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Short Communication

Genetic variation and relationship in *Staphylococcus aureus* isolates from human and food samples using random amplified polymorphic DNAs

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A genetic characterization of 18 different isolates of *Staphylococcus aureus* using random amplified polymorphic DNAs (RAPDs) was carried out. Out of one hundred primers tested, ten showed polymorphism. The amplification reactions with the 10 primers generated 88 bands, 51 of which is polymorphic with band size ranging between 200 and 3,000 bp. Variation and relatedness between different isolates were determined by converting RAPD data into a Jaccard similarity matrix and analysed by UPGMA (unweighted pair-group method, arithmetic average) to produce completely twelve different groups at 100% Jaccard similarity and at 50% coefficient of similarity. The isolates were classified into two major groups, the first comprises of mildly and weakly virulence, while the other group are the highly virulence *Staphylococci*. The results demonstrated that the RAPD technique may be of great use in the classification of *S. aureus.*

Key words: *Staphylococcus aureus*, Genetic classification, RAPD technique.

INTRODUCTION

Staphylococcus aureus are Gram positive cocci in clusters. They cause a variety of superficial and deep infections, in most cases pus-forming in man. They are frequently found as contaminants in clinical specimens taken from the body surfaces, for example, swab from skin, nose, throat, wounds, burns and bed-sores. Occasionally, *S. aureus* acts as opportunistic pathogens and cause infections of the urinary tract, respiratory tract and intestinal tract (Coltman, 1981). The pathogenicity of *S. aureus* as indicated by Stokes and Ridgway (1980) include, abscesses, boils, conjunctivitis especially in newborn, cross-infections in hospitals septicaemia, mastitis and food poisoning (of meats, milk and milk products). *S. aureus* produces enzymes and toxins which include coagulase, an enzyme that clots plasma and coats staphylococcal cells, which prevents the cells from being phagocytosed and destroyed by macrophages. Sng et al. *(*1981) presented those characteristics useful for identification of *S. aureus*, as golden colony, pigmentation, production of coagulase, deoxy-ribonulease, phosphatase, α-, β-, and δ-heamolytic toxins, leucocidin, fibrinolysin and hyaluronidase.

All the identification and classification methods of characterizing *S. aureus* are complex, time-consuming and requiring basic knowledge of biochemistry or molecular biology of the species being studied (Thottappilly et al., 1999). Kloos and Schleifer (1981) described *S. aureus* as a variable bacterium with many morphological variants. It is of importance in epidemiology and ecology to be able to identify bacterial species and strains accurately. Rapid identification and classification of bacteria is normally carried out by morphology, nutritional requirements, antibiotic resistance, isoenzyme comparison, phage sensitivity (Eisenstein, 1990) and more recently DNA based methods, particularly rRNA sequences (Woese,1986) and strain-specific fluorescent oligonucleotides (Delong et al.,1990). Each of these methods has specific applications and advantages.

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Table 1. *S. aureus* isolates used in this study.

S/N	Isolate Code	Host	Source	Virulence	Disease symptom
1	1SaUCH	Man	Skin wound	Mv	Milky pus
2	5SaUCH	Woman	Skin wound	Mv	Milky pus
3	6SaUCH	Man	Skin wound	Mv	Milky pus
4	17SaUCH	Woman	Nose swab	Hv	Yellowish mucus
5	19SaUCH	Man	Nose swab	Mv	Yellowish mucus
6	20SaUCH	Man	Nose swab	Mv	Yellowish mucus
7	12SaUCH	Woman	Vagina swab	Hv	Whitish discharge
8	13SaUCH	Woman	Vagina swab	Hv	Whitish discharge
9	14SaUCH	Woman	Vagina swab	Mv	Whitish discharge
10	25SaUCH	Man	Ear swab	Wv	Brownish pus
11	26SaUCH	Woman	Ear swab	Mv	Brownish pus
12	28SaUCH	Man	Ear swab	Mv	Brownish pus
13	4SaUCH	Man	Urine	Hv	Brownish pus
14	7SaUCH	Woman	Urine	Mv	Brownish pus
15	8SaUCH	Man	Urine	Hv	Brownish pus
16	33SaUCH	Man	Stool	Hv	Watery stool
17	34SaUCH	Man	Stool	Hv	Watery stool
18	14IT	Soybean	Soy-milk	Mv	Watery stool

Keyword: Hv---highly virulence.
Mv---mildly virulence.
Wv---weakly virulence.

However, closely related isolates are difficult to identify and differentiate using the biochemical methods. For effective chemotherapeutic treatments of infections or disease caused by this organism, the degree of virulence of different strains needed to be determined. The objective of this study is to carry out a genetic characterization of different isolates of S. aureus using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). This RAPD procedure works with anonymous genomic markers, requires only small amounts of DNA and when compared with the biochemical methods, is simpler, very sensitive, cheaper, faster and less labour intensive than other DNA maker methodologies.

MATERIALS AND METHODS

Source of *S. aureus*

Of the 18 pure isolates of *S. aureus* used in this work, 17 were human clinical samples obtained from the Microbiology Section of the University College Teaching Hospital (UCH) Ibadan, Nigeria and the 18th from the Seed Health Unit, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria (Table 1). They were stored according to the method of Gore and Walsh (1964).

Cell Propagation

S. aureus isolates were first propagated using a modified procedure developed by Kado and Heskett (1970). About 75 ml of nutrient broth (pH 7.5) was prepared inside a conical flask. About 200 µl of nutrient broth containing the isolate was transferred into the freshly prepared broth and kept under constant shaking at 37ºC for 24 h for bacterial growth. The bacterial cell was removed by centrifugation, washed with 0.1 mM Tris- EDTA and kept at -20ºC for DNA extraction.

Genomic DNA Extraction

DNA extraction was according to Roeder and Broda (1987) with some modifications. Approximately 0.3 g of washed bacterial cell was suspended in 200 µl of 2X CTAB buffer (50 mM Tris, pH 8.0, 0.7 mM NaCl, 10 mM EDTA, 2% hexadecyltri-methylammonium bromide, 0.1% 2-mercaptoethanol), followed by 100 µl of 20% sodium dodecyl sulfate and incubated at 65ºC for 20 min. DNA was purified by two extraction with phenol: chloroform: isoamyl alcohol (24:25:1) and precipitated with -20ºC absolute ethanol. After washing with 70% ethanol, the DNA was dried and re-suspended in 200 µl of sterile distilled water. DNA concentration was measured using a DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerto CA, USA) at 260nm. To check for degradation of the DNA, the samples were loaded on a 1% agarose gel 1X TAE (45 mM Tris-acetate, 1 mM EDTA, pH 8) and electrophoresed.

RAPD-PCR analysis

RAPD-PCR analysis was according to Guthrie et al. (1992). DNA primers tested in this study were purchased from Operon Technologies (Alamada, California, USA) and each is 10 nucleotides long. Two concentrations of each template DNA (24 ng and 96 ng per reaction) were used to test reproducibility and eliminate sporadic amplification products from the analysis. One hundred (100) primers OPA, OPY, OPQ, OPX, and OPW series

Table 2. Oligonucleotide primers that showed genetic discrimination among the isolates.

S/N	Operon code	Nucleotide sequence (5' to 3')	No of fragments amplified	No of polymorphic bands
1	OPX-04	CCGCTACCGA	12	6
2	OPX-12	TCGCCAGCCA	14	9
3	OPX-17	GACACGGACC	15	9
4	OPX-20	CCCAGCTAGA	7	5
5	OPY-01	GGTGGCATCT	8	3
6	OPW-07	CTGGACGTCA	5	3
7	OPW-09	GTGACCGAGT	7	5
8	OPW-10	TCGCATCCCT	6	2
9	OPW-11	CTGATGCGTG	6	3
10	OPW-13	CACAGCGACA	8	6
		Total	88	51

were screened with two isolates (1SaUCH and 4IT) for their to amplify the *S. aureus* DNA. Ten of these primers were found useful and gave polymorphism. These were used in amplifying the DNA from all the isolates. Amplifications were performed in 25 µl reaction mixture consisting of genomic DNA, 1X reaction buffer (Promega), 100 µM each of dATP, dCTP, dGTP, and dTTP, 0.2 µM primer, 2.5 µM $MgCl_2$ and 1 U of Taq polymerase (Boehringer, Germany). A single primer was used in each reaction. The reaction mixture was overlaid with 50 µl of mineral oil to prevent evaporation. Amplification was performed in a thermowell microtiter plate (Costa Corporation) using a Perkin Elmer programmable Thermal Controller (Model 4800 and 9600). The cycing program was (i) 1 cycle of 94ºC for 3 min; (ii) 45 cycles of 94ºC for 1 min for de-naturation, 35ºC for 1 min for annealing of primer and 72ºC for 2 min for extension; and (iii) a final extension at 72ºC for 7 min. Amplification products were maintained at 4ºC until electrophoresis.

The reaction products were resolved by electrophoresis in a 1.4% agarose gel using 1X TAE buffer at 150 V for 2 h. A 1-kb ladder (Life Technologies,Gaithersburg, MD, USA) was included as molecular size marker. Gels were visualized by staining with ethidium bromide solution (0.5 µg/ml) and banding patterns were photographed over UV light using a red filter.

Data analysis

Position of unequivocally scorable RAPD bands were transformed into a binary character matrix ("1" for the presence and "0" for the absence of a band at a particular position). Pairwise distance matrices were compiled by the NTSYS-PC 2.0 software packages (Rohlf, 1993), using the Jaccard coefficient of similarity (Jaccard, 1908). Dendorgrams were created by UPGMA cluster analysis.

RESULTS AND DISCUSSION

The amplification reactions with the 10 primers generated 88 bands, 51 of which was polymorphic (Table 2) with size range of between 200 and 3,000 bp. According to the pairwise genetic distances among the isolates analyzed at 100% similarity, all the isolates were classified completely into twelve different groups (Figure 1). At 50% coefficient of similarity, all the isolates were classified in two major groups. The first major group comprised of mildly and weakly virulent isolates while the second group comprised of highly virulent ones. It was hypothesized that the frequent occurrence of mutants might be responsible for the high level of variation among the isolates.

The results demonstrated that the RAPD technique may be of great use for the classification of *S. aureus*. Obviously, for these patterns to have a practical meaning in the areas of medicine, population biology and epidemiology, specific DNA bands must be related with the virulence genes (Welsh et al., 1990). This could be accomplished by a systematic comparison of DNA band patterns among bacteria contrasting for the different virulence genes. Similar approach has been used to differentiate aggressive from non-aggressive isolates of the oilseed rape pathogen *Phoma lingam* (Schafer and Wostmeyer, 1992).

The DNA fingerprint defined for each race of *S. aureus* could be useful for the surveillance of epidemiological revolution strategies, medical diagnoses and in the identification of new strains and isolates. The next step would to clone the isolates specific bands (bands with different virulence genes), sequence them and design specify primers for the development of sequence characterized amplified regions which would be much more specific and easier to apply. More research involving additional molecular techniques is needed to confirm if further sub-grouping would be appropriate.

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