

Acid, alkali and heat adaptation studies of Bacilli isolated from alkali cleaning solution in a Dairy Plant

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Abstract: *In this study bacteria were isolated from alkali cleaning solution used for cleaning of pasteurization in a Dairy Plant. The thermoduric bacteria from these samples were isolated after laboratory pasteurization in nutrient agar. The isolated samples were identified as genus Bacillus by morphology, cultural characters and biochemical reactions. The isolates were subjected to various pH treatments ranging from 4.5 to 10.5 and temperature range of 60 to 80°C. The growth of organisms estimated by enumeration of organisms at different times and the growth rate of each isolates at each pH values were calculated and plotted as a function of time.*

Key Words: *acid adaptation, alkali adaptation, heat adaptation, Thermoduric Bacilli*

I. Introduction

The pH environment of the milk contact surfaces fluctuates between acidic (4) and alkaline (9) values during Cleaning In Place (CIP) procedures used to clean the dairy processing equipment (Alvarez et al., 1998). The temperature of the cleaning procedure usually ranges from 60-70⁰C. Alkaline cleaners are those that have a pH higher than 7. They are commonly used in the dairy industry because they saponify fat and convert the fat to soap and can be removed with water. These are usually consist of sodium hydroxide (caustic soda) potassium hydroxide (caustic potash), sodium carbonate (soda ash), and sodium silicates. The most common acids found in dairy cleaning are the inorganic acids, such as phosphoric acid and nitric acid, and the organic acids, such as citric acid. The organisms surviving the acid and alkaline pH conditions will cause subsequent contamination of the pasteurized milk. Hence a study was conducted to isolate the organisms present in the alkaline cleaning solution to detect out their alkaline, acidic and heat adaptability.

II. Materials And Methods

2.1 Alkaline Adaptation Of Isolates

2.1.1 Preparation of samples

The isolated thermoduric bacilli were used for this study. The bacteria were grown overnight in nutrient broth (pH 7.4) at 37°C in an incubator. For alkaline adaptation studies pH of nutrient broth were adjusted with 1N NaOH to 8.5, 9.5 and 10.5. The tubes were inoculated with one per cent of the above prepared overnight broth culture. These tubes containing pH adjusted broth culture were used for alkaline adaptation studies.

2.1.1. Enumeration of Alkaline adapted cells

The enumeration (Mortan 2001) of the prepared samples were carried out at 0, 15, 30, 45 and 60 minutes of incubation at room temperature. The growth rate (cfu) of each isolate at each pH value was calculated and plotted as a function of time (Lindsaya et al., 2000).

2.2. Acid Adptation

2.2.1. Preparation of samples

The isolated thermoduric bacilli were used for this study. The bacteria were grown overnight in nutrient broth (pH 7.4) at 37°C in an incubator. For acid adaptation studies pH of nutrient broth were adjusted with 1N Hydrochloric acid (HCl) to 4.9, 5.5 and 6.3. The tubes were inoculated with one per cent of the above prepared overnight broth culture. These tubes containing pH adjusted broth culture were used for acid adaptation studies.

2.2.2. Enumeration of acid adapted cells

Enumeration (Mortan 2001) of the prepared samples were carried out at 0, 15, 30, 45 and 60 minutes of incubation at room temperature. The growth rate (cfu) of each isolate at each pH value was calculated and plotted as a function of time (Lindsaya et al., 2000).

2.3. Heat Adaptation

The isolated thermoduric bacilli were used for this study. The bacteria were grown overnight in nutrient broth (pH 7.4) at 37°C in an incubator. For heat adaptation studies the overnight broth cultures were inoculated into nutrient broth at 1% level and subjected to different heat treatments 65°C, 70°C and 80°C. Enumeration

(Mortan 2001) of the prepared samples were carried out at 0, 15, 30, 45 and 60 minutes of incubation. The growth rate (cfu) of each isolate at each pH value was calculated and plotted as a function of time (Lindsay et al., 2000).

III. Results

The growth curve of the isolated organisms at three different alkaline pH is given in the Figure 1. A reduction in the growth rate of the organisms at all the three alkaline pH was observed. The growth curve of the isolated organisms at three different acidic pH is given in the Figure 2. There is reduction in the growth rate of the organisms at all the three acidic pH and the highest reduction was at a pH of 6.3. The growth curve of the isolated organisms at three different temperature is given in the Figure 3. There is significant reduction in the growth rate of the organisms at all the temperature and the highest reduction was at 70°C.

Fig. 1 Growth curve of acid adapted organisms

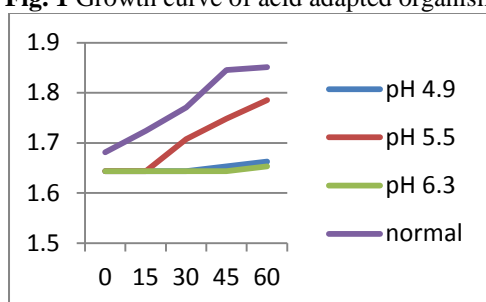


Fig. 2 Growth curve of alkali adapted organisms

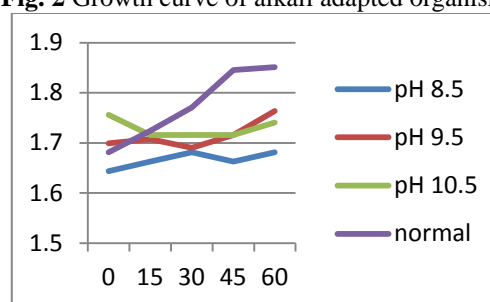
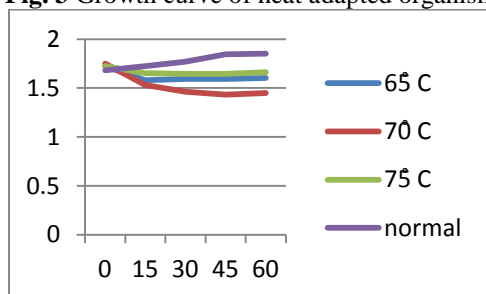


Fig. 3 Growth curve of heat adapted organisms



IV. Discussion

The maximum growth was obtained at a pH of 5.5 followed by 4.9 and 6.3. This is similar to the findings of Lindsay et al. (2000), in which the maximum growth was occurred at a pH of 5.8 for *B. pumilus* and growth rate decreased from 5.9 to 7. As the maximum inhibition to the growth rate was observed at a pH of 6.3, the concentration of acidic solution for cleaning can be adjusted to this pH for more cleaning efficiency. The alkaline adaptation studies by enumeration shows that the growth of the organism was almost similar at pH 9.5 and 10.5. However, at a pH 8.5 the growth rate of organism was much more reduced. This is not in agreement with the findings of the Lindsay et al. (2000), who reported a maximum growth rate at a pH of 7.8 followed by 8.9. In contrast to that in the present study at pH 8.5 maximum inhibition of growth occurred and as pH increasing the growth rate is increased. The enumeration of the heat adaptation of the isolated organisms showed that the growth of the organisms was maximum inhibited at 70°C followed by 65°C and 75°C.

V. Conclusion

For the maximum inhibition of the Bacilli which is predominant in the various samples of alkali cleaning solution tested, it is recommended to use minimum of 70°C for its destruction. The preferred pH for alkaline and acid cleaning solution were found to be 8.5 and 6.3 respectively.

References

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