SUSCEPTIBILITY OF *AEROMONAS HYDROPHILA* TO MEDIUM-CHAIN FATTY ACIDS AND THEIR MONOESTERS

L. Malá[1,3], K. Laloučková[1,3], P. Hovorková[2], E. Skřivanová[1,3]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Prague, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Excellent Team for Mitigation, Prague, Czech Republic
[3]Institute of Animal Science, Department of Nutritional Physiology and Animal Product Quality, Prague-Uhříněves, Czech Republic

Medium-chain fatty acids (MCFAs) and their monoesters were tested for their antibacterial activity against the Gram-negative pathogen *Aeromonas hydrophila*. The antimicrobial effect was evaluated at two temperatures (4 °C and 37 °C) using a standardized microdilution method in a 96-well microtitration plate. The minimum inhibitory concentrations of selected MCFAs were determined as the lowest concentration limiting the growth of *A. hydrophila* in wells compared to a positive control of ≥ 80%. The results indicated that the most effective compound against *A. hydrophila* was sucrose monocaprate after incubation at 37 °C (0.625 mg ml^{-1}), whereas monocaprylin was the most effective compound after incubation at 4 °C (1.25 mg ml^{-1}). Free MCFAs showed no antibacterial effects towards this bacterium. Low solubility and sensory properties could limit the use of fatty acids in aquatic environment, which should be the subject of further studies.

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INTRODUCTION

The ubiquitous pathogen *Aeromonas hydrophila* is a rod-shaped, freshwater, Gram-negative bacterium belonging to the phylum of Proteobacteria, class Gammaproteobacterias, order Aeromonadales and family *Aeromonadaceae*, which consists of 17 different hybridization groups (Garrity et al., 2005; Rasmussen-Ivey et al., 2016; Fernandez-Bravo, Figueras, 2020). Virulence factors of motile aeromonads, such as adhesins, cytotoxins, and lipases, can lead to multifactorial diseases (Beaz-Hidalgo et al., 2013). *Aeromonas* spp. can cause both intraintestinal and extraintestinal diseases, and syndromes, ranging from relatively mild illnesses to life-threatening conditions, including septicaemia, necrotizing fasciitis, and myonecrosis (Janda, Abbott, 2010; Arslan, Kucuksari 2015; Palma-Martinez et al., 2016; Banerjee et al., 2017). Aeromonads have also been linked with gastroenteritis demonstrated mainly as diarrhoea in children under the age of five, with the so-called traveller's diarrhoea and with food poisoning (Ottaviani et al., 2013; Qamar et al., 2016; Tsheten et al., 2016). The main sources of foodborne infections caused by *Aeromonas* spp., as identified by Stratev, Odeyemi (2016), are sea-

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food, meat and meat products, milk and dairy products, and vegetables. Aeromonads can grow at temperatures ranging from 4 to 42 °C. This fact suggests that *A. hydrophila* is capable of growing in foods at refrigeration temperatures (Palumbo et al., 1985). As an enteric pathogen, *Aeromonas* species have the inherent capability to grow in water distribution systems, especially in biofilms (Igbinosa et al., 2012). The transmission of infection from water, animal feed and animals can be considered as a possible threat to humans. Mainous et al. (2011) evaluated a variety of commercially available chemicals efficacy in reducing or eliminating *A. hydrophila* in water. Effective disinfectants were ethanol (50% and 70%), benzyl-4-chlorophenol/phenylphenol, sodium hypochlorite, etc. The diseases associated with the genus *Aeromonas* are often treated with antibiotics. Studies indicate that fluoroquinolones and cefotaxime are the most successful therapies for infections associated with aeromonads (Alcaide et al., 2010; Parker, Shaw, 2010).

However, treatment with synthetic antibiotics in human and animal medicine is a controversial topic due to the spread of bacterial antibiotic resistance. Different authors from all around the world have reported the multidrug resistance of *A. hydrophila* (Vivekanandhan et al., 2002; Sen, Rodgers, 2004; Kaskhedikar, Chhabra, 2010; del Castillo et al., 2013). Kelley et al. (1998) postulated that the resistance of *A. hydrophila* isolates to penicillins (including penicillin and ampicillin), streptomycin, bacitracin and tetracycline originates in poultry litters. For this reason, there is an effort to find new substances of natural character that could improve the health of both animals and humans.

Medium-chain fatty acids (MCFAs) and their monoesters are one of the possible alternatives to antibiotics. MCFAs are saturated and unbranched six to twelve carbon fatty acids. This group consists of caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0) (Zentek et al., 2011). They can be found at higher levels in milk lipids of many animal species (mouse, rat, rabbit, etc.), and in some plant oils (coconut, palm, tucuma, muru-muru and *Cuphea* oil). These oils have shown a high antibacterial activity against different microorganisms (Dierick et al., 2003; Zentek et al., 2011; Hovorkova et al., 2018). The coconut oil is rich in MCFAs, especially lauric acid. It has a strong antimicrobial effect against Gram-positive bacteria and a number of fungi and viruses (Dayrit, 2014). The antibacterial properties of coconut oil were first described in the study of Hierholzer, Kabara (1982). Recent studies have shown also the effect towards Gram-negative bacteria, such as *Helicobacter pylori* (Shino et al., 2016). The microbicidal activity of a number of lipids, especially the activity of MCFAs and their monoesters, has been reported by many research groups. In various studies, the antibacterial effect against various pathogens, such as *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, and *Listeria* spp. was proved (Batovska et al., 2009; Skrivanova et al., 2009; Yang et al., 2009; Borate et al., 2013; Loung et al., 2014; Carlson et al., 2015). Some bacteria, such as *Lactobacillus* spp., are stimulated by the presence of MCFAs. For this reason, they are more preferred than polyunsaturated fatty acids (PUFAs) in this context (Guerzoni et al., 2001).

The purpose of this study is to evaluate the susceptibility of *Aeromonas* spp. strains to MCFAs and their monoesters because of lacking adequate information regarding the effect of these substances on *A. hydrophila*.

MATERIAL AND METHODS

Bacterial strain and culture media

The antibacterial activity of MCFAs and their derivatives was determined against *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943^{AL} CCM 7232^{T} (Czech Collection of Microorganisms, Brno, Czech Republic) grown and maintained in Tryptone soya broth (TSB) (Oxoid, UK). The bacterial culture was incubated at 37 °C and at 4 °C for 48 h under aerobic conditions as recommended by the Czech Collection of Microorganisms.

Medium-chain fatty acids and their derivatives

The MCFAs and their derivatives, namely, caprylic (C8:0) acid, capric (C10:0) acid, and lauric (C12:0) acid, were purchased from Sigma-Aldrich (Czech Republic), and their derivatives monocaprin, monocaprylin, and monolaurin were purchased from VWR (Czech Republic).

Preparation of medium-chain fatty acids and their derivatives for microdilution tests

Respective MCFAs and their derivates were weighed and diluted in the same amount of dimethyl sulfoxide (DMSO) (Lach-Ner, Czech Republic) (1 : 1 v/v, active substance/DMSO ratio) and TSB was added to reach a final concentration of 5 mg ml^{-1} of each potentially active compound. The final concentration of DMSO did not exceed 0.5%, and thus it did not influence the activity of tested compounds.

Determination of the antibacterial effect *in vitro*

The antibacterial activity of the tested compounds was evaluated *in vitro* by the broth microdilution method using 96-well microtitre plates, modified according to the recommendations proposed for a more effective assessment of the anti-infective potential of

Table 1. Minimum inhibitory concentrations of MCFAs and their derivatives against *A. hydrophila* (mg ml^{-1}) after 48 h of incubation at 37 °C

Compound	Trial		
	1	2	3
Sucrose monocaprate	0.625	1.25	0.625
Monocaprylin	5	5	5
Monocaprin	2.5	1.25	1.25
Monolaurin	2.5	1.25	2.5
Caprylic acid	5	> 5	> 5
Capric acid	> 5	> 5	> 5
Lauric acid	> 5	5	> 5

Table 2. Minimum inhibitory concentrations of MCFAs and their derivatives against *A. hydrophila* (mg ml^{-1}) after 48 h of incubation at 4 °C

Compound	Trial		
	1	2	3
Sucrose monocaprate	1.25	2.5	2.5
Monocaprylin	0.625	1.25	1.25
Monocaprin	> 5	5	> 5
Monolaurin	> 5	> 5	> 5
Caprylic acid	5	> 5	> 5
Capric acid	> 5	> 5	> 5
Lauric acid	5	> 5	> 5

natural products (Cos et al., 2006). Seven two-fold dilutions were carried out from the initial solution dilutions of each compound prepared in TSB.

The bacterial inoculum was standardized to achieve a density of 5×10^5 CFU ml^{-1} using the McFarland scale and inoculated into wells (10 µl). Microplates were incubated at 37 °C for 48 h under aerobic conditions. Considering the ability of *A. hydrophila* to survive in cooler temperatures, a trial at 4 °C for 48 h was also performed.

The growth of microorganisms was assessed as the turbidity determined by an Infinite 200 PRO microplate reader (Tecan, Switzerland) at 405 nm. The minimum inhibitory concentrations (MICs) were related to the density of the growth control and expressed as the lowest compound concentrations that resulted in an 80% growth reduction compared to that of the compound-free growth control. A positive control (containing 10 µl of bacterial suspension and 90 µl of TSB), a negative control (containing 100 µl of TSB) and a control with DMSO were also prepared. All samples were tested as three independent experiments, each carried out in triplicate.

RESULTS

The most effective compound against *A. hydrophila* was sucrose monocaprate (MIC_{80} = 0.625 mg ml^{-1}) after incubation at 37 °C (Table 1), whereas monocaprylin (MIC_{80} = 1.25 mg ml^{-1}) was the most effective compound after incubation at 4 °C (Table 2), followed by sucrose monocaprate (MIC_{80} = 2.5 mg ml^{-1}). Interestingly, monocaprylin exerted only a slight effect (MIC_{80} = 5 mg ml^{-1}) at 37 °C (Table 1). Two other compounds were effective at 37 °C (Table 1), monocaprin (MIC_{80} = 1.25 mg ml^{-1}) and monolaurin (MIC_{80} = 2.5 mg ml^{-1}). Both these compounds did not exert antibacterial effects against *A. hydrophila* at 4 °C (Table 2) (MIC_{80} values > 5 mg ml^{-1}), similarly to the other tested compounds. Sucrose monocaprate, as the only one of the tested compounds, was effective at both evaluated temperatures. Contrary to monoacylglycerols, free fatty acids showed no antibacterial effects in our experiment.

DISCUSSION

Although there are many studies on the effects of MCFAs on various species of bacteria, this is the first report focused on susceptibility of *A. hydrophila* to MCFAs and their monoesters. For instance, the antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus* (Monk et al., 1996; Nobmann et al., 2009), *Bacillus cereus* (Karlova et al., 2010), *Campylobacter jejuni* (Thormar et al., 2006) and *Clostridium perfringens* (Skrivanova et al., 2014) was studied. *L. monocytogenes* is well known for its ability to grow at low temperatures, similar to *A. hydrophila* (White et al., 2002). However, Monk et al. (1996) and Nobmann et al. (2009) did not test *L. monocytogenes* at low temperatures, only the antibacterial effect of compounds like free fatty acids and their esters was tested. The esters of lauric acid showed the highest inhibitory effect. Zentek et al. (2011) confirmed the considerable antibacterial activity of MCFAs (caproic, caprylic, capric and lauric acid) towards *Candida albicans* and *Lactobacillus acidophilus*.

The exact mode of antibacterial action of fatty acids has not yet been proven. However, it has been reported that fatty acids and monoglycerides produce their killing/inactivating effects by lysing the plasma membrane lipid bilayer of microorganisms (Fife, 2013), resulting in a change of membrane permeability that can lead to cell death (Altieri et al., 2009; Desbois, Smith, 2010; Kim, Rhee, 2013).

The practical use of MCFAs, or their natural form (plant oils) can be limited due to their low solubility in water. Although MCFAs and their monoesters have considerably higher water solubility than long-chain fatty acids (LCFAs), there are still some limitations that need to be addressed and further tested.

Another possible limitation can be seen in the sensory traits of MCFAs. Some free fatty acids have an unpleasant odour/taste. On the other hand, the sucrose esters of fatty acids tend not to have an unsavoury taste; thus, they are more suitable for use in practice. As in the case of fatty acids and their monoglycerols, sucrose esters are also known to be non-toxic compounds (Habulin et al., 2008).

Based on our results, free MCFAs did not show antibacterial effect against *A. hydrophila*. On the other hand, effects of their monoesters at concentrations of 0.625–5 mg ml^{-1} were observed. The sucrose monocaprate was effective at both measurement temperatures. Sucrose monoesters are commercially used for inhibiting the growth of spore-forming bacteria as antibacterial agents (Ye et al., 2010). Sugar fatty acid esters may affect cell membranes at low concentrations, leading to a change in the permeability of cell membranes (Rodriguez et al., 2004). Zhang et al. (2014) determined the antimicrobial activities of sugars (sucrose, maltose, lactose) and fatty acids (caprylic, capric and lauric acid) in sugar ester compounds against three common pathogens, *S. aureus*, *Escherichia coli* O157:H7 and *Candida albicans* (yeast). Sucrose and maltose monoesters showed a higher antibacterial activity than lactose monoesters. Thus, the antimicrobial activity could be influenced by the carbon chain length, degree of esterification and hydrophilic groups. Furthermore, the results showed that all of the tested monoesters were effective against *S. aureus*. Yang et al. (2003) demonstrated that sucrose and methylglucose esters with medium- and long-chain fatty acids suppressed the growth of two microorganisms (*Zygosaccharomyces bailii* and *Lactobacillus fructivorans*) involved in the spoilage of salad dressings. Sucrose monoesters were more effective than methylglucose esters.

Other factors can also affect the antimicrobial efficacy of fatty acids (e.g., the number of double bonds, bacterial strains, etc.) (Kabara et al., 1972). In particular, the monoglycerides monocaprin and monolaurin were identified as active antibacterial agents in several studies; however, the results of their effectiveness do not always correspond. For example, monolaurin was shown to be over 200 times more effective than lauric acid in killing *S. aureus* and *Streptococcus pyogenes*, in which the two compounds have the same number of carbons but lauric acid lacks the glycerols present in the monoglyceride (Schlievert, Peterson, 2012). Sun et al. (2003) suggested that the high antibacterial activity of MCFA derivatives could be caused by their higher stability compared to the free MCFAs. Moreover, monoglycerides exhibit higher antibacterial activity than their corresponding free fatty acids because their efficacy is independent of environmental pH (Bergsson et al., 2001; Thormar et al., 2006).

Temperature is another important factor affecting the antimicrobial effect of some compounds. In this report, the antibacterial activity of free MCFAs and their monoesters toward *A. hydrophila* was evaluated after incubation at 37 °C and 4 °C. The values were different at these measurement temperatures. It is curious, that monocaprylin was the most active compound after incubation at 4 °C. Nair et al. (2004) determined the antibacterial effect of caprylic acid and its monoglyceride, monocaprylin, on *L. monocytogenes* and *E.coli* O157:H7 in whole milk. Both these compounds were tested at 37, 8 and 4° C. Monocaprylin was the most effective in killing *L. monocytogenes* at 4 °C. This study corresponds with different efficacy of monocaprylin in our research. A possible reason for this temperature-dependent effect can be the changes of fatty acid profile and fluidity of bacterial cell membrane at lower temperatures, or different solubility of each MCFA (Nair et al., 2004).

In summary, sugar esters and monoesters of medium-chain fatty acids possess significant antimicrobial effects towards various types of Gram-positive and Gram-negative bacteria. Their potency is highly associated with the type of sugar and type of fatty acid (Nobmann et al., 2009; Karlova et al., 2010; Ye, Hayes, 2017).

CONCLUSION

In this study, a pronounced susceptibility of *Aeromonas hydrophila* strain to medium-chain fatty acids and their monoesters was observed. Given the results of our *in vitro* research, MCFAs monoesters have the potential to be effective against the Gram-negative pathogen *A. hydrophila*.

REFERENCES

Alcaide E, Blasco MD, Esteve C (2010): Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water and eels. Research in Microbiology, 161, 40–45. doi: 10.1016/j.resmic.2009.10.006.

Altieri C, Bevilacqua A, Cardillo D, Sinigaglia M (2009): Effectiveness of fatty acids and their monoglycerides against gram negative pathogens. International Journal of Food Science and Technology, 44, 359–366. doi: 10.1111/j.1365-2621.2008.01744.x.

Arslan S, Kucuksari R (2015): Phenotypic and genotypic virulence factors and antimicrobial resistance of motile *Aeromonas* spp. from fish and ground beef. Journal of Food Safety, 35, 551–559. doi: 10.1111/jfs.12205.

Banerjee B, Madiyal M, Ramchandra L, Mukhopadhyay C, Garg R, Chawla K (2017): Unusual severe extra-intestinal manifestations of a common enteric pathogen – *Aeromonas* spp. Journal of Clinical and Diagnostic Research, 11, 3. doi: 10.7860/JCDR/2017/26600.9787.

Batovska DI, Todorova IT, Tsvetkova IV, Najdenski HM (2009): Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: Individual effects and synergistic relationships. Polish Journal of Microbiology, 58, 43–47.

Beaz-Hidalgo R, Martinez-Murcia A, Figueras MJ (2013): Reclassification of *Aeromonas hydrophila* subsp. *dhakensis* Huys et al. 2002 and *Aeromonas aquariorum* Martínez-Murcia et al. 2008 as *Aeromonas dhakensis* sp. nov. comb nov. and emendation of the species *Aeromonas hydrophila*. Systematic and Applied Microbiology, 36, 171–176. doi: 10.1016/j.syapm.2012.12.007.

Bergsson G, Arnfinnsson J, Steingrimsson O, Thormar H (2001): *In vitro* killing of *Candida albicans* by fatty acids and monoglycerides. Antimicrobial Agents and Chemotherapy, 45, 3209–3212. doi: 10.1128/AAC.45.11.3209-3212.2001.

Borate PP, Disale SD, Ghalme RS (2013): Studies on isolation, analysis and antimicrobial properties of coconut shell oil. International Journal of Advanced Scientific and Technical Research, 2, 146–157.

Carlson SJ, Nandivada P, Chang MI, Mitchell PD, O'Loughlin A, Cowan E, Gura KM, Nose V, Bistrian BR, Puder M (2015): The addition of medium-chain triglycerides to a purified fish oil-based diet alters inflammatory profiles in mice. Metabolism, 64, 274–282. doi: 10.1016/j.metabol.2014.10.005.

Cos P, Vlietinck AJ, Vandenv BD, Maes L (2006): Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof-of-concept". Journal of Ethnopharmacology, 106, 290–302. doi: 10.1016/j.jep.2006.04.003.

Dayrit FM (2014): The properties of lauric acid and their significance in coconut oil. Journal of the American Oil Chemists' Society, 92, 1–15. doi: 10.1007/s11746-014-2562-7.

del Castillo CS, Hikima JI, Jang HB, Nho SW, Jung TS, Wongtavatchai J, Kondo H, Hirono I, Takeyama H, Aoki T (2013): Comparative sequence analysis of a multidrug-resistant plasmid from *Aeromonas hydrophila*. Antimicrobial Agents and Chemotherapy, 57, 120–129. doi: 10.1128/AAC.01239-12.

Desbois AP, Smith VJ (2010): Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology, 85, 1629–1642. doi: 10.1007/s00253-009-2355-3.

Dierick NA, Decuypere JA, Degeyter I (2003): The combined use of whole *Cuphea* seeds containing medium-chain fatty acids and an exogenous lipase in piglet nutrition. Archives of Animal Nutrition, 57, 49–63. doi: 10.1080/0003942031000086626.

Fernandez-Bravo A, Figueras MJ (2020): An update of the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. Microorganisms, 8, 129. doi: 10.3390/microorganisms8010129.

Fife B (2013): Health properties of coconut oil. Agro FOOD Industry Hi Tech, 24, 4–7.

Garrity G, Brenner DJ, Krieg NR, Staley JT (eds) (2005): The *Proteobacteria.* Part C: The Alpha-, Beta-, Delta-, and *Epsilonproteobacteria*. In: Bergey's Manual of Systematic Bacteriology, Vol. 2. Springer, New York, 1–1194.

Guerzoni ME, Lanciotti R, Vannini L, Galgano F, Favati F, Gardini F, Suzzi G (2001): Variability of the lipolytic activity in *Yarrowia lipolytica* and its dependence on environmental conditions. International Journal of Food Microbiology, 69, 79–89. doi: 10.1016/S0168-1605(01)00575-X.

Habulin M, Sabeder S, Knez Z (2008): Enzymatic synthesis of sugar fatty acid esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. The Journal of Supercritical Fluids, 45, 338–345. doi: 10.1016/j.supflu.2008.01.002.

Hierholzer JC, Kabara JJ (1982): *In vitro* effects of monolaurin compounds on enveloped RNA and DNA viruses. Journal of Food Safety, 4, 1–12. doi: 10.1111/j.1745-4565.1982.tb00429.x.

Hovorkova P, Lalouckova K, Skrivanova E (2018): Determination of *in vitro* antibacterial activity of plant oils containing medium-chain fatty acids against Gram-positive pathogenic and gut commensal bacteria. Czech Journal of Animal Science, 63, 119–125. doi: 10.17221/70/2017-CJAS.

Igbinosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI (2012): Emerging *Aeromonas* species infections and their significance in public health. The Scientific World Journal, 2012, Article ID 625023. doi: 10.1100/2012/625023.

Janda JM, Abbott SL (2010): The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews, 23, 35–73. doi: 10.1128/CMR.00039-09.

Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP (1972): Fatty acids and derivatives as antimicrobial agents. Antimicrobial Agents and Chemotherapy, 2, 23–28. doi: 10.1128/AAC.2.1.23.

Karlova T, Polakova L, Smidrkal J, Filip V (2010): Antimicrobial effects of fatty acids fructose esters. Czech Journal of Food Sciences, 28, 146–149. doi: 10.17221/37/2008-CJFS.

Kaskhedikar M, Chhabra D (2010): Multiple drug resistance in *Aeromonas hydrophila* isolates of fish. Food Microbiology, 28, 157–168.

Kelley TR, Pancorbo O, Merka W, Barnhart H (1998): Antibiotic resistance of bacterial litter isolates. Poultry Science, 77, 243–247. doi: 10.1093/ps/77.2.243.

Kim SA, Rhee MS (2013): Marked synergistic bactericidal effects and mode of action of medium-chain fatty acids in combination with organic acids against *Escherichia coli* O157:H7. Applied and Environmental Microbiology, 79, 6552–6560. doi: 10.1128/AEM.02164-13.

Loung F, Silalahi J, Suryanto D (2014): Antibacterial activity of enzymatic hydrolyzed of virgin coconut oil and palm kernel oil against *Staphylococcus aureus*, *Salmonella thypi* and *Escherichia coli*. International Journal of PharmTech Research, 6, 628–633.

Mainous ME, Kuhn DD, Smith AS (2011): Efficacy of common aquaculture compounds for disinfection of *Aeromonas hydrophila*, *A. salmonicida* subsp. *salmonicida*, and *A. salmonicida* subsp. *achromogenes* at various temperatures. North American Journal of Aquaculture, 73, 456–461. doi: 10.1080/15222055.2011.630265.

Monk JD, Beuchat LR, Hathcox AK (1996): Inhibitory effects of sucrose monolaurate, alone and in combination with organic acids, on *Listeria monocytogenes* and *Staphylococcus aureus*. Journal of Applied Bacteriology, 81, 7–18. doi: 10.1111/j.1365-2672.1996.tb03276.x.

Nair MKM, Vasudevan P, Hoagland T, Venkitanarayanan K (2004): Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in milk by caprylic acid and monocaprylin. Food Microbiology, 21, 611–616. doi: 10.1016/j.fm.2004.01.003.

Nobmann P, Smith A, Dunne J, Henehan G, Bourke P (2009): The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms. International Journal of Food Microbiology, 128, 440–445. doi: 10.1016/j.ijfoodmicro.2008.10.008.

Ottaviani D, Leoni F, Rocchegiani E, Santarelli S, Masini L, D'Annibale ML, Pianetti A, Carraturo A (2013): A severe case of *Aeromonas veronii* biovar sobria travellers' diarrhoea characterized by *Vibrio* parahaemolyticus co-isolation. Journal of Medical Microbiology, 62, 161–164. doi: 10.1099/jmm.0.044743-0.

Palma-Martinez I, Guerrero-Mandujano A, Ruiz-Ruiz MJ, Hernandez-Cortez C, Molina-Lopez J, Bocanegra-Garcia V, Castro-Escarpulli G (2016): Active shiga-like toxin produced by some *Aeromonas* spp. isolated in Mexico City. Frontiers in Microbiology, 7, Article 1522. doi: 10.3389/fmicb.2016.01522.

Palumbo SA, Morgan DR, Buchanan RL (1985): Influence of temperature, NaCl, and pH on the growth of *Aeromonas hydrophila.* Journal of Food Science, 50, 1417–1421. doi: 10.1111/j.1365-2621.1985.tb10490.x.

Parker JL, Shaw JG (2010): *Aeromonas* spp. clinical microbiology and disease. Journal of Infection, 62, 109–118. doi: 10.1016/j.jinf.2010.12.003.

Qamar FN, Nisar MI, Quadri F, Shakoor S, Sow SO, Nasrin D, Blackwelder WC, Wu Y, Farag T, Panchalingham S, Sur D, Qureshi S, Faruque ASG, Saha D, Alonso PL, Breiman RF, Bassat Q, Tamboura B, Ramamurthy T, Kanungo S, Ahmed S, Hossain A, Das SK, Antonio M, Hossain MJ, Mandomando I, Tennant SM, Kotloff KL, Levine MM, Zaidi AKM (2016): *Aeromonas*-associated diarrhea in children under 5 years: The GEMS experience. American Journal of Tropical Medicine and Hygiene, 95, 774–780. doi: 10.4269/ajtmh.16-0321.

Rasmussen-Ivey CR, Figueras MJ, McGarey D, Liles MR (2016): Virulence factors of *Aeromonas hydrophila*: In the wake of reclassification. Frontiers in Microbiology, 7, Article 1337. doi: 10.3389/fmicb.2016.01337.

Rodriguez E, Seguer J, Rocabayera X, Manresa A (2004): Cellular effects of monohydrochloride of L-arginine, N^{α}-lauroyl ethylester (LAE) on exposure to *Salmonella typhimurium* and *Staphylococcus aureus*. Journal of Applied Microbiology, 96, 903–912. doi: 10.1111/j.1365-2672.2004.02207.x.

Schlievert PM, Peterson ML (2012): Glycerol monolaurate antibacterial activity in broth and biofilm cultures. PLoS ONE, 7, e40350. doi: 10.1371/journal.pone.0040350.

Sen K, Rodgers M (2004): Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. Journal of Applied Microbiology, 97, 1077–1086. doi: 10.1111/j.1365-2672.2004.02398.x.

Shino B, Peedikayil FC, Jaiprakash SR, Bijapur GA, Kottayi S, Jose D (2016): Comparison of antimicrobial activity of chlorhexidine, coconut oil, probiotics, and ketoconazole on *Candida albicans* isolated in children with early childhood caries: An *in vitro* study. Scientifica, 2016, Article ID 7061587. doi: 10.1155/2016/7061587.

Skrivanova E, Molatova Z, Skrivanova V, Marounek M (2009): Inhibitory activity of rabbit milk and medium-chain fatty acids against enteropathogenic *Escherichia coli* O128. Veterinary Microbiology, 135, 358–362. doi: 10.1016/j.vetmic.2008.09.083.

Skrivanova E, Prazakova S, Benada O, Hovorkova P, Marounek M (2014): Susceptibility of *Escherichia coli* and *Clostridium perfringens* to sucrose monoesters of capric and lauric acid. Czech Journal of Animal Science, 59, 374–380.

Stratev D, Odeyemi OA (2016): Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: a mini-review. Journal of Infection and Public Health, 9, 535–544. doi: 10.1016/j.jiph.2015.10.006.

Sun CQ, O'Connor CJ, Roberton AM (2003): Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. FEMS Immunology and Medical Microbiology, 36, 9–17. doi: 10.1016/S0928-8244(03)00008-7.

Thormar H, Hilmarsson H, Bergsson G (2006): Stable concentrated emulsions of the 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the foodborne bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. Applied and Environmental Microbiology, 72, 522–526. doi: 10.1128/AEM.72.1.522-526.2006.

Tsheten T, Tshering D, Gyem K, Dorji S, Wangchuk S, Tenzin T, Norbu L, Jamtsho T (2016): An outbreak of *Aeromonas hydrophila* food poisoning in Deptsang village, Samdrup Jongkhar, Bhutan, 2016. Journal of Research in Health Sciences, 16, 224–227.

Vivekanandhan G, Savithamani K, Hatha AAM, Lakshmanaperumalsamy P (2002): Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. International Journal of Food Microbiology, 76, 165–168. doi: 10.1016/S0168-1605(02)00009-0.

White DG, Zhao S, Simjee S, Wagner DD, McDermott PF (2002): Antimicrobial resistance of foodborne pathogens. Microbes and Infection, 4, 405–412. doi: 10.1016/S1286-4579(02)01554-X.

Yang CM, Luedecke LO, Swanson BG, Davidson PM (2003): Inhibition of microorganisms in salad dressing by sucrose and methylglucose fatty acid monoesters. Journal of Food Processing and Preservation, 27, 285–298. doi: 10.1111/j.1745-4549.2003.tb00518.x.

Yang D, Pornpattananangkul D, Nakatsuji T, Chan M, Carson D, Huang CM, Zhang L (2009): The antimicrobial activity of liposomal lauric acids against *Propionibacterium acnes*. Biomaterials, 30, 6035–6040. doi: 10.1016/j.biomaterials.2009.07.033.

Ye R, Hayes DG (2017): Bioactive properties of sugar fatty acid esters. Frontiers in Bioactive Compounds, 2, 124–145. doi: 10.2174/9781681084299117020009.

Ye R, Pyo S-H, Hayes DG (2010): Lipase-catalyzed synthesis of saccharide–fatty acid esters using suspensions of saccharide crystals in solvent-free media. Journal of the American Oil Chemists' Society, 87, 281–293. doi: 10.1007/s11746-009-1504-2.

Zentek J, Buchheit-Renko S, Ferrara F, Vahjen W, Van Kessel AG, Pieper R (2011): Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Animal Health Research Reviews, 12, 83–93. doi: 10.1017/S1466252311000089.

Zhang X, Song F, Taxipalati M, Wei W, Feng F (2014): Comparative study of surface-active properties and antimicrobial activities of disaccharide monoesters. PLoS ONE, 9, e114845. doi: 10.1371/journal.pone.0114845.*:*

Corresponding Author:

Prof. MVDr. Eva S k ř i v a n o v á , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 678, e-mail: skrivanovae@af.czu.cz