SUSCEPTIBILITY OF MORGANELLA MORGANII TO VARIOUS ENVIRONMENTAL STRESSES AFTER COLD AND HEAT SHOCK TREATMENTS

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Received for Publication October 31, 2012
Accepted for Publication February 3, 2013
doi: 10.1111/jfs.12029

ABSTRACT

Morganella morganii was subjected to cold shock treatment at 10C for 2 h or heat shock treatment at 42C for 45 min. The effect of these shock treatments on the viability of M. morganii under adverse conditions was investigated. Viability of M. morganii cells during storage at 4C was slightly affected by cold shock treatment under the conditions tested (P > 0.05). However, a significant increase in the viable population of the cold-shocked cells was found in the subsequent challenge of ~18C, as compared with the nonshocked cells (P < 0.05). In addition, cold-shocked cells became more susceptible to high incubation temperature (P < 0.05). Nevertheless, cold shock treatment did not alter the susceptibility of this bacterium to 1.0 mM H2O2, 20% NaCl and low pH (pH, 3.5). On the contrary, heat shock treatment increased the susceptibility of M. morganii to low incubation temperatures and H2O2, while made the cells more resistant to high temperature. As in the cold shock treatment, heat shock treatment under the conditions tested did not affect the susceptibility of M. morganii to NaCl and low pH.

PRACTICAL APPLICATIONS

These findings may be of significant importance with regard to food safety because cooling chains and heating regimes do exist in the food industry, which could potentially induce a cold or heat shock phenomenon.

INTRODUCTION

Morganella morganii has been identified as the most prolific histamine former and has been isolated from fish incriminated in scombroid poisoning. (Kim et al. 2000, 2001a,b, 2002; Lorca et al. 2001). All isolates of M. morganii are capable of producing >1,000 ppm histamine in culture broth (Kim et al. 2001a). M. morganii has the typical characteristics of mesophilic bacteria and temperature plays an important role in affecting the growth and histamine production of this bacterium (Eitenmiller et al. 1981; Behling and Taylor 1982; Kim et al. 2002). In addition, M. morganii is an opportunistic secondary invader that was originally thought to be the cause of summer diarrhea. In 1986, Muller isolated M. morganii significantly more often from patients with gastrointestinal disease than from healthy controls (Muller 1986). Although rare, case reports implicating this organism as a cause of disease are scattered throughout the literature. For example, M. morganii has caused neonatal sepsis in an 11-day-old boy (Salen and Eppes 1997), a brain abscess in a neonate (Verboon-Maciolek et al. 1995), a tubo-ovarian abscess in a 15-year-old girl (Pomeranz et al. 1997) and arthritis in diabetic patients (Gautam et al. 2003; Cetin et al. 2008). Reports involving M. morganii infections in immunocompromised individuals include chorioamnionitis and neonatal seizures in a pregnant woman (Johnson and Feingold 1998), a postoperative foot infection in a diabetic (Gebhart-Mueller et al. 1998), and pyomyositis (Arranz-Caso et al. 1996) and meningitis (Mastroianni et al. 1994) in AIDS patients.

During the preparation and processing of food, microorganisms are usually subjected to various stresses such as cold, heat, acid, sanitizers and preservatives. These stresses cause the injury and death of microorganisms and therefore are considered to hinder their proliferation resulting in longer and safer food preservation. However, stress...
hardening, a phenomenon referring to the increased resistance to other lethal stresses after adaptation to one sublethal environmental stress, has been observed in various microorganisms (Lou and Yousef 1997; Browne and Dowds 2002; Chiang et al. 2008). Stress hardening may counteract the effectiveness of food control measures and compromise food safety. Therefore, the stress-hardening phenomenon deserves more attention and should be taken into account in the development of food preservation measures (Chiang et al. 2008).

Low-temperature preservation is usually used in food processing to control the growth and survival of spoilage and pathogenic microorganisms. However, it is reported that microorganisms, adapted to a period of low growth temperature (cold shock) may promote survival in similar or other adverse environments (Lin et al. 2004). For example, after a transfer of a cell population from 37°C to various lower temperatures, Vibrio cholerae developed an increased ability to survive and grow at temperatures down to 15°C (Datta and Bhadra 2003). Cultures of V. parahaemolyticus subjected to cold shock treatment at 15 or 20°C were able to survival better at 5 or −18°C (Lin et al. 2004). Pretreatment of Bacillus subtilis cells at 10°C affected the heat resistance of the spores formed subsequently (Movahedi and Waite 2002).

In food processing, foods are subjected to various degrees of heat treatment to inactivate the pathogenic and spoilage microorganisms. Microorganisms exposed to sublethal heat treatment, a temperature a few degree Centigrade above their growth temperature, may develop cells with changed resistance to subsequent heat challenge or other stress. This phenomenon has been termed the heat shock response. Heat shock response of microorganisms has been documented by various investigators (Lin and Chou 2004; Chiang et al. 2005; Hassani et al. 2007).

To the best of our knowledge, there is no information about the response of M. morganii to cold and heat shock treatments. Hence, in the present study, the effect of cold and heat shock treatments on the susceptibility of M. morganii to various stress conditions was investigated.

MATERIALS AND METHODS

Microorganism

M. morganii (PTCC 1078) was supplied by the Persian Type Culture Collection. The stock culture was stored at −20°C in Tryptic Soy Broth (Merck, Darmstadt, Germany) supplemented with 25% (v/v) sterile glycerol (Merck). Test organism was first activated by two successive transfers in trypticase soy broth (TSB) at 35°C for 24 h. To prepare the inoculum, 0.1 mL of the activated culture was added to 10 mL of TSB and incubated at 35°C for 18 h. This stationary phase culture used for the preparation of shocked cells and the controls (nonshock cells).

Preparation of Cold-Shocked, Heat-Shocked and the Control Cells

To prepare cold-shocked and heat-shocked cells of M. morganii, 0.1 mL of the culture was added to 10 mL of phosphate buffer (pH 7.0) which was precooled at 10°C (cold-shocked cells) or preheated at 42°C (heat-shocked cells). These were incubated at 10°C for a period of 2 h or at 42°C for a period of 45 min for cold shock or heat shock treatments, respectively. Control cells were also prepared in the same way by adding 0.1 mL of the culture to 10 mL phosphate buffered saline (PBS) (pH 7.0), which were incubated at room temperature (c. 25°C).

Challenge Studies

To examine the effect of cold and heat shock treatments on the survival of M. morganii at 4°C, 1.0 mL of the shocked cells or the control cells was inoculated into 9.0 mL of the precooled PBS (pH 7.0) at an initial population of c. 10⁶ cfu/mL. They were then incubated at 4°C for a period of 5 days. Viability was determined before and at different intervals during cold storage. To investigate the effect of cold and heat shock treatments on the survival at −18°C, 0.5 mL of the shocked or control cells was inoculated into each of the culture tubes containing 4.5 mL of the precooled PBS (pH 7.0) at an initial population of c. 10⁶ cfu/mL. Cells were then stored at −18°C in a freezer for 5 days. At various storage times, tubes were removed from the freezer and contents were thawed under running tap water. The viability of the cells was then determined.

When the effect of cold and heat shock treatments on the thermal tolerance of the test organism was examined, 1.0 mL of the shocked or control cells of M. morganii was inoculated into 9.0 mL PBS (preheated at 50°C) at an initial population of c. 10⁶ cfu/mL and incubated at 50°C for 30 min. Samples were taken at different time intervals and the survival of the cells was subsequently determined.

To determine susceptibility to 20% NaCl or 1.0 mM H₂O₂, 1.0 mL of the shocked or control cells of M. morganii was inoculated into 9.0 mL PBS (preheated at 50°C) at an initial population of c. 10⁶ cfu/mL and incubated at room temperature (c. 25°C) for 4 h. Samples were taken at different time intervals and the survival of the cells was subsequently determined.

To investigate the susceptibility of test organism to acidic condition, 1.0 mL of the shocked or control cells of M. morganii was inoculated into 9.0 mL PBS containing either 20% NaCl or 1.0 mM H₂O₂ at an initial population of c. 10⁶ cfu/mL and incubated at room temperature (c. 25°C) for 4 h. Samples were taken at different time intervals and the survival of the cells was subsequently determined.
4 h. Samples were taken at different time intervals and the survival of the cells was subsequently determined.

**Enumeration of *M. morganii***

To determine the viable population of *M. morganii*, samples were first serially diluted with physiological saline solution. Viable counts were then made by surface plating 0.1 mL of the decimal diluted sample each on duplicate tryptic soy agar plates. Colonies were counted after 36 h of incubation at 35°C.

**Statistical Analysis**

All experiments were performed in triplicate. Results were analyzed using the repeated measure analysis of variance (ANOVA) and one-way ANOVA (Statistical Package for the Social Sciences [SPSS] 16, SPSS Inc., Chicago, IL). The significance levels are expressed at a 95% confidence level (*P* ≤ 0.05) throughout.

**RESULTS AND DISCUSSION**

**Effect of Cold and Heat Shock Treatments on the Viability of *M. morganii* at 4°C**

As shown in Fig. 1, viable population of the cold-shocked cells was slightly higher than the controls during the storage at 4°C. While in the controls, about 0.92 log cfu/mL decrease in the viable population was observed after 5 days of storage at 4°C, the population of the cold-shocked cells was decreased by 0.45 log cfu/mL within the same period of time (*P* > 0.05). On the contrary, Lin et al. (2004), found a significant decrease in the survival percentage of non-cold-shocked *V. parahaemolyticus* during the storage at 4°C compared with the cold-shocked cells. They reported that the time and temperature of the cold shock treatment may affect the cold shock response of bacteria.

Unlike the cold-shocked cells, heat-shocked cells of *M. morganii* became more susceptible to incubation at 4°C, where at the end of the storage period; the viable population of the heat-shocked cells was approximately 1.2 and 1.7 log cfu/mL lower than the respective values in the control and cold-shocked cells, respectively (*P* < 0.05). The same result has been reported for *V. parahaemolyticus* (Chang et al. 2004).

**Effect of Cold and Heat Shock Treatments on the Viability of *M. morganii* at −18°C**

As shown in Fig. 2, viable population of the cold-shocked, heat-shocked and the control cells of *M. morganii* all showed a decreasing trend upon extending the storage time. However, the magnitude of reduction in the viable population was less obvious with the cold-shocked cells than the control and the heat-shocked cells. At the end of the 5-day storage period, the viable population of the cold-shocked cells was approximately 1.2 and 2.75 log cfu/mL higher than the control and heat-shocked cells, respectively (*P* < 0.05). In addition, heat shock treatment was found to reduce the resistance of this organism at −18°C and a significantly lower viable population was observed with the heat-shocked cells than the control cells after 5 days of storage (*P* < 0.05). In agreement with these observations, cold shock treatment made *V. parahaemolyticus* more resistant to freezing temperature (Lin et al. 2004), while heat shock treatment made this organism more susceptible (Chang et al. 2004).

Cells of *M. morganii* with or without cold or heat shock declined to a greater extent at −18°C (Fig. 2) than those at 4°C (Fig. 1). Formation of ice crystals and solute concentration effect, which occur at frozen temperature, are detrimental to microorganisms (Marth 1973; Davies and...
same results have been reported previously for *V. parahaemolyticus* (Skandamis et al. 2004) and *COLD AND HEAT SHOCK RESPONSE IN M. MORGANII* where at the end of the incubation period; the viable population of the heat-shocked cells was significantly higher than the control and cold-shocked cells (*P* < 0.05). The decreased thermal tolerance of *M. morganii* after cold shock treatment observed in the present study is consistent with the similar effect observed on *V. parahaemolyticus* (Lin et al. 2004). On the contrary, an increase in the thermal tolerance of *M. morganii* was observed after heat shock treatment, where at the end of the incubation period; the viable population of the heat-shocked cells was significantly higher than the control and cold-shocked cells (*P* < 0.05). The same results have been reported previously for *V. parahaemolyticus* (Lin and Chou 2004) and *L. monocytogenes* (Skandamis et al. 2008).

**Effect of Cold and Heat Shock Treatments on the Susceptibility of *M. morganii***

Being a strong oxidizing agent, H2O2 can oxidize –SH groups and thus inactivate enzyme activity (Banwart 1989). It is also reported that H2O2 may react with iron to form the more toxic hydroxyl radical (–OH) that facilitates bactericidal effect of hydrogen peroxide (Repine et al. 1981). Therefore, hydrogen peroxide has been used as a food preservative. In combination with heat, it was also employed in milk pasteurization and sugar processing (Jay 2000). Lin et al. (2004) observed that the susceptibility of *V. parahaemolyticus* cells to H2O2 increased after cold shock treatment. However, in the present study, no significant difference (*P* > 0.05) was observed in the resistance of *M. morganii* to H2O2 between cold-shocked cells and the control cells (Fig. 4). Contrary to this observation, heat-shocked cells of *M. morganii* showed a significantly lower viability than the control and cold-shocked cells (*P* < 0.05). Increased tolerance of heat-shocked *V. parahaemolyticus* and decreased tolerance of heat-shocked *L. monocytogenes* to H2O2 have been reported previously (Chang et al. 2004; Lin and Chou 2004).

**Effect of Cold and Heat Shock Treatments on the Susceptibility of *M. morganii* to NaCl***

NaCl in high concentration may cause plasmolysis, the injury and death of microorganisms (Jay 2000). Therefore, in addition to imparting salty flavor, NaCl is commonly used in various steps of food preparation to inhibit the growth of spoilage and pathogenic bacteria. It has been reported previously that environmental stress might affect the susceptibility of microorganism to high salt content. For example, *V. parahaemolyticus* becomes more susceptible to high NaCl content after exposure to ethanol shock (Chiang et al. 2008) or heat shock treatments (Chiang et al. 2005). In the present study, the cells of *M. morganii* were exposed to PBS containing 20% NaCl. In general, *M. morganii* showed a high level of resistance to NaCl and the viability did not affect significantly in the presence of 20% NaCl (Fig. 5). Furthermore, neither cold nor heat shock treatments induced a noticeable change in the viability of *M. morganii* in the presence of 20% NaCl (*P* > 0.05). According to these results, extensive research will be undertaken to generate information on the osmotic tolerance of *M. morganii*.
Effect of Cold and Heat Shock Treatments on the Acid Tolerance of M. morganii

The ability of bacteria to tolerate low pH is a very important trait to survive in a variety of environmental niches. Environmental stresses have been reported to change the susceptibility of microorganisms to acidic conditions. However, this effect varied with the type and condition of the stress and kind of microorganism. For example, increased acid tolerance of *V. parahaemolyticus* has been reported with acid adaptation (Wong et al. 1998; Koga et al. 1999) and decreased acid tolerance of *V. parahaemolyticus* has been reported with cold shock (Lin et al. 2004) and heat shock treatments (Chiang et al. 2005). However, in the present study (Fig. 6), viability of *M. morganii* in acidic environment did not affect significantly by cold shock or heat shock treatments (*P* > 0.05). This may be attributed to the high acid resistance of *M. morganii*. According to Park and Diez-Gonzalez (2004), strains belonging to the Proteae tribe such as *M. morganii* might have a glutamate dependent acid-resistance mechanism.

CONCLUSION

In conclusion, the results of the present study demonstrated that the viability of *M. morganii* cells during storage at 4°C was slightly affected by cold shock treatment under the conditions tested (*P* > 0.05). However, a significant increase in the viable population of the cold-shocked cells was observed in the subsequent challenge of –18°C, as compared with the nonshocked cells (*P* < 0.05). In addition, cold-shocked cells became more susceptible (*P* < 0.05) to high incubation temperature (50°C). On the contrary, heat-shocked cells became more susceptible to low temperatures (4 and –18°C) and more resistant to high incubation temperature (*P* < 0.05). Although, cold shock treatment under the conditions tested, did not alter the susceptibility of *M. morganii* to H₂O₂, heat shock treatment at 42°C for 45 min made the cells more susceptible to 1.0 mM H₂O₂ (*P* < 0.05). However, cold shock and heat shock treatments under the conditions tested did not affect the susceptibility of *M. morganii* to NaCl and low pH. These findings may be of significant importance with regard to food safety because cooling chains and heating regimes do exist in the food industry, which could potentially induce a cold or heat shock phenomenon. Because temperature and time of the cold or heat shock treatments as well as the growth phase of the culture may affect the extent of shock response of bacteria (Lin et al. 2004; Chiang and Chou 2009), more research is needed to better understand the response of *M. morganii* to cold and heat shock treatments as a function of different times and temperatures of the shock treatments and growth phases of the culture.

ACKNOWLEDGMENTS

This study was supported by the research grant provided by Shahid Chamran University of Ahvaz. The authors would like to thank Mrs. P. Esfahani for her kind assistance.

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