Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (1) 56-58

Short Communication

Antagonism Among Skin Bacterial Isolates

Azuka Romanus Akpe*[a], Ifeoma Betsy Enweani[b], Frederick Ikechukwu Esumeh[a], Peter Usuoge[a], Helen Obiazi[a], Rachael Ngozi Osagiea and Agbokhaode Oshogwemoh[a]

[a]Department of Microbiology, Ambrose Alli University PMB 14, Ekpoma Edo State, Nigeria

[b]Department of Medical Laboratory Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria

(received June 16, 2011; revised January 24, 2012; accepted February 6, 2012)

Abstract. A study on the inhibitory activity or antagonism among skin bacterial isolates was carried out by observing if the isolates inhibited the growth of one another. Five bacterial species were isolated from the 40 swabs of the different part of the skin used in this study. The isolates in order of decreasing frequency of isolation were *Staphylococcus epidermidis* 11(25.00%), *Micrococcus roseus* 11(25.00%), *Bacillus subtilis* 9(20.46%), *Staphylococcus aureus* 8(18.18%) and *Pseudomonas aeruginosa 5*(11.36%). *M. roseus* and *B. subtilis* were strongly inhibited by *S. epidermidis, S. aureus* and *P. aeruginosa. S. aureus* was inhibited by *S. epidermidis.* Furthermore, *M. roseus, S. epidermidis* and *P. aeruginosa* failed to inhibit the growth of each other.

Keywords: antagonism**,** bacteriocin, human skin, bacterial isolates

A property of microorganisms which enables one microorganism to kill, injure or inhibit the growth of a different microorganism. Some of the inhibitory substances produced by bacteria includes fatty acids, antibiotics and bacteriocins among others (Eijsink *et al,.* 2002; Nes and Holo, 2000; Black, 1996). These bacteria harbor extra chromosomal elements known as bacteriocinogenic factors. It is known that bacteriocins genes can be harbored into chromo-somes, plasmids and transposons (Dufour *et al.,* 2007). Like phages, bacteriocins attach to specific outer membrane receptors, and they can select for resistant mutants which lack effective receptors (Bernard *et al.,* 1980).

In recent years, several reports of the formation of bacteriocins by various strains of gram positive bacteria found on human skin have been published (Jack *et al.,* 1995). Some of these bacteriocins especially from staphylococci have been highly purified and they often have antibacterial activity towards other genera e.g. diphtheroids (Fabio *et al.,* 1987; Gagliano and Handsdill, 1970).

The bacteriocins from gram negative bacteria which have received the most attention are the colicins (produced by *Escherichia coli*) of which a larger number exist (Joklik and Willet, 1976). While bacteriocins of lactic acid bacteria are the most studied in gram positive bacteria (De Vuyst and Vandamme, 1994) and some have been intensively studied for application in food preservation (Gibbs, 1987). Other examples of bacteriocins are marcescins produced by *Serretia* and pyocins produced by strains of *Pseudomonas* (Jawetz *et al.,* 1995).

Collection of samples. 40 swab samples were randomly collected from students and other people in the premises of Ambrose Alli University, Ekpoma Nigeria. Sterile cotton wool swabs (moistened in sterile peptone water immediately before use) were used to swab the skin surface of the various parts of the body (such as neck, arm, armpit, foot, pinna, face, hand and leg). This was immediately transferred to the microbiology laboratory of Ambrose Alli University, Ekpoma Nigeria for bacteriological analysis.

Cultural methods. Nutrient agar and Mac Conkey agar were used in this study. The agar plates were inoculated by streaking out the swab samples on them. It was then incubated at 37 °C for 24 h. Subcultures were made on fresh plates and pure colonies were transferred to nutrient agar slant and stored at 4 °C for further characterization and identification.

Characteristics and identification of isolates. All isolates were characterized and identified on the basis of colonial appearance on solid media and biochemical tests as earlier described by Cowan and Steel (1984).

*Author for correspondence; E-mail: lordromis@yahoo.com.uk

Screening for production of inhibitory substances. A modified version of the macrocolony technique, described by Aslim *et al*. (2005) was used to test for production of inhibitory substances. In this method, the test isolates were inoculated vertically over an area of the surface of the dried agar plates. The plates were incubated at 37 °C for 48 h (this timing was to allow the test isolates produce sufficient inhibitory substances).

The plates were then exposed to chloroform vapour for 30 min before being exposed to air for one hour. The other isolates (indicator organisms) were then streaked across at right angles to the original line of growth of the test isolates. The plates were then incubated at 37 °C for 18-24 h. Inhibition zones of more than 2mm were then noted.

In this study a total of 44 isolates were obtained from 40 swabs of various parts of the skin of both male and female subjects used in this study. The isolates were made up of five bacterial species namely: *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and *Micrococcus roseus*. The frequencies of occurrence of isolates in decreasing order are *M. roseus* 11(25.00%), *S. epidermidis* 11(25.00%), *B. subtilis* 9(20.46%), *S. aureus* 8(18.18%) and *P. aeruginosa* 5(11.36%) These are shown in Table 1.

The sex distribution of isolates revealed that there was no significant difference in the type and number of bacterial isolates from the skin of both sexes (Table 2).

Result of screening for production of inhibitory substances is shown in Table 3. It revealed that all isolates except *M. roseus* exhibited inhibitory activity against *B. subtilis* while *S. epidermidis* alone could inhibit *S. aureus.* Both *M. roseus* and *B. subtilis* had no inhibitory activity on any of the indicator isolates.

Table 1. Distribution and percentage frequency of isolates from different parts of human skin

Specimen source	No.examined	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*S.epidermidis*	*M. roseus*
Face	5	-	1	2	2	2
Arm	6	-	1	3	2	-
Hand	5	-	1	2	1	2
Leg	6	1	1	2	-	2
Foot	2	-	-	-	1	1
Pinna	5	4	-	-	-	2
Neck	5	-	2	-	2	1
Armpit	6	-	2	-	3	1
Total	**40**	5 (11.36%)	8 (18.18%)	9 (20.46%)	11 (25.00%)	11 (25.00%)

Table 2. Sex distribution of skin bacterial isolates

Sex	No. of samples	No. of isolates (%)	Organisms isolated
Male	22	25(56.8)	*M. roseus* *S. aureus* *S. epidermidis* *B. subtilis* *P. aeruginosa*
Female	18	19(43.2)	*M. roseus* *S. aureus* *S. epidermidis* *B. subtilis* *P. aeruginosa*
Total	40	44(100)	

Table 3. Inhibitory activities of bacterial isolates against each other

Test isolates	Indicator isolates				
	M. roseus	*S. epidermidis*	*S. aureus*	*B. subtilis*	*P. aeruginosa*
M. roseus	NA	-	-	-	-
S. epidermidis	-	NA	+	+	-
S. aureus	-	-	NA	+	-
B. subtilis	-	-	-	NA	-
P. aeruginosa	-	-	-	+	NA

+ = Antagonism detected; - = Antagonism not detected; NA = Not applicable.

Of the five bacterial isolates, only *P. aeruginosa* was gram negative while others were gram positive. This agrees with the earlier report of Selwyn and Ellis (1972) and Jack *et al.* (1995). They observed that gram positive organisms dominate the skin surface. This could be as a result of some form of antagonism of gram positive organisms normally resident on the skin against gram negative ones.

Substances produced by *S. epidermidis, S. aureus* and *P. aeruginosa* inhibited the growth of *B. subtilis.* This may be because *B. subtilis* is not part of the normal bacterial flora of the skin and hence other organisms were able to prevent it from multiplying and establishing itself as part of the normal flora of the skin.

The result also showed that *S. aureus* which one would expect to be readily adaptable to the dry environment on the skin is strongly inhibited by *S. epidermidis* and this may explain why most strains of *S. aureus* are only transient members of the skin microflora. This could

be explained by the findings of Otto *et al.* (2001) that the predominance of *S. aureus* and *S. epidermidis* is as a result of competition by means of quorum sensing cross talk in favour of *S. epidermidis*.

The frequency of isolation of *S. epidermidis* and *M. roseus* (25.00%) each also agrees with the observation of Dunny and Leonard (1997) and Reeves (1972). They observed that coagulase negative staphylococci and micrococci dominate the skin surface. These bacteria may dominate the skin surface by virtue of the bacteriocins that they produce. The observations that *M. roseus* did not inhibit the growth of *S. aureus* does not imply that it did not produce any inhibitory substance, but it could possibly be that the strain of *S. aureus* is resistant to the action of the inhibitory substance produced by *M. roseus.* This phenomenon has been observed by Skinner and Carr (1974).

The non-detection of antagonism between *P. aeruginosa, S. epidermidis* and *M. roseus* could probably be explained by the fact that they are members of the normal resident bacterial flora of the skin.

Although, there is no direct evidence that the antagonism detected among the isolates studied were due to bacteriocin production, there however lies a strong possibility that their inhibitory activities were bacteriocin based.

References

Aslim, B., Yuksekdag, Z.N., Sarikaya, E., Beyatli, Y. 2005. Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. *Swiss Society of Food Science and Technology,* **38:** 691-694.

Bernard, D.D., Renato, D., Herman, N.E., Harold, S.G. 1980. *Microbiology,* 3rd edition. pp. 220-222, Herper and RowPublisher. Philadelphia, Pennsylvania, USA.

Black, J.G. 1996. *Microbiology: Principles and Application.* 3rd edition, pp. 392-397, Prentice Hall, Upper Saddle River New Jersey, USA.

Cowan, S.T., Steel, K.J. 1984. *Manual for the Identification of Medical Bacteria.* 8th edition, 238 pp., Cambridge University Press, UK.

De Vuyst, L., Vandamme, E.J. 1994. Antimicrobial potential of lactic acid bacteria. In: *Bacteriocins of Lactic Acid Bacteria,* L De Vuyst, Vandamme and E.J. Wandamme, (eds.), pp. 91-142, *Glasgow: Blackie Academic & Professional.* UK.

Dufour, A., Hindre, T., Haras, D., Le Pennec, J.P. 2007. The biology of lantibiotics from the lacticin 481 group is coming of age. *FEMS Microbiological Review,* **31:** 134-167.

Dunny, G.M., Leonard, B.A. 1997. Cell-cell communication in Gram positive bacteria. *Annual Review of Microbiology,* **51:** 527-564.

Eijsink, V.G.H., Axelsson, L., Diep, D.B., Havarstein, L. S., Holo, H., Nes, I.F. 2002. Production of class II bacteriocin by lactic acid bacteria; an example of biological warfare and communication. *Antonie Leeuwenhoek,* **81:** 639-654.

Fabio, U., Bondi,M., Manicardi, G., Messi, P., Neglia, R. 1987. Production of bacteriocin-like substances by human oral streptococci. *Microbiologica,* **10:** 363-370.

Gagliano, V.J., Hindsdill, R.D. 1970. Characterization of a *Staphylococcus aureus* bacteriocin. *Journal of Bacteriology,* **104:** 117.

Gibbs, P.A. 1987. Novel uses for lactic acid fermentation in food preservation. *Journal of Applied Bacteriology. Symposium Supplement,* 515-585.

Jack, R.W., Tagg, J.R., Ray, B. 1995. Bacteriocins of Gram positive bacteria. *Microbiological Review,* **59:** 171-200.

Jawetz, E., Melnick, J.L., Adelberg, A.E. 1995. *Medical Microbiology.* 20th edition, 209 pp., Appleton and Lange, USA.

Joklik, W.K., Willet, P.H, 1976. *Zinsser Microbiology.* 16th edition, pp. 159-160*,* Appleton-century-crofts, New York, USA.

Nes, I. F., Holo, H. 2002. Class II antimicrobial peptide from lactic acid bacteria. *Biopolymers,* **55:** 50-61.

Otto, M., Echner, H., Voelter, W., Gotz, F. 2001. Pheromone cross-inhibition between *Staphylococcus aureus* and *Staphylococcus epidermidis. Infection and Immunity,* **69:** 1957-1960.

Reeves, P. 1972. *The Bacteriocins.* pp. 42-48, Chapman and Hall, London, UK.

Selwyn, S., Ellis, H. 1972. Skin bacteria and skin disinfection reconsidered. *British Medical Journal,* **1:** 136.