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Control of *Staphylococcus aureus* Sensitivity to Sweet Pepper (*Capsicum annum*) by a Chemical Mutation

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Abstract: Chemical mutation was carried out on *Staphylococcus aureus* using ethylmethyl sulphonate. The bacterium and its mutants were tested for sensitivity to the extract of *Capsicum annum* (sweet pepper). Mutants with varying degrees of sensitivity to the extract were obtained. On the basis of zone of inhibition, mutants were classified as Non-sensitive (NS), Slightly-sensitive (SS), Fairly-sensitive (FS), Normal-sensitive (NMS) and Super-sensitive (SUS) mutants with zones of growth inhibition on Mannitol Salt Agar (MSA) in the range of 0.00-0.09, 0.10-1.09, 1.10-2.09, 2.10-3.09 and 3.10-4.09 mm respectively. About 26, 7 and 23.5% mutants were screened as NS, SS and FS respectively. Other mutants, NS and SS constituted 27 and 16.5% of the total mutants population. Some of the mutants appeared bacteriostatic, bactericidal and bacteriolytic in actions. Also, mutation caused a change in colour of MSA from red to yellow. Some mutants completely changed the colour (complete colour change, CCC mutants) with zone of colour change of 9.00 mm. Size of colour change exhibited by other mutants ranged from 2.40 to 3.20 mm (slight colour change, SCC), 3.50 to 4.90 mm (strong colour change, STCC) and 0.00 mm (no colour change, NCC) relative to the wild-type which showed 4.70 mm zone of colour change.

Key words: *Staphylococcus aureus*, mutation, sweet pepper effect

INTRODUCTION

Capsicum annum (sweet pepper) belongs to the night shade family of Solanaceae introduced into India by the Portuguese in the 17th century. It is a native of tropical America and West Indies; cultivated in many tropical countries including Nigeria. It is used for flavouring and giving taste to curries, chutneys, salads etc. (Dutta, 1981). Traditionally, it is used to treat various diseases including dropsy, colic, diarrhoea, asthma, arthritis, muscle cramps, tooth aches, high and low blood pressures, apoplexy, gangrene or mortification and asphyxia. It is a powerful stimulant, carminative, anti-oxidant, anti-inflammatory and anti-cancer agent (Demmig-Adams *et al*., 1996; Kelloff *et al*., 2000). Al-Qarawi and Adams (2003) reported that *C. annum* helped to lower human blood pressure; a property, which has been applied locally.

Capsicum annum possesses antibacterial, antifungal and antiviral activities (Cereaga *et al*., 2003). It is active against both gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aureginosa* (Barber *et al*., 2000; Dorantes *et al*., 2000). Its antifungal action is directed towards many fungi like *Candida tropicalis* and *Saccharomyces cerevisiae* ATCC 9763 (Kivanc and Akgul, 1998). Farag *et al*. (2003) showed that *C. annum* enhanced the production of neutralizing antibodies to combat viral infections including Herpes zooster virus.

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In a previous research done by Boboye *et al*. (2007), it was noted that *Capsicum annum* and *C. frutescens* inhibited the growth of *Klebsiella pneumoniae*, *Streptococcus faecalis*, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa* and *Escherichia coli*. It was also demonstrated that the minimum inhibitory concentrations of the pepper on gram-positive, *Staph. aureus* and gram-negative, *Ps. aureginosa* were 402 and 335 mg mL^{-1} respectively (Boboye, 2004). Recently, we carried out a chemical mutation with ethylmethyl sulphonate (EMS) on *Staph. aureus* to study its sensitivity to sweet pepper. This forms a genetical basis for the reaction of *Staph. aureus* to antibacterial agent like sweet pepper and also gives a clue to the mode of action of the *Capsicum* on the bacterium.

MATERIALS AND METHODS

Capsicum annum and *Staphylococcus aureus* were obtained from 'Oba' market and State Specialist Hospital, Akure, Nigeria respectively. The bacterium was stored on nutrient agar slant.

Experiment on Mutation

Fresh culture of *Staph. aureus* was inoculated into nutrient broth and grown at 37°C for 18 h. It was pour plated on Mannitol Salt Agar (MSA) and incubated for 24 h. Chemical mutation by EMS was performed using method of Parkinson (1976) with slight modification as described by Boboye and Alao (2008). Mutational rate was calculated and mutants were used for further studies.

Preparation of Pepper Extract

The extract of pepper was prepared according to the method of Boboye and Dayo-Owoyemi (2004) with little change. Sweet pepper was ground with sterile water using sterile mortar/pestle and blender to obtain 667 mg mL^{-1}. The ground material was filtered to constitute the extract.

Test for Growth Inhibition

Agar diffusion method was employed. An 18 h grown culture containing 26×10^7 cells was pour plated in MSA. A well was bored into the agar with a 17 mm diameter cork borer and filled with 1 mL of the extract. Incubation was carried out and distance of growth restriction formed around the well bored was measured at 24 and 48 h. This was repeated for every mutant and the wild-type strain. Isolate's ability to change colour of the agar from red to yellow was recorded in distance (mm) covered by the colour from the well.

RESULTS AND DISCUSSION

Growth Effect of *Capsicum annum* on the *Staphylococcus aureus*

Mutational rate of the mutagen was 1.44% survival of the *Staph aureus*. Two hundred mutants screened exhibited various levels of growth inhibition to the *Capsicum annum*. The mutants were grouped into five classes (Table 1). Some mutants were resistant without any appreciable clear distance (0.00 to 0.09 mm) created between the holed extract and the grown cells. These are the Non-sensitive mutants (class 1) with 52 members (25% of the total mutants screened). In contrast, many mutants were restricted in growth towards the extract with a clear gap ranging from 0.10 to 4.09 mm between the agar well and the grown cells. These mutants were rated as Slightly- sensitive (SS) class 2 (0.10-1.09 mm), Fairly-sensitive (FS) class 3 (1.10-2.09 mm), Normal-sensitive (NMS) class 4 (2.10-3.09 mm) and Super-sensitive (SS) class 5 (3.10-4.09 mm) with 14, 47, 54 and 33 members respectively. The wild-type grew 3.00 mm away from the extract.

Table 1: Effect of *Capsicum annum* on the growth of mutants and wild-type of *Staphylococcus aureus*

Range of zone of inhibition (mm)	Mutant No.	Representative member/Zone of inhibition (mm)	Class/Description
0.00-0.09	12, 15, 19, 24, 26, 31, 32, 40, 45, 49, 50, 55, 57, 64, 69, 70, 77, 81, 85, 119, 123, 137, 140, 142, 144, 147, 150, 151, 155, 156, 157, 160, 163, 165, 172, 174, 175, 176, 177, 178, 181, 182, 184, 185, 188, 189, 191, 193, 194, 195, 197, 198.	12 = 0.00	1 Non-sensitive
0.10-1.09	6, 10, 25, 65, 66, 76, 82, 84, 86, 166, 168, 179, 187, 200.	82 = 0.80	2 Slightly-sensitive
1.10-2.09	2, 3, 9, 14, 16, 22, 23, 29, 34, 35, 36, 38, 43, 52, 56, 58, 61, 67, 71, 74, 75, 79, 80, 83, 87, 88, 91, 93, 95, 96, 97, 98, 99, 129, 141, 146, 149, 152, 158, 159, 170, 171, 183, 186, 190, 196, 199.	87 = 1.80	3 Fairly-sensitive
2.10-3.09	1, 4, 7, 8, 13, 17, 18, 20, 27, 28, 30, 33, 37, 39, 41, 42, 44, 46, 48, 54, 59, 62, 63, 68, 78, 89, 90, 94, 100, 101, 103, 104, 106, 108, 117, 124, 125, 126, 128, 132, 133, 135, 139, 143, 145, 148, 153, 154, 162, 167, 169, 173, 180, 192.	90 = 2.80	4 Normal-sensitive
3.10-4.09	5, 11, 21, 47, 51, 53, 60, 72, 73, 92, 102, 105, 107, 109, 110, 111, 112, 113, 114, 115, 116, 118, 120, 121, 122, 127, 130, 131, 134, 136, 138, 161, 164.	116 = 3.90	5 Super-sensitive

Table 2: Growth resumption of the mutants of *Staphylococcus aureus* after 24 h treatment with the extract of *Capsicum annum*

Class	Mutant No.	Zone of inhibition (mm) 24 h	48 h
2	10	0.90	0.70
3	34	1.80	1.60
3	43	2.00	1.80
3	75	1.70	1.50
3	149	1.60	0.00
4	1	2.20	1.80
4	7	2.60	1.40
4	18	2.30	2.10
4	41	2.30	2.10
4	46	2.10	1.90
4	145	2.00	0.00
5	53	3.20	3.00

Growth Resumption of the Mutants of *Staphylococcus aureus*

At 48 h of incubation, growth inhibition zone of the wild-type strain was observed to increase from 3.00 to 3.10 mm while many of the mutants (above 85%) retained their zones of inhibition. Other mutants in classes 2, 3, 4 and 5 resumed growth (Table 2). These constituted 7.14, 8.51, 11.11 and 3.03% mutants of the classes respectively. This action implies that the pepper was bacteriostatic to the mutants; they are thus termed pepper-bacteriostatic-respondents. On the other hand, all the Non-sensitive mutants in class 1 with the exception of MU 24, 26, 32, 64 and 81, remained resistant to the

Table 3: Colour change of growth medium by the mutants of *Staphylococcus aureus*

Zone of colour change (mm)	Mutant No.	Description of mutant
0	24, 26, 31, 32, 55, 64, 69, 70, 74, 101, 102, 103, 104, 105, 106, 108, 111, 112, 113, 116, 118, 119, 122, 123, 124, 137, 138, 139, 140, 141, 143, 144, 188, 191, 193, 195, 197, 198.	No colour change (NCC mutants)
2.40-3.20	1, 10, 12, 13, 14, 57, 66, 76, 80, 82, 83, 86, 107, 109, 114, 115, 117, 120, 121, 128, 145, 146, 148, 149, 152, 158, 159, 160, 165, 166, 170, 171, 172, 183, 184, 186, 187, 190, 192, 196, 199, 200.	Slight colour change (SLC mutants)
3.50-4.90	2, 3, 4, 5, 6, 7, 9, 11, 18, 21, 22, 23, 25, 27, 28, 29, 30, 33, 34, 35, 36, 37, 39, 41, 42, 44, 46, 47, 48, 49, 50, 51, 52, 53, 54, 56, 59, 60, 61, 62, 63, 65, 66, 67, 68, 71, 72, 73, 75, 76, 78, 79, 80, 81, 82, 84, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 129, 133, 135, 136, 150, 153, 156, 161, 162, 167, 168, 169.	Normal colour change (NMCC mutants)
9.0	8, 16, 17, 18, 20, 45, 50, 77, 110, 126, 127, 130, 131, 132, 134, 147, 151, 157, 163, 164, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182.	Complete colour change (CCC mutants)

inhibitory power of the pepper extract. Clear zones of growth inhibition measuring 0.30, 0.20, 0.80, 0.20 and 0.40 mm were observed around wells of pepper extract on agar seeded with the MU 24, 26, 32, 64 and 81 mutants, respectively after 48 h of incubation. This indicates exhibition of delayed sensitivity to the extract. These mutants are pepper-bactericidal-respondents.

The pepper appeared to inhibit growth of wild-type *Staph. aureus* by killing the cells; the cells were intact microscopically although dead. This indicates that the pepper is naturally bactericidal in action to the *Staph. aureus*. This mechanism was altered to bacteriostatic and bacteriolytic by mutation as depicted in many mutants. Microscopic examination showed that the cells of some mutants have ruptured appearing as particles making the mutants pepper-bacteriolytic-respondents. Bacteriostatic agent binds loosely to ribosomes during which there is growth inhibition; later when the agent becomes free from the ribosomes; growth is resumed. This pepper acting as bacteriostatic agent must have-inhibited protein synthesis but did not kill the organism. Bactericidal and bacteriolytic agents bind tightly to cellular target of the relevant organism. The former agent leave cells unbroken while the latter induced killing by lysis which was observed in this experiment as turbidity in the MSA after growth. They inhibit cell membrane and cell wall synthesis (Brock *et al*., 1984; Madigan *et al*., 2001).

Medium Colour Change Effect of Mutation on *Staphylococcus aureus*

In this experiment, it was also observed that the wild-type strain changed the colour of the MSA growth medium to yellow from red with a zone of 4.70 mm yellow colour. The mutants exhibited colour change at various levels. This variation range from red colour change to complete yellow (CCC mutants) through Normal colour change (NMCC mutants), Slight colour change (SLC mutants) to No colour change (NCC mutants) (Table 3). This change in colour implies that many of the mutants fermented the mannitol to release acid as the wild-type. Variation in the growth inhibition

of the cells, mode of action of the pepper and medium colour change was caused by random hits of the mutagen (EMS) on the genes encoding these properties. The genes in some cells of class 1 mutants were hit to inactivate the genes causing varying sensitivity of the mutants to inhibitory action of the pepper with concomitant differing levels in growth inhibition and fermentation of mannitol by the mutant cells. The results obtained in this work are similar to that of Vivjer *et al*. (2001) who reported that *Staph. aureus* treated with EMS formed mutants with various pathogenic virulence relative to the wild-type strain. Ethylmethane sulphonate causes mutation by transforming DNA strand. It introduces a methyl group to various points on G nucleotide. This causes guanine to faulty pairs with thymine resulting in GC:AT transition (Suzuki *et al*., 2001).

Spontaneous mutation and transfer of resistant genes among microbes in the same environment could cause resistance in microorganisms to antibiotic; this however occurs rarely (10^6 or 10^9 times) (Brock *et al*., 1984). Resistance to antimicrobial agent occurs in nature after a long use of the agent. This research has revealed that *Capsicum annum* can act differently on *Staphylococcus aureus* when subjected to mutation. This suggests that sweet pepper can be used effectively to cure diseases caused by *Staph. aureus* and the bacterium can be made resistant and sensitive like any other microorganism pointing to future possible occurrence after along use.

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REFERENCES

Al-Qarawi, A.A. and S.E. Adams, 2003. Effect of combination of *Capsicum annum* and lutein on growth, haematological and pathophysiological parameter of rats. J. Qafaz Univ., 5: 34-36.

Barber, M.S., V.S. Mc Connell and B.S. De Caux, 2000. Antimicrobial intermediates of general phenylpropanoids and lignin specific pathway. Phytochemistry, 54 (1): 53-65.

Boboye, B., 2004. Antibacterial effect of *Capsicum annum* on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Biosci. Biotech. Res. Asia, 1 (2): 49-50.

Boboye, B. and I. Dayo-Owoyemi, 2004. Antibacterial effect and minimum inhibitory concentrations of garlic (*Allium sativum*) extract on some human pathogenic bacteria. Biosci. Biotech. Res. Asia, 1 (2): 37-40.

Boboye, B., T. Babatunde and A. Onoriode, 2007. Antibacterial activities of some plants used as condiments and spices in Nigeria. World Curr. Environ., 2 (2): 171-174.

Boboye, B. and A. Alao, 2008. Effect of mutation on trehalose-catabolic-enzyme synthesized by a tropical *Rhizobium* species F1. Res. J. Microbiol., 3 (4): 269-275.

Brock, T.D., D.W. Smith and M.T. Madigan, 1984. Biology of Microorganisms. 4th Edn. Prentice-Hall International Inc., New Jersey, USA., pp: 295-297.

Cereaga, M., E. Fernandezi, L. Dorantes, L. Mota, M.E. Jaramillo and H. Hernandez-Sanchez, 2003. Antibacterial activity of *Capsicum annum* extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. Int. J. Food Microbiol., 83 (3): 331-335.

Demmig-Adams, B., A. Gilmore and W.W. Adams, 1996. *In vivo* function of carotenoids in higher plants. FASEB. J., 10 (4): 403-412.

Dorantes, L., R. Colmenero, H. Hernandez, L. Mora, M.E. Jaramillo, E. Fernandez and C. Solano, 2000. Inhibition of growth of some food borne pathogenic bacteria by *Capsicum annum*. Int. J. Food Microbiol., 57 (1-2): 125-128.

Dutta, A.C., 1981. Botany for Degree Students. 5th Edn. Oxford University Press, England, pp: 825.

Farag, R.S., Z.Y. Dawz, F.M. Hewedi and G.S. El-Barotyl, 2003. Antimicrobial activity of some Egyptian Spice essential oils. J. Food Prot., 52 (9): 665-667.

Kelloff, G.J., J.A. Growell and V.A. Steele, 2000. Progression of cancer chemoprevention: Development of diet derived thermopreventive agents. J. Nutr., 130 (2S Suppl): 467S-471S.

Kivanc, M. and A. Akgul, 1986. Antibacterial activity of essential oils from Turkish spices and citrus. Flavour and Fragrance J., 1 (4-5): 175-179.

Madigan, M., P. Martino and J. Parker, 2001. Brocks Biology of Microorganisms. 9th Edn. Prentice-Hall International Inc. London, pp: 204-208.

Parkinson, J.S., 1976. Che A, Che B and Che C genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol., 126 (2): 758-770.

Suzuki, D.T., J.E. Griffin, H.J. Miller and C.R. Lewontin, 2001. An Introduction to Genetic Analysis. 7th Edn. Freeman Publishers, USA., pp: 101-105.

Vivjer, J.C., M.M. Es-Boon and M.F. Micheal, 2001. Introduction to mutation in *Staphylococcus aureus* by ethylmethane sulphonate. Acta Microbiological Polonica, 3: 151-162.