Screening cattle dung and poultry faeces for isolation of lactic acid bacteria in Umuahia, Abia State, Nigeria

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Abstract: A total of 40 samples, 20 of cattle dung and 20 of poultry faeces were collected from various sites in Umuahia metropolis, Abia State, Nigeria, and were analyzed using pour plate method to screen for the presence of lactic acid bacteria (LAB). Forty eight organisms belonging to three genera and four species were isolated. The four species were *Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus salivarius, Lactobacillus sakei* .The total viable LAB count ranged from $2.60x10^6$ -$3.75x10^6$ CFUs/ml for cattle dung and $1.56x10^6$ -$3.60x10^6$ CFUs/ml for poultry faeces. The mean viable LAB counts were $3.13x10^6$ CFUs/ml and $2.58x10^6$ CFUs/ml for cattle dung and poultry faeces, respectively. However, there was no significant difference between the mean viable LAB counts for the two types of specimens. Cattle dung and poultry faeces represent sources from which potentially useful LAB organisms could be isolated and exploited for biotechnological applications. [Uche RC, Ekundayo E O**.** Screening cattle dung and poultry faeces for isolation of lactic acid bacteria in Umuahia, Abia State, Nigeria *Academ Arena* 2013;5(11):1-4] (ISSN 1553-992X). http://www.sciencepub.net/academia. 1

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1. Introduction

The isolation and screening of microorganisms from natural sources has always been the most powerful means for obtaining useful and genetically stable strains for industrially important products. Lactic acid bacteria (LAB) are important in the food and dairy industries because the lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavour enhancers. Many members of the Lactobacillales produced lactic acid as their major or sole fermentation product and are sometimes collectively called lactic acid bacteria. *Streptococcus, Enterococcus, Lactococcus, Lactobacillus,* and *Leuconostoc* are all members of this group. Lactic acid bacteria are non-sporing and usually non motile (Prescott *et al*., 2006).

Lactic acid bacteria find increasing acceptance as probiotics which aid in stimulating immune responses, preventing infection by enteropathogenic bacteria, and treating and preventing diarrhoea (Reid, 1999). Lactic acid bacteria are found in a variety of habitats, also in the gastrointestinal (GI) tract of human. Lactic acid bacteria have a long history as generally regarded as safe (GRAS) organisms and especially members of genus *Lactobacillus*, *Lactococcus* and *Streptococcus* are widely used in fermentation industry (Fuller, 1989). Several species of genus *Lactobacillus* have been used in food products as probiotic organisms. Probiotic strains are selected for potential application on the basis of particular physiological and functional properties. Probiotic performance of species cannot be yet made based on scientific evidence, therefore probiotic properties are assumed to be strain-specific (Sanders *et al*., 1999). This is one of the reasons for continuous search for new strains with better probiotic properties.

Lactic acid bacteria are present in the microbiota of mammals and birds (Fuller, 1989), and those originating in the intestine have undergone intensive study for their potential probiotic properties and their rapid establishment as bacterial communities for the prevention of colonization by pathogenic bacteria. Different studies aimed to identify the microbiota of the gastrointestinal tract of poultry pointed out the predominance of lactobacilli such as *Lactobacillus crispatus,* which was isolated from chicken crops and intestine (Beasley *et al*., 2004); *Lactobacillus rhamnosus* TB1, from the intestinal tract of chicken and exhibiting good adherence and in vivo colonization (Bouzaine *et al*., 2005); *Lactobacillus salivarius* with antagonism against *Escherichia coli* and *Salmonella* Enteritidis were found in gastrointestinal tracts of chicks. The objective of this study was to screen poultry faeces and cattle dung for isolation of lactic acid bacteria in Umuahia, Abia State, Nigeria.

2. Sample collection and culture preparation

Poultry faeces were collected from different poultry farms and cattle dung were collected from different cattle herds in Umuahia metropolis. Twenty samples each of cattle dung and poultry faeces were collected. Samples were transported to the Microbiology laboratory of Michael Okpara University of Agriculture Umudike, Abia State for analysis. De Man Rogosa Sharpe (MRS) agar was prepared from dehydrated powder at a concentration of 68.4g per litre and sterilized by autoclaving at 121°C for 15 minutes according to the manufacturer's instructions.

2.1 Isolation of lactic acid bacteria

One gram of each of the samples was homogenized in 9ml of sterile normal saline. Ten-fold serial dilutions of the suspension were made by transferring 1ml of the suspension into 9ml of sterile normal saline in a test tube up to 10^{-5} dilution. The pour plate method was used for bacterial isolation and enumeration by adding 0.2ml of the appropriate dilutions to 20ml of molten MRS agar cooled to 45°C in McCartney bottles. The suspension was mixed and poured into sterile 90mm plastic Petri dishes. The agar was allowed to gel and the plates were incubated aerobically at 37°C for 48 h. After incubation, the colonial morphology and characteristics were recorded and Gram staining technique was performed on representative colonies. Further biochemical tests were carried out according to the methods described by Buchanam and Gibbon (1975) and Cheesbrough (2000).

3. Result Analysis

3.1 Recovery of LAB isolates

A total of 48 LAB isolates were recovered from the samples, 24 from cattle dung and 24 from poultry faeces. Four species belonging to three genera were isolated. The frequency of isolation of each species is shown in Table 1. *Lactococcus lactis* was the most frequently isolated species from cattle dung while *Lactobacillus salvarius* was the isolate with highest percentage occurrence in poultry faeces. Overall, *Leuconostoc mesenteroides* which was isolated from both cattle dung and poultry feaces occurred most frequently (35.4%). *Lactobacillus salivarius* was not isolated from cattle dung just as *Lactobacillus sakei* was not isolated from poultry faeces.

3.2 Total viable lactic acid bacterial counts

The total viable lactic acid bacterial counts in samples are shown in Table 2. The total viable LAB counts ranged from $2.60\text{x}10^6$ -$3.75\text{x}10^6$ CFUs/ml for cattle dung and $1.58\text{x}10^6$ -$3.60\text{x}10^6$ CFUs/ml for poultry faeces. The mean viable LAB counts were 3.13 $\text{x}10^6$ CFUs/ml and 2.58 $\text{x}10^6$ CFUs/ml for cattle dung and poultry faeces, respectively. The viable LAB counts did not differ.

Table 1. Occurrence of Lactic acid Bacteria (LAB) in cattle dung and poultry faeces in Umuahia.

Sample Type	*№ of Samples Analyzed*	*Species of LAB isolated*	*№ of Isolates*	*Occurrence in sample type (%)*	*Occurrence in both types of samples (%)*
Cattle Dung	20	*Lactobacillus sakei*	5	20.83	10.42
		Leuconostoc mesenteroides	9	37.50	35.42
		Lactococcus lactis	10	41.67	33.33
Poultry faeces	20	*Lactobacillus salvarius*	10	41.67	20.80
		Leuconostoc mesenteroides	8	33.33	35.42
		Lactococcus lactis	6	25.00	33.33

Table 2 Total viable lactic acid bacterial counts in samples of Cattle dung and Poultry faeces.

Sample Type	**Mean Total Viable Count of LAB(CFU/ml) $\text{x}10^6$**	**Range of Total Viable Count $\text{X}10^6$**
Cattle dung	3.13	2.60 - 3.75
Poultry faeces	2.58	1.56 – 3.60

4. Discussion

In this study, a total of 48 isolates of lactic acid bacteria(LAB) belonging to 3 genera and 4 species were obtained from 40 samples made up of 20 samples of cattle dung and 20 samples of poultry faeces collected from various sites in Umuahia metropolis . Twenty four (24) isolates were recovered from each of the two types of samples. The organisms isolated were *Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus salivarius* and *Lactobacillus sakei.* Isolation of various species of LAB from faeces or gastrointestinal tracts of various animals has been reported: rat (Patil *et al*., 2006), dogs (Beasley *et al*., 2006, poultry (Nazef *et al*., 2007, Ibourahema *et al.,* 2008), piglets (Petsuriyawong and Khunajakr, 2010) and cow (Fungsin *et al*., 2013).

The number of species and isolates obtained in this study were small compared to that of Beasley *et al.* (2006) who recovered 13 species and 153 strains from 21 canine faecal samples examined. Fungsin *et al*. (2013) reported isolation of 240 strains from 31 samples of cow dung whereas Ibourahema *et al*. (2008) reported a total of 30 isolates in 5 species from poultry faeces and soil from poultry houses in Senegal. The differences in geographical locations, animal host species and different microbiological techniques employed may be responsible for the wide variability in the number and types of LAB from different studies. Another factor that could have contributed to the low number of isolates in our study is the fact that our cultures were only incubated aerobically. Lactic acid bacteria are facultative anaerobes and the incubation under strict aerobic condition could have limited the growth of those strains that are less tolerant to the presence of Oxygen. In terms of total viable bacterial counts, the average bacterial counts of $3.13x10^6$ CFU/ml in cattle and $2.58x10^6$ CFU/ml in poultry faeces appeared to be higher than 5.8×10^5 CFU/ml reported by Beasley *et al.* (2006). *Lactococcus lactis and Leuconostoc mesenteriodes* were more frequently isolated; 35% and 34%, respectively and the least *Lactobacillus sakei (*10.4%). The LAB load of the sample analyzed revealed that cattle dung has a higher LAB count ($3.75x10^6$-$2.60x10^6$ CFU/ml) than poultry faeces ($3.60x10^6$-$1.56x10^6$ CFU/ml). The frequency of isolation of particular strains from an animal species and the quantity of organisms are part of the important considerations in the selection of LAB for potential probiotic evaluation (Fuller, 1989). While Ibourahema *et al*. (2006) isolated *Lactobacillus casei* and *Lactococcus lactis* from the poultry samples; we did not isolate *L. casei* from our samples. *Lactococcus lactis* was the most frequently isolated species from cattle dung while *Lactobacillus salvarius* was the most commonly isolated from cattle dung. Elsewhere, the isolation of *L. salvarius* has been reported (Fungsin *et al*., 2013. *L. salvarius* isolated from canine faeces showed properties of priobiotic potential (Beasley *et al.,* 2006). *Lactobacillus sakei* was isolated from cattle dung in this study. This organism is traditionally associated with meat sausage and has been used for preservation of fresh cut meat due to its antilisterial properties.

We recognize the limitations of the characterization of our isolates based on physiological characteristics. Molecular –based characterization might have identified the isolates differently. For example, Baele *et al*. (2003) showed that *L. ingluviei* isolated from pigeon was phenotypically similar to *L. salvarius*.

Lactic acid bacteria are among the major groups of microorganisms being exploited for their probiotic potentials. Probiotic organisms in the gastrointestinal tracts of farm animals beneficially affect the host animal by improving its intestinal microbial balance, stimulating immune responses and preventing infection by enteropathogenic bacteria either by competitive exclusion or by producing substances such as biocin which are deleterious to pathogenic organisms (Fuller, 1989). The knowledge of type of LAB found in the gastrointestinal tracts of farm animals and the contribution these organisms make to the health of the animals is important. This can contribute to the development of health enhancing products that can be used specifically for each species. Different animal groups have their characteristic gut microflora. The study of these gut flora can result in the identification of organisms with probiotic potentials which can be developed for specific application in the animal species (Beasley *et al*., 2006). As pointed out by Fuller (1989), the attachment to epithelial cells is very host-specific; hence the most effective probiotic will likely be developed from organisms isolated from a particular species.

This preliminary screening of cattle dung and poultry faeces for isolation of LAB has shown that these samples represent potential sources of LAB strains with probiotic potentials. It is therefore recommended that more detailed characterization of the probiotic properties of LAB strains from these sources be undertaken.

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