the cell wall composition i.e. the presence of peptidoglycan. Gentamycin and Ofloxacin were effective against both Gram positive and Gram negative bacteria. Roger *et al.*[12] reported similar result. The active components of such antibiotics are well refined and concentrated; no impurities to lower their effectiveness. The values obtained for *E. coli* sensitivity correspond relatively with the standard Minimum Inhibitory Concentration (MIC) specified by Abraham *et al.*[13].

Plates 1 and 2 showed the migration of eight different plasmids, two fragments each of *P. aeruginosa*, *B. sphaericus*, *B. pumilus* and one fragment each of *P. flouresens* and *B. subtilis*. A few *Pseudomonas* are pathogenic among which is *P. aeruginosa*, an obligate parasite. The organism is naturally resistant to many widely used antibiotics. Resistance is frequently due to a resistance factor-R factor which is a plasmid carrying gene coding for detoxication of various antibiotics or a gene whose product is an enzyme that destroys a specific antibiotic. The resistance of *B. pumilus* to tetracycline

Table 1:Antibiotic sensitivity patterns of bacterial isolates

	Diameter of inhibition zone (mm)											
Organisms	COT	CHL	CXC	ERY	AMP	NIT	NAL	OFL	GEN	AUG	TET	AMX
Bacillus sphaericus	0	6	1	0	1	8	2	10	6	6	5	0
Bacillus subtilis	14	7	3	3	0	6	0	13	8	10	12	10
Bacillus pumilus	0	3	0	0	0	0	0	12	3	2	0	0
Pseudomonas flourescens	0	3	0	0	0	0	0	12	3	0	0	0
Escherichia coli	0	7	0	7	ND	8	2	9	8	1	2	1

*Plates 1 and 2 (Pages 57 and 58)

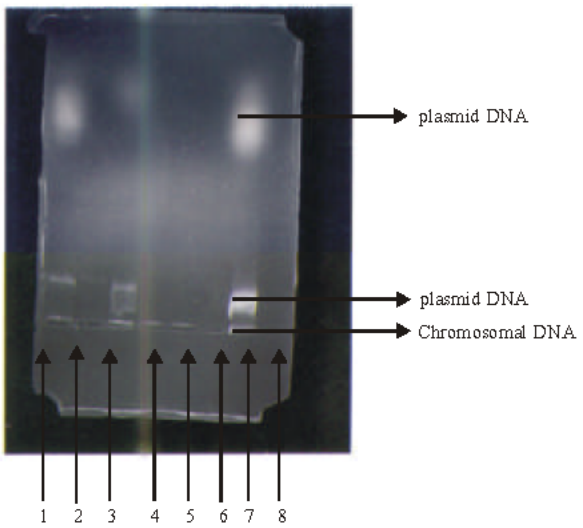

Plate 1: A gel electrophoregram showing the migration of six different plasmids, two fragments each of *P. fluorescens*, *B. sphaericus* and *B. pumilus*. Movement is from bottom to top. Each circular column (lane) represent a single plasmid

1. DNA marker, 2. *Pseudomonas fluorescens*,
3. *Klebsiella aerogenes*, 4. *Bacillus sphaericus*
5. *Staphylococcus aureus*, 6. *Escherichia coli*
7. *Lactobacillus brevis*, 8. *Bacillus pumilus*

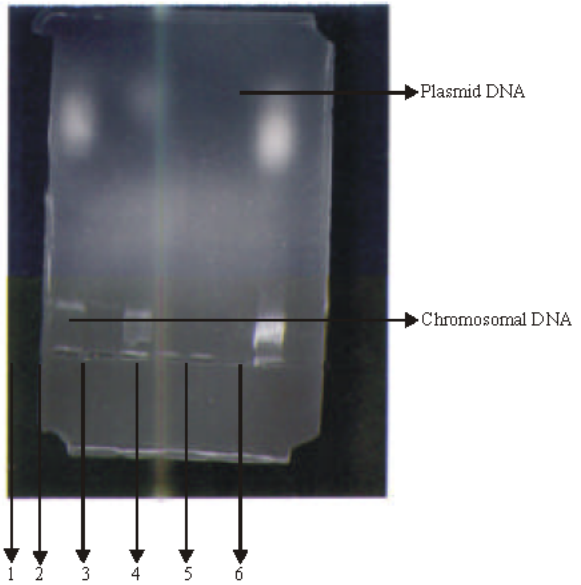

Plate 2: A gel electrophoregram showing the migration of two different plasmids, one fragment each of *P. aeruginosa* and *B. subtilis*. Movement is from bottom to top. Each circular column (lane) represent a single plasmid

1. DNA maker, 2. *Leuconostoc mesenteroides*
3. *Pseudomonas fluorescens*, 4.*Corynebacterium* sp.
5. *Proteus* sp., 6. *Bacillus subtilis*

probably resulted from the acquisition of plasmids[14]. The results suggest that the resistance of the bacterial isolates to a number of antibiotics resulted from acquisition of resistance plasmids DNA. The antimicrobial susceptibility patterns showed that the screened isolates were all susceptible to Ofloxacin, Gentamycin and Chloramphenicol employed in this study. Therefore, a discriminate use of any of these antibiotics will effectuate treatment or control for any resulted infection.

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