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Original Research Article

BIOFILM PRODUCTION AMONG BACTERIAL ISOLATES FROM *CLARIAS GARIEPINUS*

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Abstract

Isolation and characterization of bacteria was carried out on three organs (skin, gut and gills) of cultivated African catfish **(***Clarias gariepinus*). A total of sixty six bacteria were isolated; 63 (95.5%) were Gram negatives and 3 (4.5%) were Gram positives. Total average bacterial count recorded gut at 7.1 x 10^5 cfu/ml; gills at 6.3 x 10^5 cfu/ml; and skin at 1.3 x 10^5 cfu/ml. *Escherichia coli* had the highest occurrence at 17 (26%), followed by *Klebsiella oxytoca* at 10 (15%) of all isolates. Forty nine (73.1%) of all isolates were positive for biofilm production on CRA, while 36 (54.5%) were haemolytic on blood agar. Biofilm and haemolysin production are known pathogenic factors of bacteria in man and animals, hence, a need for very good hygiene level in cultivation and consumption of fish and its products.

Keywords: catfish, biofilm, haemolysin, pathogenic.

Introduction

Microbial adaptations to the ever changing environment have taken to different measures in order to overcome the stress factors. While some of these measures are extrinsic, others are intrinsic. Biofilm, a synergistic means of survival and adaptation between different species of bacteria, is one of the major challenges faced in food and drink-producing industries, as well as in medical institutions. Biofilm is a densely packed multicellular communities of microorganisms attached irreversibly to a surface or interface (Donlon *et al*., 2002). These micro-colonies may enclose communities of bacterial cells that may be composed of one or more species, and depending on the species involved; the micro-colony may be composed of 10 – 25% of cells and 75 – 90% of extracellular polymeric substances (EPS) matrix (Costerton *et al*., 1987). Biofilm formation begins with the adhesion of microbes to surfaces (be it biotic or abiotic) and subsequent processes established the microorganisms in an irreversible adhesion (Duddridge and Pritchard, 1983; Marques, 2005). The advantages of biofilm are numerous to bacteria, especially in regards to protection from antibiotics, disinfectants

and dynamic environments (Costerton *et al*., 1987). Biofilms are also extraordinarily resistant to phagocytosis, which makes their eradication from living hosts difficult (Amorena *et al.,* 1999; Jefferson, 2004). Antibiotic and immune response to biofilm producers rarely resolve the effects of biofilms on living hosts (Loo, 2003; Baldassarri *et al.,* 2001), and may even cause immune complex damage to the surrounding tissues (Deighton and Borland, 1993).

Of high importance to food industry are biofilms as they occur on various food contact surfaces like stainless steel, rubber, glass, conveyor belts etc. Many pathogenic biofilm formers have been reported as common contaminants in food industries (Criado *et al.,* 1994; Mettler and Carpentier, 1998; Parizzi, 1999; Pompermayer and Gaylarde, 2000; Suikho *et al.,* 2002) and in human medicine, bacteria in biofilms have been reported to cause therapy resistance, recurrent and chronic nosocomial infections (Vuong and Otto, 2002), while in veterinary medicine, a host of biofilm formers have been reported to resist very potent antibiotics either in combinations or singly (Clutterbuck *et al*., 2007).

In most developing countries, agriculture is a promising field of life sustenance to many as it provides food, energy, employment, and transportation. The cultivation of *Clarias gariepinus*, the most widely distributed African catfish (Skelton, 2001), is one of such enterprises. The *Clariid* fish, which is the second largest in size in Africa, is known to tolerate and survive in extremes of environmental conditions such as low oxygen, low/no water, temperatures between 8 and 35^{o}C, salinities of 0 – 10 ppm and a wide range of pH (Safriel and Bruton, 1984). Catfish is well cultivated in Nigeria (Williams and Michael, 2007) and commonly consumed as a component of 'pepper soup', a delicacy for many, may however serve to convey pathogens to unsuspecting consumers. The presence of both pathogenic and non-pathogenic bacteria species on most fishes, which seldom cause infection on the fish, are however of great importance to public health (Shawn, 1997; Adebayo-Tayo *et al.,* 2009). These pathogens like *Bacillus sp., Salmonella sp., Shigella sp., Escherichia coli, Pseudomonas sp.,* and *Staphylococcus aureus* are known common causes of food poisoning in humans, as well as common resistant isolates to most used antibiotics (Adebayo-Tayo *et al.,* 2009).

This study examined biofilm production among isolates of bacteria from gills, gut and skin of *C. gariepinus* bought from fish farm housing over 500,000 fishes in more than a hundred fishponds in Ado Ekiti, Ekiti Sate, Nigeria.

Material and Methods

Sample collection and analysis

Fishes were bought in sterile receptacle and conveyed to the laboratory for analysis. Each of the specimens was dissected aseptically to remove the gut, gills and skin. Each organ was placed in sterile beaker containing 5ml sterile distilled water and vigorously shaken to allow the content dissociate in water.

Bacteria count and isolation

For bacteria count, 1ml was taken and serially diluted in ten folds up to 10^{-5} from which pour plate method was carried out using cooled-molten nutrient agar. After incubation at 37^{o}C for 24 hours, counts were taken and expressed in colony forming units (CFU) per milliliter (ml).

A loopful of the original suspension was streaked on the surfaces of freshly prepared Eosin Methylene blue agar (EMB), Trypticase soy agar (TSA), and Macconkey agar (MAC) respectively. The plates were incubated aerobically at 37˚C for 24hours and representative colonies emerging from the plates were grouped according to their

cultural characteristics, purified by repeated sub–culturing and maintained on appropriate agar slants as stock culture. All isolates were characterized using standard microbiological and biochemical tests as described by Barrow and Feltham (1993) and Cheesbrough (2006). Bacterial isolates were identified with the help of Bergey's Manual of Determinative Bacteriology and online Gideon Informatics (1994-2015).

Isolates were also tested for the possible production of haemolysin on 5% blood agar, incubated at 37 □ C for 24 hours and observed for clear zone or no clear zone around the colonies (Cheesbrough, 2006).

Biofilm Detection

Each identified isolate was subjected to biofilm detection using Congo Red Agar (CRA) medium as described by Freeman (1989).

Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) at $p< .05$ significance level.

Results and Discussion

A total of sixty-six bacteria isolates were identified; sixty-three (95.5%) Gram negatives and three (4.5%) Gram positives. Most of the Gram negative organisms isolated belong to the Family *Enterobacteriaceae* (79.1%). *Escherichia coli* had the highest occurrence at 17 (26%), followed by *Klebsiella oxytoca* at 10 (15%) of all isolates. Organisms isolated and their frequencies from the fish organs are detailed in Table 1.

Table 1: Frequency distribution of bacteria isolated from the skin, gills and gut of *C. gariepinus*

Isolated bacteria	Fish Organs			Total Percentage occurence(n=66)
	Gut (%)	**Skin(%)**	**Gills(%)**	
Escherichia coli	6 (28.5)	5 (22.7)	6 (25)	25.76
Klebsiella oxytoca	5 (23.7)	3 (13.7)	2 (8.3)	15.15
Proteus vulgaris	3 (14.3)	0 (0)	1 (4.2)	6.06
Enterobacter aerogenes	1 (4.8)	0 (0)	1 (4.2)	3.03
Shigella sonnei	1 (4.8)	1 (4.5)	1 (4.2)	4.55
Enterobacter cloacae	1(4.8)	1 (4.5)	0 (0)	3.03
Shigella flexneri	3 (14.3)	2 (9.2)	3 (12.4)	12.12
Prevotella pallens	0 (0)	2 (9.2)	1 (4.2)	4.55
Chromobacterium violaceum	0 (0)	1 (4.5)	0 (0)	1.52
Providencia rettgeri	0 (0)	2 (9.2)	2 (8.3)	6.06
Porphyromonas macacae	0 (0)	1 (4.5)	0 (0)	1.52
Pantoea agglomerans	0 (0)	1 (4.5)	1 (4.2)	3.03
Pseudomonas oryzihabitans	0 (0)	1 (4.5)	0 (0)	1.52
Chryseobacterium indologenes	0 (0)	0 (0)	1 (4.2)	1.52
Erwinia chrysanthemi	0 (0)	0 (0)	1 (4.2)	1.52
Citrobacter koseri	0 (0)	0 (0)	3 (12.4)	4.55
Rhodococcus gordoniae	1 (4.8)	0 (0)	0 (0)	1.52
Kytococcus schroeteri	0 (0)	1 (4.5)	0 (0)	1.52
Luteococcus sanguinis	0 (0)	1 (4.5)	1 (4.2)	3.03
TOTAL	21[a]	22[a]	23[a]	

Figures with same superscript have no significant difference at P< 0.05

Some bacteria isolate produced heamolysis on blood agar (52.2%), a common feature of pathogenic isolates; 40.3% showed beta haemolysis while 11.9% showed alpha haemolysis (Table 2).

Table 2: Haemolytic isolates on blood agar

Isolated bacteria	Gut		Skin		Gills	
	β	***α***	***B***	***α***	***B***	***α***
Escherichia coli	4	1	3	-	2	1
Klebsiella oxytoca	5	-	1	-	-	-
Proteus vulgaris	1	2	-	-	1	-
Enterobacter aerogenes	1	-	-	-	-	-
Shigella sonnei	-	-	-	-	-	1
Enterobacter cloacae	-	-	-	1	-	-
Shigella flexneri	-	1	1	-	1	1
Prevotella pallens	-	-	2	-	1	-
Chromobacterium violaceum	-	-	-	-	1	-
Providencia rettgeri	-	-	2	-	-	-
Erwinia chrysanthemi	-	-	-	-	1	-
Citrobacter koseri	-	-	-	-	1	-

Legend: *β*- beta haemolysis, *α*- alpha haemolysis

Total average bacterial count recorded in the organs are as follow; gut at 7.1 x 10^5 cfu/ml; gills at 6.3 x 10^5 cfu/ml; and skin at 1.3 x 10^5 cfu/ml. Statistical analysis showed no statistical difference (p<.05) in bacterial counts of gut and gills, but difference was statistically significant with the count on skin when compared with the other organs. Isolates' population per organ showed no significant difference in the average means.

A total of forty nine (74.2%) bacteria isolates produced rough black colonies on Congo red agar. Analysis of isolates that produce biofilm shows that high percentages were also haemolytic on red blood agar (Figure1).

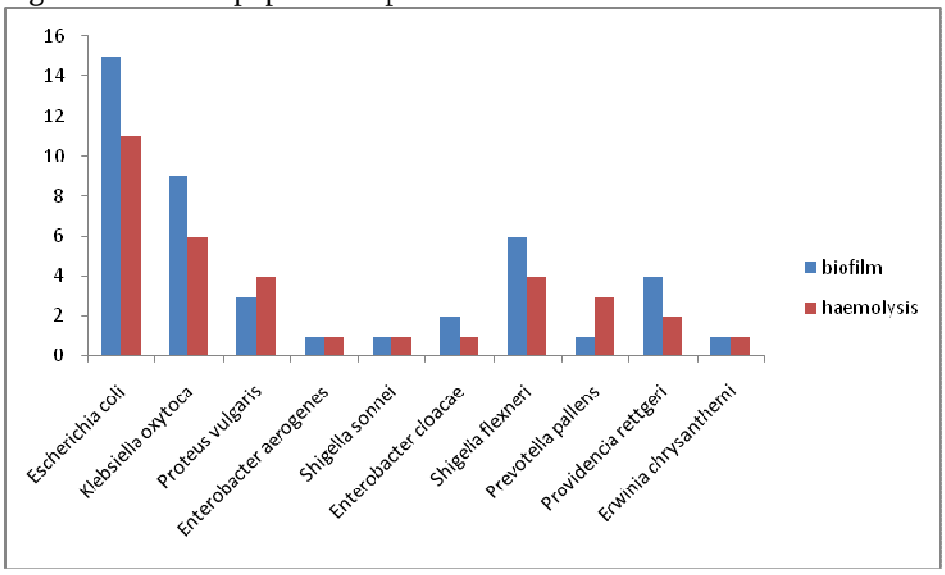

Figure 1: Biofilm and haemolysin producers

The bacteria isolated in this study are common isolates of fresh water catfish as reported by other researchers (Shewan and Hobbs, 1990; Sugita *et al*., 1997; Shewan, 2000; Okaeme, 2006) isolated from the skin, gut and gills including *Bacillus* species from the skin of sea water fish. Sugita *et al*. (2002) reported that *Staphylococcus spp*,

Escherichia coli were isolated frequently from the skin of fresh water fish and concluded that the skin of fresh water fish were the natural habitat of these bacteria. Some of these organisms in the genera *Escherichia, Proteus, Shigella, Klebsiella, Enterobacter* have been implicated as fish pathogens (Starliper, 2001; Brenkman *et al*., 2008; Akoachere *et al*., 2009; Loch *et al*., 2012).

From this study, members of the family *Enterobacteriaceae* were found to be dominant at 87.5%, 61.5%, and 46.1% in the intestines, gills and skin respectively. Because of the high preponderance of these organisms to cause disease in humans through various routes, their presence in fish is unwanted, as there are numerous reports of infections associated with the members of *Enterobacteriaceae* in humans (Holt *et al*., 2000; Yagoub, 2009).

The ability of bacteria to form biofilms helps to survive in hostile environments within the host and is considered to be responsible for chronic or persistent infections (Christensen *et al*., 1985). Several studies have shown that the formation of slime and biofilms by organisms causing catheter-associated and nosocomial infections is associated with the presence of the icaA and icaD genes (Ziebuhr *et al*., 1997; Arciola *et al*., 2001, 2002). A total of forty nine (74.2%) bacteria isolates were detected as biofilm producing using Congo red agar method. Jain and Agarwal (2009) evaluated the phenotypic Congo Red Agar and microplate test in biofilm detection and concluded that both tests demonstrated good sensitivity and specificity in the detection of microorganisms that produced biofilms. Production of biofilm and haemolysin by isolates in this study suggest that they are pathogenic and may cause undesired health problems if consumed via contamination.

Conclusion and Recommendation

For profitable fish farming and healthy consumption, a very good level of hygiene is required to avoid the loss of the fishes due bacterial infection and onward possible contamination of food products for consumption by man. We also recommend the use of Congo red agar for routine testing of biofilm production in bacteria isolates.

Competing Interests

There are no competing interests on this study.

Authors Contributions

Oyinloye and Olagbemide supervised the work and wrote this manuscript, while Omajugho and Diyaolu carried out the work.

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