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MICROBIAL DYNAMICS DURING CUCUMBER FERMENTATION

By

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Cucumber *(cucumis sativus)* is one of the primary vegetables often fermented to obtain pickles. The cucumber pickle is an example of a product of a mixed microbial fermentation in which desirable and undesirable bacteria and fungi interact and compete during the initial stages of the fermentation. The harvested cucumbers were subjected to fermentation in 10%(w/v) NaCl solution. The changes in microflora of the fermenting cucumber in brine solution were studied for 15 days. The results showed initial counts of 4.2 x 10^6cfu/ml, 1.4 x 10^3cfu/ml, 5.0 x 10^6cfu/ml and 1.2 x 10^3cfu/ml for lactic acid bacteria (LAB), fungi, indicator organisms and salmonella-shigella respectively in the fermenting brine solution. There was sharp increase in counts of LAB and indicator organisms in the brine on the 3rd day and thereafter they started to decline but the indicators were finally inhibited by the 12th day. The salmonella-shigella showed very slight increase on the 3rd day but finally inhibited by the 12th day. The fungi counts fluctuated and reached their peak on the 6th day, but were finally inhibited also by the 15th day. LAB persisted to the end of the fermentation with the total count of 2.1x10cfu/ml. The results of the cucumber fruit showed a different pattern. There were no growth of salmonella-shigella within the fruit for the period of fermentation. On the third day of fermentation only LAB and indicator organisms grew with counts of 5x10^3cfu/g and 3x10^3cfu/g respectively. The indicator organisms were inhibited within the cucumber by the 6th day. The aerobic plate counts (APC) and lactic acid bacteria (LAB) maintained steady colony counts of 3.7 log_{10} cfu/g to 4.3log_{10}cfu/g and 5.0 log_{10}cfu/g to 3.6log_{10}cfu/g respectively to the end of the fermentation period. The changes in the microbial flora in the brine and fruit could have been due to the competitive nature of the microorganisms in any environment. The fermentation process has proved to be effective in eliminating pathogenic organisms and other contaminants and also preserved the produce.

Keywords: Fermentation, pickles, microbial, enteropathogenic microflora.

Introduction

Slicing or table cucumber is usually served raw in salads, sandwiches, and drinks to add crunch, but they can also be cooked like zucchini (cook *et al*., 1998; Booth, 1971). Various microorganisms are usually associated with fresh fruits and vegetables. Such as normal flora transit flora, spoilage and pathogenic organisms. Cucumber grows in contact with the soil insects, animals, during growing and harvesting in the field and therefore, the microbial flora will include soil microorganisms, contaminated irrigation water, direct contamination by wild animals, birds and insects, during transportation with contaminated containers (Reina, *et al.,* 2002; Mcdonald, *et al*., 1991, Heaton and Jones, 2007; Williamson, *et al.,* 2003). The common microorganisms usually isolated from cucumber are enteropathogenic bacteria, lactic acid bacteria (LAB), *Pseudomonas* spp, Erwinia carotovora and some fungi and the heterotrophic plate counts in the produce range between 4.0x10^2 to 5.7x10^2 CFUg-1 (Nahaisi, *et al.,* 2005).

. The predominating microbial changes occurring in certain brined and salted vegetables undergoing natural fermentation was studied by Etchells and Jones (1943; 1946). The normal microbial flora and the contaminants are usually not removed before natural fermentation of the produce commences(Battock and Azam-Ali 1998). Early studies of cucumber fermentation indicated that three groups of microorganisms were often actively involved (Rosen and Fabian, 1953; Etchells *et al*., 1952). There is usually succession of LAB during fermentation of pickled cucumber. The epiphytic LAB which occur naturally on the surface of the cucumber initiate fermentation. These bacteria effectively control the microbial ecology of the fermentation by consuming the glucose and fructose present, producing lactic acid and lowering the brine pH. The low pH favors *Lb. plantarum, a* homofermentative, acid-tolerant LAB which takes over the fermentation. Cucumber fermentations typically undergo a homolactic acid fermentation, which does not result in production of carbon dioxide from sugars. Fermentation is carried out primarily by *Lactobacillus plantarum,*. Currently, starter cultures of *Lb. plantarum* are rarely used. Most commercial cucumber fermentations rely upon growth of the epiphytic LAB which occur naturally on the surface of cucumbers. These bacteria effectively control the microbial ecology of the fermentation by consuming the glucose and fructose present, producing lactic acid, lowering the brine end fruit pH. The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the cucumber. The CO_2 produced replaces the oxygen creating an anaerobic environment that help to preserve the color of the cucumber. In addition to CO_2 and lactic acid, LAB produce a variety of metabolites like bacteriocins, peroxides and peptides that can inhibit other bacteria (DeVuyst and Vandamme, 1994; Etchells and Jones, 1943; Sapers and Annous, 2004). Many factors therefore contribute to the changes in microbial flora in both the fermenting cucumber brine and within the fruit. Safety of any food product is paramount. The perishable nature and potential hazard associated with cucumber has necessitated this method of fermentation. This study is aimed at the determining the microbial ecology of fermenting cucumber in brine solution, the elimination and succession of the groups of microorganisms. With an increase in foodborne infections associated with the consumption of fresh fruits and vegetables, and also the perishable nature of these produce, it is the aim of this study to determine the effect of simple fermentation technology on spoilage and pathogenic microorganisms. Also, the microbial interactions, succession and those that play active roles during fermentation are determined. The results from this study will determine if this produce can be used in the rural areas to effectively preserve it and also prevent food poisoning resulting from eating raw fruits and vegetables.

Materials and methods

Cucumber samples were harvested aseptically, ie making sure that no new microbes are introduced onto the samples, from farms in Odukpani Local Government Area of Cross River State and placed in food grade sampling bags. These were put into a cool box (temp. 4^oC-8^oC) (APHA, 1998) to avoid physiological shock of the organisms (Williamson *et al*., 2003) and transported to the laboratory. Eight small cucumber samples (40g) were each placed in wide mouthed glass bottles of 2 litres capacity containing 10%(w/v) sodium chloride (Morton Salt, Chicago, USA) solution. The bottles were closed with caps fitted with rubber septums to allow for sampling with syringes (Olsen and Perez-D'iaz, 2009) The bottles were shaken properly and a sterile syringe was carefully used to take the brine solution (10mls) from it through the rubber septum at the start of the fermentation. A ten fold dilution of this was prepared. Five grams of cucumber fruit were obtained from the bottle and surface sterilized with 70% ethanol (Difco) and placed in a sterile blender. The blender was also sterilizedusing 70% erhanol. This was blended and 1g portion of the produce was placed in 9ml peptone-saline diluent and further dilutions were made up to 10^{-5}. Duplicates of 0.1ml each from the brine dilution and the fruit dilution were pipetted from the 10^{-4} and 10^{-5} dilutions and plated on deMan Ragosa Sharpe (MRS) agar for isolation and enumeration of acid producing bacteria, MacConkey agar for the coliform bacteria, Sabauraud dextrose agar for fungi and Salmonella-Shigella agar for isolation of *Salmonella* spp and *Shigella* spp. All the culture media were obtained from Becton Dickinson Co.,MD, USA. These were incubated at 37^0C for 18-24hrs except the SDA plates which were incubated at 27^0C for 2 days. At the end of the incubation, the colony forming units on the various media were counted from the duplicate plates and the averages taken for each medium. Using the dilution factors and the amounts plated, the microbial loads for the different groups of the organisms were calculated. The cucumber fruits were allowed to ferment at room temperature (25^oC-27^oC). Cucumber fermentation at room temperature (27^0C was carried out for a period of 15 days during which samples of brine solution and fruits were aseptically taken out at intervals of 3 days and the above procedures repeated.

Results:

Four groups of microorganisms were isolated and identified in the fermenting brine and 3 groups from the fruit. These were the LAB, indicators (*E.coli*), yeasts and pathogens (Salmonella and Shigella) from brine, and LAB, Indicators and fungi from the fruit. The microbial changes in the brine solution and cucumber fruit during fermentation are shown in Figure 1 and Table 1 respectively. The average lactic acid bacteria and indicator organisms (*E.coli*) in the fermenting cucumber brine solution as indicated in Fig. 1 increased to a maximum on the 3^{rd} day and gradually declined thereafter as fermentation continued. The fungi population decreased to a minimum on the 3^{rd} day, and increased to a maximum on the 6^{th} day and declined thereafter. By the 15^{th} day the fungi were totally inhibited. The indicator organisms increased to a maximum on the 3^{rd} day and declined to total inhibition by day 12. *Salmonella* and *Shigella* were also inhibited by day 12.

The fermenting cucumber fruit showed a different pattern of microbial population changes. There was no growth of *Salmonella* and *Shigella* species in the fruit for the period of the fermentation, while indicator organism *(E. coli)* was detected on the third day as shown in Table 1. This is in line with the finding of Samish *et al*., (1963) who stated that the inner tissues of fruits and vegetables are usually not sterile. Also, *E. coli* was probably in the brine solution and could have gotten access into the tissues of the cucumber through any cut on the produce.

Other than the first day of fermentation, the LAB maintained steady counts up to 15 days of fermentation while the aerobic plate count decreased to 3.6 log cfu/g from 5 logcfu/g. The pH of the cucumber fruit dropped from 6.75 to 3.26, while the pH of the brine solution dropped from 6.98 to 3.39 on the final day of the fermentation. Table 1 shows the categories of microbial changes within the fermenting cucumber

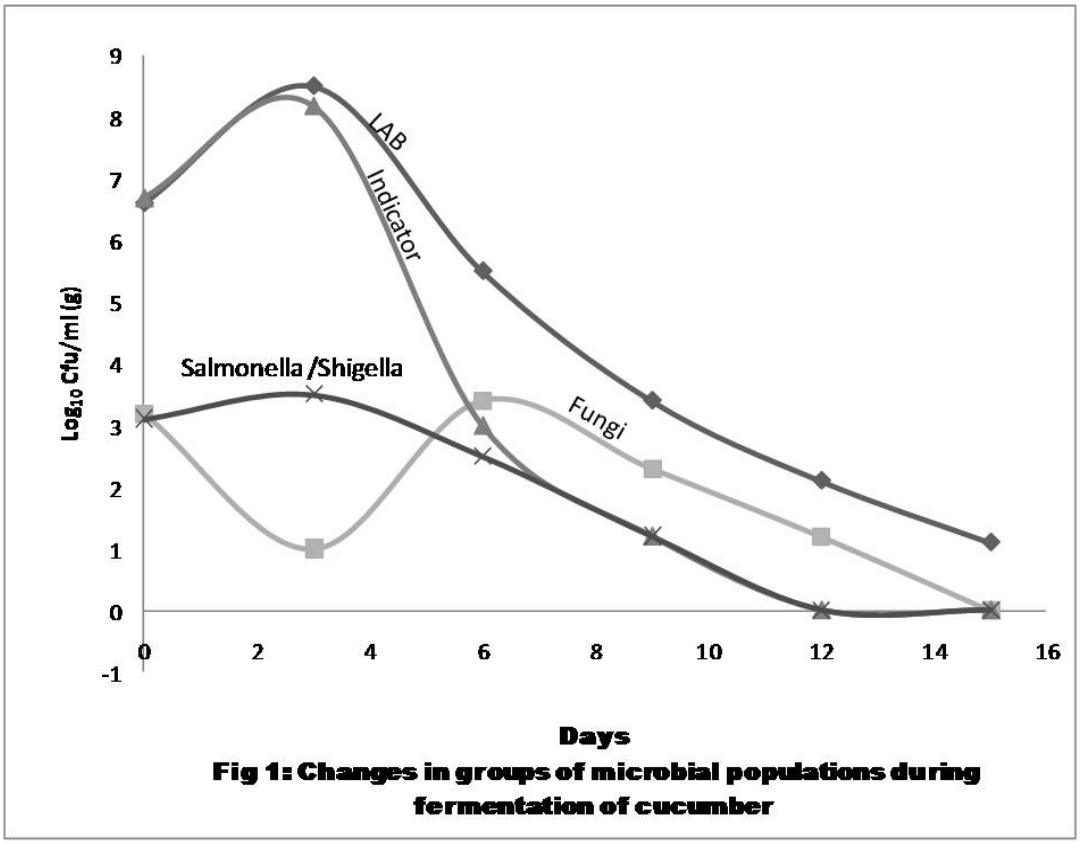

Fig 1:Changes in groups of microbial populations during fermentation of cucumber

Table 1: Bacterial and pH changes within cucumber during fermentation for 15 days at room temperature

	Bacterial Counts (log cfu/g)				
	pH	S.S	LAB	Fungi	Indicator organisms
Day 1	5.75	NG	NG	NG	NG
Day 3	4.60	NG	3.7(± 0.2)	5(± 0.6)	3.5(± 0.3)
Day 6	3.45	NG	4.4(± 0.1)	4.7(± 0.2)	NG
Day 9	3.27	NG	4.3(± 0.4)	3.7(± 0.3)	NG
Day 12	3.25	NG	4.2(± 0.7)	3.5(± 0.2)	NG
Day 15	3.26	NG	4.3(± 0.5)	3.6(± 0.3)	NG

S.S Salmonella and shigella, LAB-lactic acid bacteria, NG-No growth

Discussion

The interactions of the groups of microorganisms during the fermentation of the cucumber resulted in the elimination of fungi, indicator organisms (*E, coli*) and salmonella/shigella at different stages of the fermentation period. The pattern of LAB growth was reported by Costilow and Fabian, (1953). They reported a sharp increase of LAB in the first few days of fermentation and steady decline as the fermentation progressed. This could have been due to the less acid tolerant species of the LAB that die off and then succeeded by the more acid tolerant LAB species (McDonald et al., 1990). The fungi, indicator organisms (*E.coli*) and salmonella/shigella could not compete favourably with LAB due to the unfavourable condition during the fermentation and therefore were eliminated (Pederson and Albury,2005; Costilow and Fabian, 1953, (De Vuyst and Vandamme, 1994; McDonald, *et al.,* 1990; Fleming, *et al.,* 2002). Their roles during the fermentation were not known, but it is assumed that they were contaminants on the cucumber before fermentation.

Spoilage and pathogenic organisms are a serious problem in fruits and vegetables and the results of this study has shown that the fruit was safe for consumption and also can be kept for extended period of time. The absence of food-borne pathogens and indicator organisms in the fermented cucumber is a positive development and this corroborates the findings of Daeschel, *et al.,* (1984). The natural controls which affect the microbial populations of the fermenting cucumber could have been due to the concentration of the salt, the temperature of the brine, the availability of fermentable materials and the numbers and types of micro-organisms present at the start of fermentation. The decrease and total inhibition of both the indicator and pathogenic organisms were positive developments.

Microbial interactions in brine solution of fermenting cucumber are interesting phenomena. This simple technology is a breakthrough in the preservation of fruits and vegetables in this part of the world. Also, the elimination of pathogenic food borne organisms makes safe the produce for consumption. The need for chemical preservatives will no more be there, and also, further treatment because of food borne pathogens will not be necessary.

Conclusion

This study has been able to show that there were heterofermentative LAB that produced enough acid and were succeeded by the more acid-tolerant homofermentative LAB. The high acid could have created uncondusive environment that inhibited the growth of the other categories of organisms that were isolated in the beginning of the fermentation. The four groups of organisms isolated from the brine solution of the fermenting cucumber were inhibited as fermentation progressed except lactic acid bacteria. This study is significant in that this simple technology has been able to prolong the shelf life of the cucumber and made it safe for consumption. The interactions among the groups of organisms in the fermenting brine solution and fruit and the subsequent elimination of some can be explored further. This will serve as a reference point in this part of the world and can be used to preserve other fresh produce and make them safe for consumption.

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