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Bioinhibition of diarrhogenic Gram-positive bacterial pathogens by potential indigenous probiotics

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Abstract

High level infant mortality rates and onset of drug resistance has led into the possible development of indigenous probiotics as alternative bacteriotherapy in the control of infantile bacterial diarrhoea. This study was to determine the in vitro inhibitory potential of four probiotic candidates obtained from Nigerian indigenous fermented foods and beverages and from faecal specimens of healthy infants on infantile Gram-positive diarrhogenic bacterial pathogens. Potential probiotic candidates, AAOOL4, L. reuteri AAOOCH1, L. plantarum AAOO25NN and L. delbrueckii AAOOT20 were assayed for in vitro bactericidal effects on diarrhogenic bacterial test strains- Bacillus cereus 25S, B. cereus 32S, B. licheniformis 26S and B. licheniformis 39S. All the test strains inoculated into an industrial infant weaning food already seeded with the probiotic strains were significantly inhibited within 96 hours. L. acidophilus AAOOL4, L. reuteri AAOOCH1, L. plantarum AAOO25NN and L. delbrueckii AAOOT20 had in vitro bactericidal effects on bacteri isolates implicated in infantile diarrhoea, indicating the probiotic potential of the candidates.

Keywords: bactericidal; biotherapy; diarrhoea; infantile; probiotics; tropics

INTRODUCTION

The alarming rise in antibiotic resistance among bacterial isolates in developing countries has been continually reported and it is an established fact that the onset of drug resistance threatens virtually all classes of antibacterial agents [1, 2, 3, 4]. There is also prediction of a worsening situation, especially in paediatric chemotherapy [5] and coupled with various adverse effects exhibited by antibiotics, this type of therapy is of clinical significance in children, especially neonates and infants. It is also known that antibiotic therapy breaks down some of the normal host defence systems, leaving individuals more susceptible to infection with opportunistic pathogens [6]. The various side effects of antibiotics in children also include allergic reactions and some other forms of severe discomforts. The need therefore, for a new antimicrobial drug has become an obvious scientific challenge, since inappropriate use and clinical conditions have also favoured selection for strains resistant to an increasing number of antibiotics [7].

For more than a century, researchers have suggested that live bacterial cultures, such as those found in yogurt, might be useful in the prevention and treatment of gastrointestinal disorders [8]. The development of innovative biodrugs by using living microorganisms which are active in the human digestive environment has also been considered by various workers [9, 10, 11, 12, 13, 14, 15]. The potential medical applications of such biodrugs in few developed countries are numerous, however, studies on probiotics are still very scanty and yet mostly undocumented in Nigeria. This preliminary study is therefore, undertaken to investigate the in vitro bactericidal effects of locally developed probiotics on Gram-positive bacterial isolates implicated in infantile diarrhoea, using

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an industrial infant weaning food as a basal medium.

MATERIALS AND METHODS

Bacterial species

Diarrhogenic stool and vomitus specimens were obtained from patients at Oni Memorial Children Hospital, Ibadan, and Department of Paediatrics, University College Hospital (UCH), Ibadan, Nigeria. Final microbiological analyses were carried out at the Nigeria Institute of Medical Research (NIMR), Lagos and Department of Botany and Microbiology, University of Ibadan laboratories.

Lactobacillus isolates from infantile faecal samples of healthy children and Nigerian indigenous fermented foods and beverages were examined microscopically, and initial confirmation and grouping of the lactobacilli was based on Gram's reaction, catalase reaction with hydrogen peroxide [16], growth at 15℃ and 45℃ in MRS and medium; gas and acid production from glucose, and fermentation of lactose, sucrose, arabinose, fructose and mannitol [17]. The isolates that met the results for these preliminary identification criteria were separately grown in replicates overnight (18-24 hours) in 10 ml Rogosa broth at 35℃ until the weight of the cell mass obtained was 0. 05-0. 1g. The purity of the strains was checked, and the cells were then washed twice in 0. 9% sterile NaCl solution after centrifuging. The isolates were stored at 40C in Hogness freezing buffer (3. 6mM K_2HPO_4; 1. 3mM KH_2PO_4; 2. 0mM Na-citrate; 1. 0mM $MgSO_4$; 12% glycerol) and kept frozen. Identification of the bacterial isolates combined the standard phenotypic taxonomic tools which include morphological, biochemical and physiological characteristics.

Determination of probiotic potential

The Lactobacillus strains were assayed for their resistance to physiological bile, survival at simulated gastric and intestinal pH (pH 1-13) and minimal resistance to antibiotics.

Survival of diarrhogenic bacteria in infant weaning food:

The diarrhogenic bacterial species and the Lactobacillus strains were separately grown using standard procedures and appropriate culture media. For each set up, the inoculum was prepared by separately washing the bacterial strains several times with sterile phosphate-buffer saline. The microbial load of the freshly prepared inoculum was adjusted to give about 105cfu ml-1 (diarrhogenic bacteria) and 107cfu ml-1 (Lactobacillus strains). For the preparation of the industrial weaning food, 50g of Nestle Nutriend maize formula was dissolved in 150 ml of boiled but cool water (50℃) as specified by the manufacturer. The appropriate volume of tap water was brought to a vigorous boil and allowed to cool slightly before the required quantity of the weaning food powder was added while stirring to avoid lumps.

The probiotic candidates were then introduced at concentrations of 107cfu ml-1 into the cool, prepared weaning food in the culture flasks (10% v/v) and left for about 6h. Ten ml of the diarrhogenic cell suspensions (105 cfuml-1) were then added to the culture flasks and the contents (10% v/v) were thoroughly homogenised to ensure even distribution of the inoculum. Incubation was at 35℃ for 96 hours. Both the control samples (no single or mixed probiotic cultures) and the probiotic culture samples contained same inocula levels at 0h. Growth of the diarrhogenic indicator bacteria was monitored throughout the experimental period. The quantitative determination of the surviving diarrhogenic bacteria was a modification method of Olukoya et al. [18].

RESULTS

Inoculation of the test diarrhogenic isolates B. cereus 25S, B. cereus 32S, B. licheniformis 26S and B. licheniformis 39S into the prepared infant weaning food that was previously inoculated with the probiotic candidates (L. acidophilus AAOOL4, L. reuteri AAOOCH1, L. plantarum AAOO25NN and L. delbrueckii AAOOT20) as single and mixed cultures showed significant inhibition of the various diarrhogenic test isolates between 24 and 96h, with the highest inhibition mostly produced by the mixed probiotic cultures (Figs. 1a-1d).

A similar decline trend was observed among the growth kinetics of the diarrhogenic indicator isolates. It was noticeable that the indicator isolates in the probiotic pre-inoculated infant weaning food reached significantly lower levels faster than the uninoculated industrial infant weaning food samples, however, the inhibitory activities of the potential probiotics were not diarrhogenic-species specific.

DISCUSSION

The premium placed on children in African societies is so high, yet, even in the 21st century, the childhood mortality rates in majority of African countries remain disturbingly high and is still increasing [19, 20]. It is a well known fact that diarrhoeal diseases are a principal cause of childhood morbidity and mortality in the developing countries and are responsible for the death of more than 4 million children each year [21, 22, 23, 24] and which can account for as much as 25% of their national healthcare costs [22]. Paediatric diarrhoea has also been reported as the most common cause of global mortality in children under five years of age [25]. Several workers have reported diarrhoeal diseases as being of infective aetiology, and similarly, the isolated Bacillus species [26, 27, 28] obtained in this study have been implicated in causing infantile and children diarrhoea in previous studies [22, 24, 25, 29, 30, 31, 32].

High level infant mortality rates and onset of drug resistance being exhibited by the aetiologic microorganisms responsible for diarrhoea[3] has therefore, led into the possible development of Lactobacillus probiotics as alternative biotherapy in the control of infantile bacterial diarrhoea. It is however, necessary to determine the bacteriostatic or bactericidal effects of the probiotic candidates on the infectious pathogens, using an industrial infant weaning food as a basal material, since children are mostly weaned on industrial infant weaning foods in Nigeria in recent times.

Oral consumption of health-promoting Lactobacillus or probiotics has been associated with the prevention, alleviation, or cure of diverse intestinal disorders such as viral and bacteria gastroenteritis [33, 34, 35, 36, 37, 38, 39]. Much of the early evidence on the actual health effects of probiotics was anecdotal but during the last few years, data based on rigorous clinical studies indicating health-promoting properties of certain well-characterised Lactobacillus strains have started to accumulate. However, fundamental laboratory research is necessary as the primary prerequisite in probiotic therapy, especially, since diet, geographical location and host factors have been found to have effects on selection of probiotic candidates.

The growth kinetics (log cfu ml-1) of the diarrhogenic bacteria inoculated into the industrial infant weaning food, which already contain single and mixed probiotic candidates indicated that a decline trend was observed among the diarrhogenic indicator isolates. There was a significant decrease in the number of the surviving indicator organisms in the probiotic inoculated samples compared with the uninoculated infant weaning food samples, with the highest inhibition of the indicator bacteria mostly produced by the mixed probiotic cultures. Similar results had been previously reported by Kaila et al. [40], Castagliuolo et al. [41], McFarland [42] and other workers. The inhibitory activities recorded in this study were quite lower than that recorded in the previous study of Ogunshe [14]. This may not be unconnected with the fact that the probiotic candidates were inoculated into de Man, Rogosa and Sharpe (MRS) medium which had a very low ph value of about 5.0. The pH range of the MRS medium would have also contributed to the inhibitory activities of the probiotic candidates; however, the significant reduction obtained in the current study also indicated that the infant weaning food sample would support the inhibitory activities of the potential probiotics in the control of infantile diarrhoea.

This study has exploited the in vitro advantage of lactobacilli as probiotics in a particular weaning food and the advantages of the application of probiotics as determined in this study are the broad range antimicrobial activity of the probiotics against diarrhogenic bacterial agents leading to a possible prophylactic and therapeutic value as earlier suggested by Fuller [43], Olukoya et al. [18] and Shornikova [44]. However, further studies are on-going in the determination of the supportive effects of all the industrial infant weaning foods in Nigeria in the inhibitory activities of the probiotic candidates, as well as the determination of the effect of diets and sources of the lactobacilli on the probiotic activities of the probiotic candidates, to confirm if geographical locations has any effect on the inhibitory activities of probiotics.

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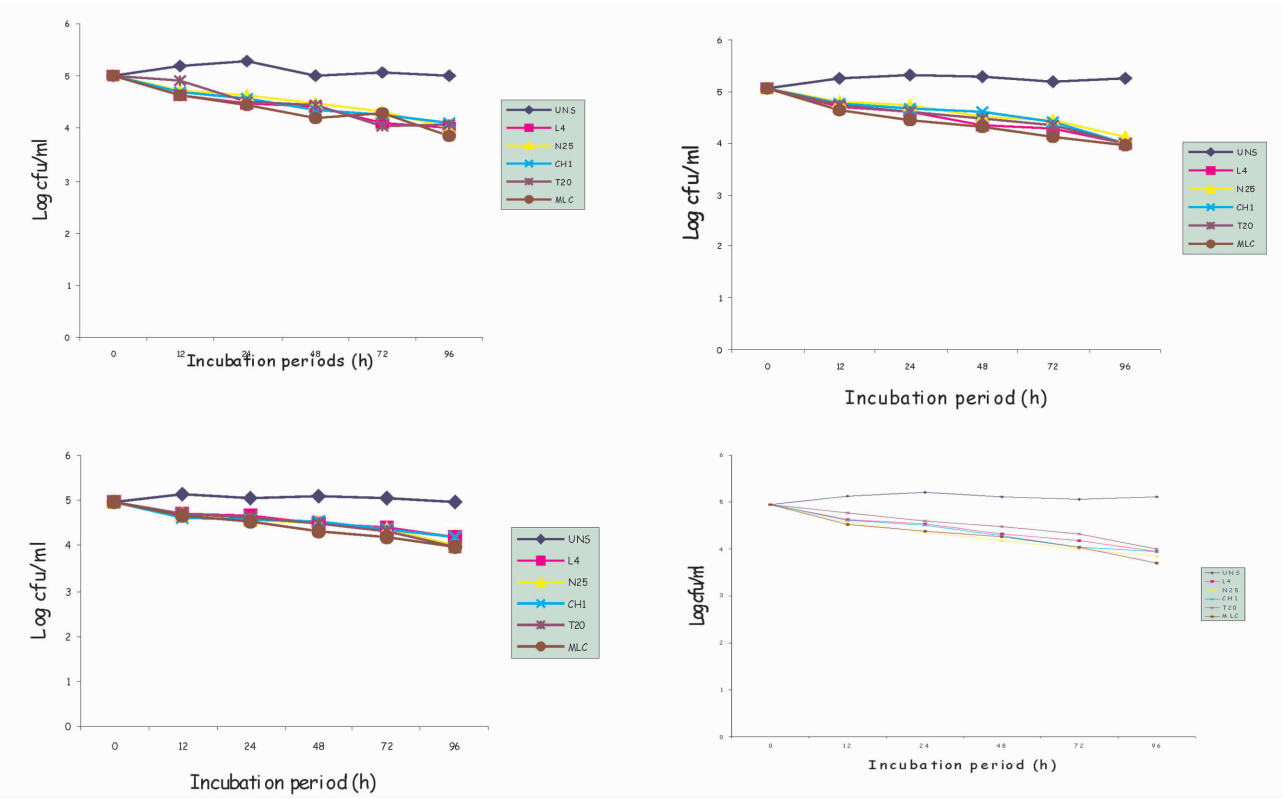

Fig. 1: Growth kinetics (log cfu/ml) of diarrhogenic Gram-positive bacteria inoculated into an industrial weaning food

Keys: L4 = Lb. acidophilus AAOOIRL4; N25 = Lb plantarum AAOONN25; CH1 = Lb. reuteri AAOOCH1; T20 = Lb. delbrueckii SOT20; MLC = Mixed Lactobacillus culturesValues represent mean scores (n = 2)

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