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Effect of natural preservatives on the growth of histamine producing bacteria

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Abstract: *Present study deals with the hampering of the growth of histamine producing bacteria (HPB), by using NaCl and spices which are easily available and cheaper cost wise. For this experiment, four strains of HPB viz. Vibrio parahaemolyticus, Bacillus cereus, Pseudomonas aeruginosa and Proteus mirabilis were tested against 1 to 10% concentrations of NaCl and 1 to 5% concentrations of natural preservatives (turmeric, ginger and garlic) in a basal medium. HPB showed different growth rates at different concentrations of NaCl and natural preservatives. V. parahaemolyticus, B. cereus and Ps. aeruginosa showed no growth at 10% concentration. When the HPB growth was tested with garlic, turmeric and ginger extracts, growth of all the bacteria was inhibited by garlic and turmeric extracts at 5% concentration. In ginger, V. parahaemolyticus, B. cereus and P. mirabilis were totally inhibited at 5% concentration. But Ps. aeriginosa showed very less growth at this concentration.*

Key words: *Bacteria, Sodium chloride, Garlic, Ginger, Turmeric, Natural preservative*

Introduction

Fish curing is an age old practice and is a very popular method of preservation in developed and developing countries (Shetty *et al.,* 1996). About one third of the see food in India are processed by curing (Prasad and Seenaya, 2000). Spices are widely used as curing agents in a variety of food products. Many spices including cloves, cinnamon, black pepper, turmeric, ginger, garlic and onions exhibit anioxidative activities and inhibit the microbial growth in a variety of foods (Al-Jalay *et al.,* 1987; Jurdi Haldeman *et al.,* 1987).

Sea-foods are highly perishable and can cause histamine fish poisoning through bacterial contamination, if not properly stored. Therefore, understanding about the preservation techniques to control the histamine producing bacteria in fish is essential. Though there are some techniques traditionally followed for the preservation of food fishes, yet only limited scientific information is available on these methods of preservation. Hence, the present study was carried out to find out the minimum requirements of NaCl and natural preservatives (turmeric, garlic and ginger) to inhibit the growth of histamine producing bacteria.

Materials and methods

Growth studies in different NaCl and varying concentrations of natural preservatives (ginger, garlic and turmeric) were conducted on the histamine forming bacterial isolates *viz. Vibrio parahaemolyticus, Bacillus cereus, Pseudomonas aeruginosa* and *Proteus mirabilis* which were isolated from the commercially important edible fishes *viz. Lates calcarifer, Chanos chanos, Mugil cephalus and Sillago sihama* from the Muthupettai lagoon. The inocula of these bacterial strains were prepared in seawater broth containing peptone (0.5%), yeast extract (0.5%) and maintained at room temperature (28 ±2 °C) for 24 hr. The 24 hr old cultures were then used for conducting different tests.

Growth study at different NaCl concentrations: The basal medium (broth) was supplemented with 10 different concentrations of NaCl (salt) between 1 and 10%. For each concentration, 4 sets of flasks with basal broth were aseptically prepared and kept in an autoclave at 121°C for 15 lbs and separately inoculated with the bacterial strains. The flasks were kept at room temperature (28±2 °C) for 72 hr and the optical density (O.D) was measured in a spectrophotometer (Spectronic 20 D) at 580 nm. A control for each concentration and for each bacterium was prepared (1 ml of inoculum + 100 ml of medium) at the time of taking O. D. measurements.

Preparation of natural preservative extract: The juice of young ginger, garlic and turmeric was freshly prepared by chopping and blending the edible meaty portion in a handy chopper and passing the juice through fine cloth. One ml of the stock solution with distilled water was added to the basal medium (100 ml) for the final concentration of 1 to 5%.

Growth studies in different concentrations of natural preservatives: For each concentration, 4 sets of flask with basal medium (final vol.100 ml) were taken and inoculated with the 24 hr old bacterial culture and later, the flask were kept at room temperature (28±2 °C) for 72 hr and the growth was measured at 580 nm. Control samples contained the same quantity of basal medium without juice extract of natural preservatives.

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Statistical analysis: Correlation coefficient (r) was performed between NaCl and natural preservatives and histamine producing bacteria.

Results and Discussion

Various practices are being used to preserve fish meats. For large storages, the fishes are frozen in a cold storage room at low temperature. Traditionally, fishermen are using salt but the natural preservatives such as garlic, turmeric and ginger extracts can also be used. Therefore, effectiveness of these preservatives was evaluated in the present study.

Different bacterial species tested against different concentrations of NaCl showed varying growth responses. But at the same time, all the species showed increasing growth rate at 2% concentration than 1% concentration (Fig. 1) and NaCl showed a significant negative correlation (r=0.93924) with *Ps. aeruginosa*. This reveals that the low concentration of NaCl is absolutely necessary at the initial stage for the growth of these bacterial strains.

Higher salt concentrations have long been used to preserve foods and such foods may still be spoiled by microbial growth as there are halophilic bacteria which require substantial amounts of salt for their growth. Salt range for growth may depend on environmental factors (Kushner Kamekura, 1998). Generally, bacteria are more salt dependant at higher than at lower temperatures; some times they are more specifically Na^+ dependent. Halophilic bacteria may also grow over a wide range of salt concentrations in nutritionally rich media than in poor media (Kushner, 1978; Kauri *et al*., 1990). The halophilic archaea bacteria usually have temperature optima of about 50°C for growth (Hochstein, 1988). Franzmann *et al*. (1987, 1988) have identified physchrotrophic halophilic eubacteria that can grow at 4°C and lower in hyper saline Antarctic lakes.

More detailed works have been carried out on the halopilic archaebacteria, with special reference to the effects of salts on enzyme activity and stability. Many of the enzymes of these bacteria need or can tolerate high salt (NaCl or KCl) concentrations for activity. A comparison of the aspartate transcarboxylase obtained from an extremely halophilic archaea bacterium, a moderate halophilic and a non halophile showed that salt can affect both activity and regulation (feed back inhibition) in different ways. In the absence of salts, most enzymes of the extreme halophiles are reversible or more often, immensely inactivated (Kushner, 1978).

The present study indicated that the bacterial species tested are halotolerant. However, the tolerable limits to salt concentrations may vary with different bacterial species. It has been observed that 10% NaCl is sufficient to suppress the growth of HPB population in the fish meat for preservation. But, Hussain (personal communication) has reported good growth of *Vibrio* sp., *Bacillus* sp. and *Morgonella* sp. in 1 to 10% NaCl, with higher growth in 4, 3 and 7%. Further, in the present study, *Ps. aeruginosa* alone showed a constant decrease with increasing concentrations of NaCl while all the other species showed peak and fall in growth at different concentrations. At 10% NaCl, with the exception of *Proteus* sp. which showed very little growth, rest of the bacteria were completely inhibited.

When the bacterial growth was tested at 1 to 5% of garlic extract, *Pr. mirabilis* showed higher growth at 1% concentration followed by *Ps. aeruginosa, B. cereus* and *V. parahaemolyticus* (Fig. 2). The (r) value showed negative correlation with all the four strains of histamine producing bacteria. At 5% concentration of garlic alone suppressed the growth of all the histamine producers, probably by inhibiting the coagulase activity of the bacteria. This would indicate that garlic is superior in arresting the growth of a wide range of histamine producing bacteria than turmeric and ginger. This lends support to the findings of Johnson and Vaughan (1969), who have stated that 5% extract of garlic had germicidal effect on *Staphylococcus aureus.* Garlic, which showed excellent growth restriction in this study, is also known for its antifungal effect on *Candida alficans* and other pathogenic fungi (Yamada and Azuma, 1977).

In the case of turmeric, *P. mirabilis* showed higher growth at 1% concentration followed by *Ps. aeruginosa, B. cereus* and *V. parahaemolyticus*. Growth of all the bacteria tested except *Ps. aeruginosa* was inhibited at 5% concentration (Fig. 3). The (r) values showed negative correlation with all the four strains of histamine producing bacteria. Shakila *et al.* (1996) reported that turmeric exhibited the moderate effect on the formation of toxic histamine in mackerel .

In ginger, *P. mirabilis* showed maximum growth followed by *B. cereus, Ps. aeruginosa* and *V. parahaemolyticus* at 1% concentration. *V. parahaemolyticus*, *B. cereus* and *P. mirabilis* were totally inhibited at 5% concentration of ginger extract but *Ps. aeruginosa* showed very less growth at this concentration (Fig. 4). The (r) value showed negative correlation with all the four species of histamine producing bacteria. Ramanadhan and Das (1993), have also reported that the ginger and turmeric juices were more effective than the commonly used garlic and onion juices (at 10% concentration), in inhibiting lipid oxidation in NaCl pretreated cooked ground fish.

Hence, from the present study, it is suggested that low storage temperature (-5 to 0°C) and the traditional preservatives, NaCl (10%), garlic (5%), turmeric and ginger (>5%), which are easily available and cheaper cost wise, can be used by the fisherfolk to arrest the growth of histamine producing bacteria in fishes, thereby avoiding histamine fish poisoning.

Acknowledgments

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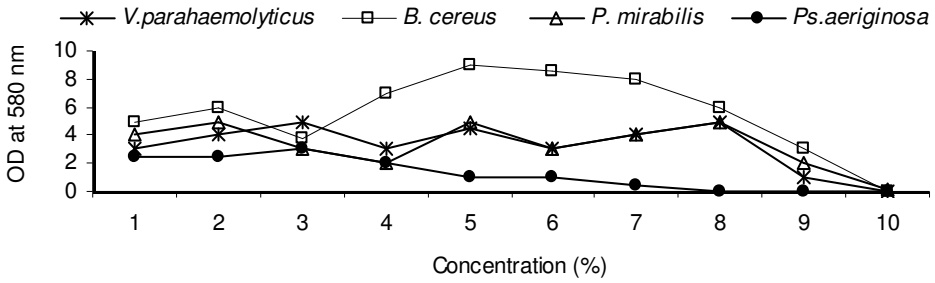

Fig. 1: Growth of histamine producing bacteria in different NaCl concentrations

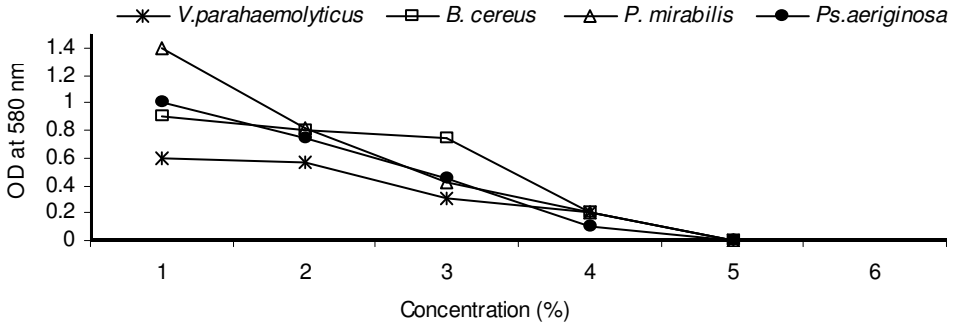

Fig. 2: Effect of traditional preservative (garlic) on histamine producing bacteria

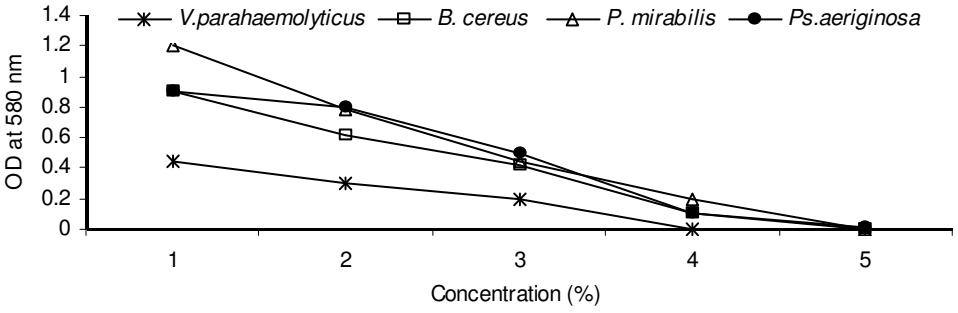

Fig. 3: Effect of traditional preservative (turmeric) on the growth of histamine producing bacteria

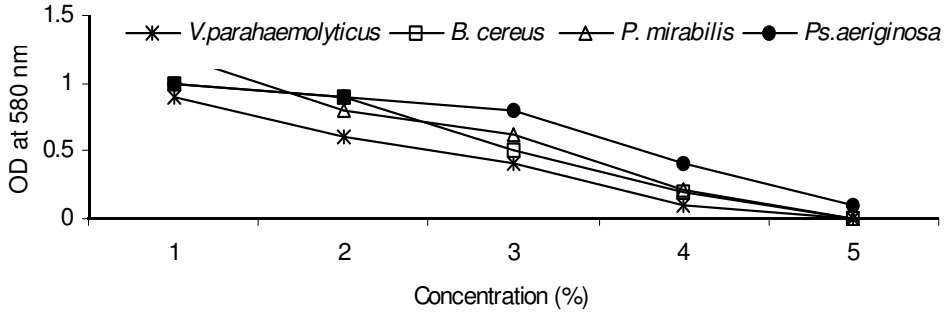

Fig. 4: Effect of traditional preservative (ginger) on the growth of histamine producing bacteria

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