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The Effect of Monolaurin on *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Microbial resistance to antibiotics, especially among staphylococcal strains is a major threat to public health. The aim of this study was to assessment of anti-bacterial effect of monolaurin on *Staphylococcus aureus* and *Escherichia coli*. In this study, colonies are cultured in nutrient broth media and incubated at 37°C for 24 h. Then, tubes chilled in the laboratory environment. Three different concentrations of monolaurin prepared and added to culture media containing BHI agar media. Then, these plates are incubated 37°C for 24 h and prepared to count. After 24 h, plates were out and colonies are counted and multiply in 100. Data were analyzed by SPSS software Version 16. Based on data revealed that at 60°C and more. There was no observed any microorganisms colonization either *Staphylococcus aureus* or *Escherichia coli* but at the 55°C, there was observed more decrease in colonization than 50°C. This finding suggests that chilling is one of the most important ways to maintenance of foods. The results also showed that temperature also increases inhibitory properties of monolaurin. The results of the study suggest that monolaurin have anti-bacterial effect against *Staphylococcus aureus* and *Escherichia coli*.

Key words: Monolaurin, *Staphylococcus aureus*, *Escherichia coli*, temperature, microorganisms colonization, BHI agar media

INTRODUCTION

Microbial resistance to antibiotics, especially among staphylococcal strains is a major threat to public health. Since, resistance by certain strains of Staphylococcus to multiple antibiotics like methicillin emerged in the late 1970's (Ayliffe, 1997), many strategies to control antibiotic resistance have been proposed (Cunha, 2002). Considering current therapeutic regimens, vancomycin usage has proven to be the most reliable to treat resistant staphylococcal infections (Edmond *et al*., 1996; Waldvogel, 1999; Burnie *et al*., 2000). However, some staphylococcal strains have become resistant, at least to some extent even to vancomycin indicating a dire need for new alternative therapeutic approaches (Burnie *et al*., 2000; Hiramatsu *et al*., 1997; Denis *et al*., 2002).

One specific strategy has been to use multiple antibiotics of the same spectrum and low resistance potential when single antibiotic substitutions are not effective. However, researchers have examined yet another strategy; use of natural products with low potential for the development of resistance. The anti-viral, anti-bacterial and anti-protozoal properties of lauric acid and monolaurin have been recognized for nearly 3 decades by only a small number of researchers. Their research, however has resulted in ≥50 research papers and numerous US and foreign patents. Kabara (1967) performed the original seminal research in this area of fat research. Kabara first patented certain Fatty Acids (FAs) and their derivatives, e.g., Monoglycerides (MGs) can have adverse effects on various microorganisms. While nontoxic and approved as a direct food additive by the FDA, monolaurin adversely affects bacteria, yeast, fungi and enveloped viruses.

Kabara found that the properties that determine the anti-infective action of lipids are related to their structure, e.g., free fatty acids and monoglycerides. The monoglycerides are active; diglycerides and triglycerides are inactive. Of the saturated fatty acids, lauric acid has greater anti-viral activity than either Caprylic acid (C-8), Capric acid (C-10) or myristic acid (C-14).

Fatty acids and monoglycerides produce their killing/inactivating effects by several mechanisms. An early postulated mechanism was the perturbing of the plasma membrane lipid bilayer. The anti-viral action attributed to monolaurin is that of fluidizing the lipids and phospholipids in the envelope of the virus causing the disintegration of the microbial membrane. More recent studies indicate that one anti-microbial effect in bacteria is related to monolaurin's interference with signal transduction/toxin formation. Another anti-microbial effect in viruses is due to lauric acid's interference with

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virus assembly and viral maturation. The third mode of action may be on the immune system itself (Witcher *et al*., 1996). Hierholzer and Kabara (1982) first reported the anti-viral activity of the monoglyceride of lauric acid (monolaurin) on viruses that affect humans.

They showed virucidal effects of monolaurin on enveloped RNA and DNA viruses. This research was done at the Center for Disease Control of the US Public Health Service. This study was carried out using selected virus prototypes or recognized representative strains of enveloped human viruses. All these viruses have a lipid membrane.

The presence of a lipid membrane on viruses makes them, especially vulnerable to lauric acid and its derivative monolaurin. These initial findings have been confirmed by many other studies. Research has shown that enveloped viruses are inactivated by added fatty acids and monoglycerides in both human and bovine milk (Isaacs and Thormar, 1991). Others (Isaacs and Thormar, 1986) have confirmed Kabara's original statements concerning the effectiveness of monolaurin.

Some of the viruses inactivated by these lipids are the measles virus, herpes simplex virus (HSV-1 and -2), herpes family members (HIV, hepatitis C, Vesicular, Stomatitis Virus (VSV), visna virus and Cyto Megalo Virus (CMV). Many of the pathogenic organisms reported to be inactivated by these anti-microbial lipids are those know to be responsible for opportunistic infections in HIV positive individuals. For example, concurrent infection with cytomegalovirus is recognized as a serious complication for HIV positive individuals.

These anti-microbial fatty acids and their derivatives are essentially nontoxic to man. According to the published research, lauric acid is one of the best inactivating fatty acids and its monoglyceride is even more effective than the fatty acid alone (Kabara, 1978; Fletcher *et al*., 1985; Kabara, 1985). The lipid-coated (envelope) viruses, bacteria and other microorganisms are dependent on host lipids for their lipid constituents. The variability of fatty acids in the foods of individuals as well as the variability from de novo synthesis accounts for the variability of fatty acids in their membranes.

Monolaurin does not appear to have an adverse effect on desirable gut bacteria but rather on only potentially pathogenic microorganisms. For example, Isaacs and Schneidman (1991) reported no inactivation of the common *Esherichia coli* or *Salmonella enteritidis* by monolaurin but major inactivation of *Hemophilus influenza*, *Staphylococcus epidermis* and group B gram positive streptococcus. The potentially pathogenic bacteria inactivated by monolaurin include *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae*, groups A, streptococci-gram-positive organisms and some gram-negative organisms (*Vibrio parahaemolyticus* and *Helicobacter pylori*). Decreased growth of *Staphylococcus aureus* and decreased production of toxic shock syndrome toxin-l was shown with monolaurin (Holland *et al*., 1994).

Monolaurin was 5000 times more inhibitory effect against Listeria monocytogenes than ethanol. *In vitro* monolaurin rapidly inactivate *Helicobacter pylori.* Of greater significance, there appears to be very little development of resistance of the organism to the bactericidal effects of these natural anti-microbials.

A number of fungi, yeast and protozoa are also inactivated or killed by monolaurin. The fungi include several species of ringworm (Isaacs and Thormar, 1991). The yeast reported to be affected is *Candida albicans* (Isaacs and Schneidman, 1991), the protozoan parasite *Giardia lamblia* is killed by monoglycerides from hydrolyzed human milk (Isaacs and Thormar, 1991). *Chlamydia trachomatis* is inactivated by monolaurin. Hydrogels containing monocaprin/monolaurin are potent *in vitro* inactivators of sexually transmitted viruses such as HSV-2 and HIV-1 and bacteria such as *Neisserian gonorrhea*. The aim of this study was to assessment of anti-bacterial effect of monolaurin on *Staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

In this study, colonies are cultured in nutrient broth media and incubated at 37°C for 24 h. Then, this culture is divided into the 3 separate tubes including:

- First tube incubated at 50°C for 10 min
- Second tube incubated at 55°C for 10 min
- Third tube incubated at 60°C for 10 min

Then tubes chilled in the laboratory environment. Three different concentrations of monolaurin prepared and added to culture media containing BHI agar media. Then these plates are incubated 37°C for 24 h and prepared to count. After 24 h, plates were out and colonies are counted and multiply in 100. Data were analyzed by SPSS software Version 16.

RESULTS AND DISCUSSION

Results are shown in Table 1 and 2. Based on Table 1 and 2 revealed that at 60°C and more, there was no observed any microorganisms colonization either *Staphylococcus aureus* or *Escherichia coli* but at the 55°C, there was observed more decrease in colonization

Table 1: Data related to *Staphylococcus aureus*

Parameters	1	2	3	4	5	6	7	8	9	10
Concentration of monolaurin (ppm)	0	0	0	5	5	5	10	10	10	100
Temperature	50	55	60	50	55	60	50	55	60	50
Repetition 1	∞	∞	0	63	57	0	56	17	0	0
Repetition 2	∞	∞	0	55	49	0	48	15	0	0
Repetition 3	∞	∞	0	49	41	0	27	12	0	0

Parameters	11	12	13	14	15	16	17	18	19	20	21
Concentration of monolaurin (ppm)	100	100	500	500	500	1000	1000	1000	2000	2000	2000
Temperature	55	60	50	55	60	50	55	60	50	55	60
Repetition 1	0	0	0	0	0	0	0	0	0	0	0
Repetition 2	0	0	0	0	0	0	0	0	0	0	0
Repetition 3	0	0	0	0	0	0	0	0	0	0	0

Table 2: Data related to *Escherichia coli*

Parameters	1	2	3	4	5	6	7	8	9	10
Concentration of monolaurin (ppm)	0	0	0	5	5	5	10	10	10	100
Temperature	50	55	60	50	55	60	50	55	60	50
Repetition 1	+++∞	++∞	0	+++∞	++∞	0	++∞	+∞	0	0
Repetition 2	+++∞	++∞	0	+++∞	++∞	0	++∞	+∞	0	0
Repetition 3	+++∞	++∞	0	+++∞	++∞	0	++∞	+∞	0	0

Parameters	11	12	13	14	15	16	17	18	19	20	21
Concentration of monolaurin (ppm)	100	100	500	500	500	1000	1000	1000	2000	2000	2000
Temperature	55	60	50	55	60	50	55	60	50	55	60
Repetition 1	0	0	0	0	0	0	0	0	0	0	0
Repetition 2	0	0	0	0	0	0	0	0	0	0	0
Repetition 3	0	0	0	0	0	0	0	0	0	0	0

than 50°C. This finding suggests that chilling is one of the most important ways to maintenance of foods. The results also showed that temperature also increases inhibitory properties of monolaurin.

The carriage and subsequent dissemination of antibiotic-resistant *Staphylococcus aureus* by hospital staff and patients is a recognized risk for nosocomial infections (Carson *et al*., 1995). Although, use of antibiotics has generally been tempered to avoid antibiotic resistance, the development of resistance was inevitable. Therefore what alternatives now exist to treat antibiotic resistant organisms? Essential oils, especially origanum oil and the fat monolaurin are natural substances reported to have the ability to kill *Staphylococcus aureus* and other microbes in culture (Carson *et al*., 1995; Hitokoto *et al*., 1980; Ismaiel and Pierson, 1990; Mansour *et al*., 1999; Kim *et al*., 1995; Kivanc *et al*., 1991). Nevertheless, *in vivo* studies designed to test the anti-microbial effects of various essential oils and monolaurin are lacking.

Many essential oils have been shown to be effective against a number of organisms. Origanum oil, cinnamon and clove were judged very active by examining their inhibitory effects on *Clostridium botulinum* 33A (Ismaiel and Pierson, 1990). In addition to effects against *Klebsiella pneumonia* and *Staphylococcus aureus*, Origanum oil is fungicidal (Manohar *et al*., 2001; Hitokoto *et al*., 1980). Also, anti-viral actions of Origanum and clove oils against RNA and DNA viruses have been reported (Siddiqui *et al*., 1996). As a potential mechanism of action, the outer protective membrane of the viruses disintegrated after exposure to the Origanum oil when viewed by electron microscopy (Siddiqui *et al*., 1996). Importantly, most essential oils of spices are classified as GRAS (Generally Recognized as Safe) indicating that consumers can eat them reasonably without fear (Ismaiel and Pierson, 1990). Accordingly, the benefits/risks ratio of essential oils in treating microbes would seem to be very high. Considering other natural products with anti-microbial properties, Kabara championed the use of certain lipids. He measured the anti-microbial activity of fatty acids and their corresponding monoglycerides and reported that the optimum chain length was C12 (Kabara *et al*., 1977). Of the saturated fatty acids, lauric acid (C12) has greater anti-viral activity than caprylic acid (C8), capric acid (C10) or myristic acid (C14). In contrast to monolaurin, the dilaurin derivative was inactive. It is now generally accepted that monoglycerides are active: diglycerides and triglycerides are inactive. When the anti-chlamydial effects of several fatty acids and monoglycerides were studied by incubating Chlamydia trachomatis bacteria, the results indicate that the lipid kills the bacteria, possibly by disrupting the membranes(s) of the elementary body (Bergsson *et al*., 1998). Corroborating evidence is available from viral studies suggesting that the bactericidal effects are via disintegration of membranes by fatty acids (Thormar *et al*., 1987; Isaacs and Thormar, 1991) similar to a report of the action of Origanum oil on viruses (Siddiqui *et al*., 1996).

CONCLUSION

The results of the study suggest that monolaurin have anti-bacterial effect against *Staphylococcus aureus* and *Escherichia coli*.

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