Advanced Journal of Microbiology Research Vol. 2003
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Genetic fingerprinting and phylogenetic diversity of *Staphylococcus aureus* isolates from Nigeria

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Accepted 23 July 2003

Genetic fingerprinting of 18 different isolates of *Staphylococcus aureus* from Nigeria using random amplified polymorphic DNA (RAPD) was carried out. Ten out of 100 Operon primers showed polymorphism among the isolates tested generating 88 bands, 51 of which were polymorphic with sizes ranging between 200 and 3,000 bp. All the isolates were classified completely into two major groups (*Sa-1* and *Sa-2*) with twelve different subgroups. *Sa-1* group originated from human while isolates from plant and animal origins formed the *Sa-2* group. The twelve different subgroups suggest adaptation of *S. aureus* in the different host cells. This indicates possible relationship between host origin and genetic variation among *S. aureus* isolates. The DNA fingerprint defined for each race of *S. aureus* could be useful in epidemiological studies, medical diagnosis and the identification of new strains and their origins.

Keywords: *Staphylococcus aureus*, foodborne-acquired infections, genetic fingerprinting; phylogenetic diversity, RAPD, polymorphism.

INTRODUCTION

Staphylococcus aureus is one of the most common causes of foodborne-acquired infections, causing a wide variety of infections, from simple abscesses to fatal sephsis, as well as endocarditis, meningitis and toxinoses including food poisoning and toxic shock syndrome. Staphylococcus pathogenic versatility is compounded by its ability to develop resistance to new antibiotics almost as fast as they are introduced. However, nosocomial infections caused by *S. aureus* are clinically serious and control of such infections requires strain typing to identify degree of virulence, the source of contamination, and resistance to commonly used antibiotics.

It is important 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 comparisons, phage sensitivity (Eisenstein, 1990; Selander et al., 1987; Aber and Mackel, 1981; Milkman, 1973) and more recently by DNA based methods, particularly rRNA sequences (Woese, 1986), strain-specific fluorescent oligonucleotides (Delong et al., 1989; Amann et al., 1990) and the polymerase chain reaction (PCR) (Mullis and Faloona, 1987; Smith and Selander, 1990; McCabe, 1990).

Detection and identification methods using the PCR to amplify DNA have been used for other organisms (Hartskeerl et al., 1989), but these require sequence

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Table 1. Isolates of *S. aureus* used in this study.

S/N	Isolate Code	Host	Source	Locality
1	Sa01	Pig	Pork	Ibadan
2	Sa05	Cow	Cooked meat	Ibadan
3	Sa06	Cow	Cooked meat	Kano
4	Sa17	Human	Stool	Ibadan
5	Sa19	Human	Stool	Ibadan
6	Sa20	Human	Stool	Ibadan
7	Sa12	Cow	Raw meat	Kano
8	Sa13	Cow	Raw meat	Ibadan
9	Sa14	Human	Urethra swab	Ibadan
10	Sa25	Human	Urethra swab	Ibadan
11	Sa26	Human	Urine	Ibadan
12	Sa28	Soya bean	Soya milk	Abuja
13	Sa04	Soya bean	Soya milk	Ibadan
14	Sa07	Soya bean	Soya milk	Ikenne
15	Sa08	Cow	Cow milk	Mokwa
16	Sa33	Commercial	Milk	Lagos
17	Sa34	Cow	Cow milk	Kano
18	Sa41	Human	Urine	Ibadan

information for specific primers. However, PCR using arbitrary primers (AP-PCR) requiring no prior sequence information has revealed DNA polymorphisms that may be useful for fingerprinting (Welsh and McClelland, 1990; Williams et al., 1990). Random amplified polymorphic DNA (RAPD) markers, which are based on the amplification of discrete DNA fragments in the genome by the use of oligonucleotide primers with random sequences, have been largely used to identify physiological races of fungi (Guthrie et al., 1992). With this technique a DNA fingerprint may define individual in a very fast and reliable way. RAPD-PCR method, when compared with biochemical methods is cheap, simple, more sensitive and faster. Apart from the study of antibiotic resistance (Ikeh, 2003), little is known concerning the genetic diversity that exists in populations of *S. aureus* isolates from human and food origins in Nigeria.

In this study genetic fingerprinting and phylogenetic diversity of isolates of *S. aureus* from Nigeria was evaluated using RAPD markers. Such information will be useful in its classification, epidemiological survey, ecology and diagnosis.

MATERIALS AND METHODS

Genetic material

S. aureus isolates (Table 1) used in this study were obtained from the University College Teaching Hospital, Ibadan, and the International Institute of Tropical Agriculture, Ibadan, Nigeria where their identity had been confirmed by coagulase biochemical test. Isolates preservation and storage were in accordance with Gore and Walsh (1964).

Isolates propagation

S. aureus isolates were first propagated using a modified procedure developed by Kado and Keskett (1970). About 200 µl *S. aureus* isolate was transferred into 75 ml of nutrient broth (pH 7.5) in a 250 ml conical flask and kept under constant shaking at 37^{O}C for 24 h. The bacterial cell was removed by centrifugation, washed with 0.1 mM Tris-EDTA and kept at -20^{O}C for DNA extraction.

Genomic DNA Extraction

DNA extraction was according to Roeder and Broda (1987) and Thottappilly et al. (1999) with some modification. 0.3 g of washed bacterial cell were suspended in 200 µl of 2xCTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethylammonium bromide; 0.1% 2-mercaptoethanol), followed by the addition of 100 µl of 20% sodium dodecyl sulfate and incubated at 65^{O}C for 20 min. DNA was purified by two extractions with phenol:chloroform:isoamyl alcohol (24:25:1) and precipitated with -20^{O}C absolute ethanol. After washing with 70% ethanol, the DNA was dried and resuspended in 200 µl of sterile distilled water. DNA concentration was measured using DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerto CA, USA) at 260 nm. DNA degradation was checked by electrophoresis on a 1% agarose gel in 1xTAE (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).

RAPD-PCR analysis

RAPD-PCR analysis was according to Guthrie et al. (1992). DNA primers tested were purchased from Operon Technologies (Alameda, California, USA) and each is 10 nucleotides long. Two concentrations of each DNA (24ng and 96ng per reaction) were used to test reproducibility and eliminate sporadic amplification products from the analysis. One hundred primers (OPA, OPY, OPA, OPX and OPW series) were screened with two isolates (Sa01 and Sa14) for their ability to amplify the *S. aureus* DNA. Ten of these

primers (Table 2) were found useful since they gave polymorphism. These were used in amplifying the DNA from all *S. aureus* 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 Operon random primer, 2.5 µM $MgCl_2$ and 1U 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 9600. The cycling program was (i) 1 cycle of 94^{o} C for 3 min; (ii) 45 cycles of 94^{o}C for 1 min for denaturation, 40^{o}C for 1 min for annealing of primer and 72^{o}C for 2 min for extension; and (iii) a final extension at 72^{o}C for 7 min. The amplification products were resolved by electrophoresis in a 1.4% agarose gel using TAE buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 100 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.

Table 2. Oligonucleotide primers that showed genetic discrimination among the *S. aureus* isolates using RAPD-PCR analysis.

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

Phylogenetic analysis

Positions 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 (Rohlf, 1993) using the Jaccard coefficient of similarity (Jaccard, 1908). Phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis (Sneath and Sokal, 1973; Swofford and Olsen, 1990).

RESULTS AND DISCUSSION

Ten primers showed polymorphisms among individuals isolates out of 100 primers tested. The amplification reactions with the 10 primers generated 88 bands, 51 of them being polymorphic (Table 2) with sizes ranging between 200 and 3,000 base pairs (Figure 1). Using 51 RAPD markers to construct phylogenetic relationship among 18 *S. aureus* isolates led to classification into two major groups (*Sa-1* and *Sa-2*) at 50% similarity coefficient while twelve different subgroups were obtained at 100% similarity coefficient (Figure 2).

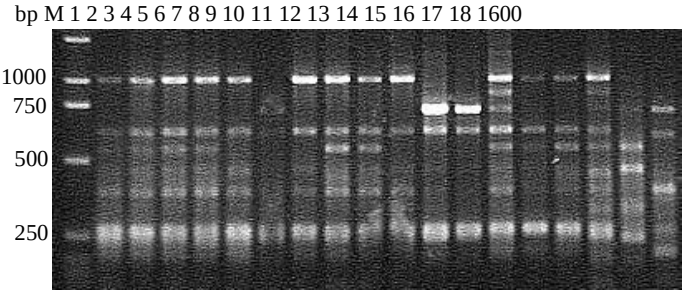

Figure 1. DNA fingerprinting patterns of 18 *S. aureus* isolates using OPX-12 RAPD primer. **M:** 1kb molecular size marker.

Genetic fingerprinting and phylogenetic diversity between different *S. aureus* isolates were determined by converting RAPD data into a Jaccard similarity matrix and analysed by UPGMA to produce a phylogenetic tree. The DNA band pattern obtained is similar to a bar code, allowing the identification of each individual. For instance, isolate Sa04 presents unique bands when its DNA amplified with most of the primers tested (Figure 1). These bands could be used to characterize and identify it. All the isolates were classified completely into two major groups (*Sa-1* and *Sa-2*) with twelve subgroups. *Sa-1* group comprised of isolates originated from human while isolates from plant and animal origins formed the *Sa-2* group. However, the twelve different subgroups obtained in this study suggests possible and frequent occurrence of mutants in *S. aureus* in different host cells.

Historically, *S. aureus* has been described as a variable bacterium with many pathogenic and antibiotic resistance variants (Coltman, 1979; Kloos and Schleifer, 1981). The limited number of morphological and cultural characters of *S. aureus*, and the lack of standardization of cultural conditions and virulence tests among different researchers have led to confusion and uncertainty in the characterization of this pathogen (Kloos and Schleifer, 1981). Distinct phenotypes usually consist of isolates that are genetically less related and such identification of isolates using biochemical, cultural and morphological techniques often lack consistency and precision (Kloos and Schleifer, 1981). In the current study, we have found that identification of genetic diversity in *S. aureus* depends on sources of isolates, different host cells and occurrence of mutants. For instance, seven isolates genotyped as *Sa-1* were originated from human while

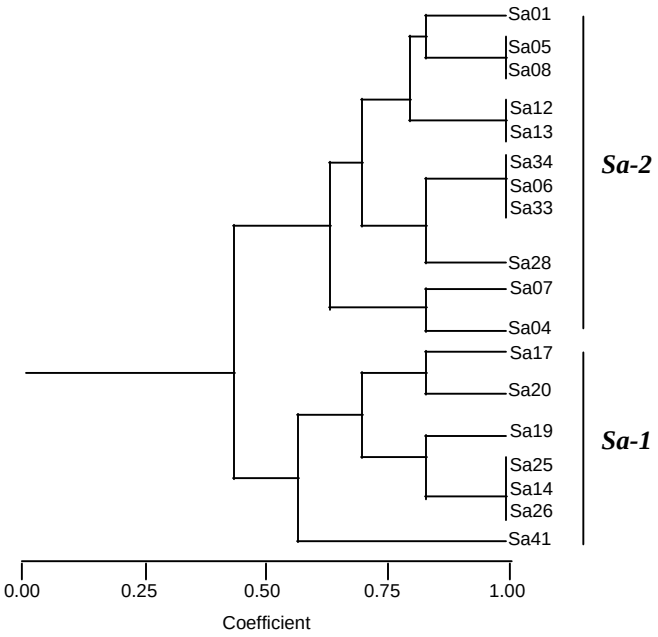

Figure 2. Phylogenetic diversity of 18 *S. aureus* isolates identified by 51 RAPD markers.

three and eight isolates respectively from plant and animal origins were genotyped as *Sa-2* (Figure 2). Besides, the possible and frequent occurrence of mutants in *S. aureus* constitutes the broad genetic variation that exists within *Sa-1* and *Sa-2* genotypes.

RAPD markers revealed possible relationship between host origin, mutation and genetic variation among *S. aureus* isolates, and this demonstrated its fingerprinting and diagnostic potential. Obviously, for these DNA bands patterns to have a practical meaning in the areas of medicine, population biology and epidemiology, specific DNA bands must be related to host origins, mutation and virulence genes (Welsh and McClelland, 1990). This could be accomplished by a systematic comparison of DNA band patterns among bacteria contrasting for the different host origins, mutation and virulence genes present. 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* should be useful for epidemiological surveys, medical diagnoses, and in the identification of new virulent strains and isolates and their origin.

ACKNOWLEDGEMENT

We thank the International Institute of Tropical Agriculture, Ibadan, Nigeria, for sponsoring this project.

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