academicJournals

Vol. 9(12), pp. 880-885, 25 March, 2015
DOI: 10.5897/AJMR2015.7384
Article Number: 7C2EA2A51860
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Characterization of probiotic bacteria isolated from regional chicken feces

S. M. Khaledur Rahman[1, 2], Biplab Kumar Dash[2]*, Forhad Karim Shoikat[2], S. M. Mahbubur Rahman[1] and Khondoker Moazzem Hossain[1]

[1]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh.
[2]Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Jessore-7408, Bangladesh.

Received 18 January, 2015; Accepted 13 March, 2015

The present study was aimed to isolate probiotic bacteria from poultry feces and to study their physiological and biochemical as well as probiotic properties. Analysis of morphological, physiological and biochemical properties confirmed that all the bacteria were Gram positive, endospore negative, catalase negative and non-motile those are the characteristics of typical probiotics. Sugar fermentation profiling of 16 important sugars ensured the presumptive identification of *Lactobacillus acidophilus*, *Lactobacillus brevis* and two *Bifidobacterium* species. All bacteria were resistant to artificial gastric juice environment at pH 2.2 and 6.6 but their resistance capacity decreased after 24 h of incubation at 37°C. These bacteria were found to multiply after 24 h of incubation at 0.3% of artificial bile salt, and to grow moderately even at 9% of NaCl. This study suggests that the isolated bacteria possess feasible physiological and biological properties to be good candidates for formulating probiotic mix for livestock and chicken.

Key words: Probiotics, chicken feces, livestock, *Lactobacillus*, *Bifidobacterium*.

INTRODUCTION

The digestive flora in avian species is frequently a complex mixture of microbial populations variously colonizing in gastrointestinal (GI) tract areas. Hundreds of diverse microorganisms are reported to recognize to subsist in the flora of the chicken's GI tract in which a few are responsible for providing nutritional benefits (Gong et al., 2002). Thus researchers put considerable attention to find out these host-friendly microorganisms and to use them directly in convenient ways. The latest inclination is the use of blend of these live bacteria with nutrients usually sugars in livestock to control undesirable intestinal pathogens, especially in view of the concern over the use of antibiotics in livestock feed. Probiotics are homo/hetero-geneous culture of live microbes that help a host to nourish nutritionally by improving the percentage of indigenous beneficial microbes in host's gut through

*Corresponding author. E-mail: bk.dash@just.edu.bd. Tel: +8801717643365.

competitive exclusion and antagonism (Fuller, 1989), by improving feed ingestion and digestion (Nahanshon et al., 1993) and by varying bacterial metabolism (Cole et al., 1987; Jin et al., 1997). Considering the significant contribution in healthy nutrition, probiotic lactic acid bacteria (LAB) especially species within the genera *Lactobacillus* and *Bifidobacterium* have been using frequently in functional food manufacturing (Holzapfel et al., 1998).

Analysis of previous studies concerning isolation of probiotic bacteria, especially LAB, showed that they can be found in dairy and meat products, sewage, plants, and in human and animal feces (Kandler and Weiss, 1986; Bayane et al., 2006). LAB isolated from chicken and poultry samples include *L. aviaries* (Fujisawa et al., 1984), *L. fermentum* sub sp. *cellobiosus* and *L. animalis* (Gusils et al., 1999) from gastrointestinal tracts of chicken, *L. gallinarum* and *L. Johnsonii* (Fujisawa et al., 1991), and *Lactobacillus casei* (Bayane et al., 2006) from chicken feces. These selected bacteria from chickens can be used as potential ingredients for chicken probiotic feed formulation intended to control salmonellosis and to improve poultry sanitation (Qin, et al., 1995; Gusils et al., 1999; Pascual, et al., 1999). The already carried out research in number of countries encouraged us to focus our aim of constituting a collection on LAB with the hope to formulate later a nutritionally effective chicken probiotic livestock feed.

MATERIALS AND METHODS

Sample collection

Chicken feces samples were collected from two local poultry farms located at Jessore and Satkhira districts of Bangladesh. Two samples were chosen from two different regional farms for maximum bacterial species variability. Feces samples were alienated from other trashes and stored at 4°C in sterile poly-bags discretely to protect from deterioration and contagion.

Isolation of LAB from sample

LAB were isolated from the samples using adapted GYP (Glucose Yeast Peptone) media at pH 6.8 according to the technique followed by Bayane et al. (2006). Five grams of sample were mixed with 100 ml of GYP broth medium to prepare suspension and was incubated anaerobically at 30°C for 24 h. Then 100 µl of the suspension was diluted up to ten logarithmic (10^{-10}) fold and spread onto GYP agar medium. The culture was incubated aerobically at 30°C for 24 h. LAB were finally purified by repetitive streaking on agar plate and by microscopic assessment.

Identification of bacterial isolates

Morphological, physiological and biochemical properties of isolated bacteria were analyzed by some common tests. Colony morphology (color, shape and size) were normally examined with open eyes, sometimes microscopic assessment was considered to separate colonies. Gram staining was carried out according to the protocol of Harley and Prescott (2002). For sugar fermentation test, bacterial culture was prepared in 10 ml MRS (De Man Rogosa and Sharpe) medium at 37°C, and further inoculation and incubation were carried out according to Erkus (2007). Motility-Indole-Lysine (MIL) partially broth medium was equipped and supplementary exemption was done according to Reller and Mirrett (1975) for motility test. Endospore test and catalase test were also executed for accuracy of categorization by Schaeffer and Fulton (1933) and Holt et al. (1994) methods correspondingly.

Analysis of probiotic properties

NaCl tolerance test was carried out using test tubes containing MRS broth furnished with special concentrations (1-10%) of NaCl, according to Hoque et al. (2010). Gastric juice tolerance capability was determined by a slight moderated procedure described by Graciela and Maria (2001) at pH 2.2 and pH 6.6. Phenol tolerance was performed in MRS broth with different concentration (0.1-0.4%) of crude phenol and 1% (v/v) of fresh overnight culture as described by Hoque et al. (2010). MRS broth medium with bile salt (0.05, 0.1, 0.3 and 0.6%) was utilized to determine the tolerance and growth rate of isolated bacteria. Agar plates were equipped by 0.5% (w/v) sodium salt of taurocholic acid to establish bile salt hydrolase activity test. To examine milk coagulation property, 1% (v/v) culture of isolated bacteria was inoculated into pure milk and incubated for 24 h.

RESULTS

Morphological, physiological and biochemical characterization

From the morphological, physiological and biochemical investigation, the isolated bacteria were identified as *Lactobacillus acidophilus, Lactobacillus brevis* and *Bifidocterium spp. L. acidophilus* and *Bifidocterium* spp. were isolated and identified from sample 1 whereas sample 2 was endowed with both *Lactobacillus brevis* and *Bifidocterium spp.* Among 16 sugars, all were fermented by *L. acidophilus* excluding sorbitol, mannitol, rhamnose while *L. brevis* did not ferment salicin, rhamnose and sorbitol. The sugar fermentation outline of *Bifidobacterium* spp. was also found positive apart from rhamnose and sorbitol.

All the four bacteria were gram positive and found non-motile during growth motivation down the inoculation line. Colony morphologies showed, very small circle shaped non-transparent colonies for *L. acidophilus* and small bar shaped non-transparent colonies for *L. brevis* (Saccaro et al., 2011). Triangular minute watery circle with white center colonies examined for *Bifidocterium* spp. were similar with the findings of expert group of Japanese association of fermented milks and fermented milk drinks. In light microscopic examination, deficient of endospores specify that all the isolates were non-endospore forming. Due to production of no gas during addition of H_2O_2, all bacterial species were claimed as catalase negative. The transformation of purple to yellow color of media was the indication of particular sugar fermentation performed by the isolated bacteria. It was examined that each bacterium had distinct carbohydrate fermentation model which has been presented in Table 1. Carbohydrate fermentations

Table 1. Carbohydrate fermentation profiles of isolated bacteria.

Sugar used	Lactose	Mannitol	Sucrose	Fructose	Salicin	Ribose	Cellubiose	Glucose	Maltose	Xylose	Rhamnose	L-Arabinose	D-Sorbitol	D-Mannose	Raffinose	Galactose
L. acidophilus	+	+/-	+	+/-	+	+	+	+	+	+	-	+	-	+	-	+
L. brevis	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+
Bifidocterium spp.(1)	+	+	+	+/-	+	+	+	+	+	+	-	+	-	+	+	+
Bifidocterium spp. (2)	+	+	+	+/-	+	+	+	+	+	+	-	+	-	+	+	+

'+'indicates good fermented; '+/-' indicates moderately fermented; '-'indicates not fermented.

Table 2. NaCl tolerance test of isolated bacteria.

NaCl (%)	*L. acidophilus*	*L. brevis*	*Bifidocterium* spp. (1)	*Bifidocterium* spp. (2)
1	++	++	++	++
2	++	++	++	++
3	++	++	++	++
4	++	++	++	++
5	++	++	++	++
6	++	++	++	++
7	++	+	++	+
8	+	+	+	+
9	-	-	+	+
10	-	-	-	-

'+' indicates low level growth; '++' indicates normal growth; '-' indicates no growth.

found little incongruity which could be association of frequent environmental factors.

Characterizations for probiotic properties

Current work showed that isolated bacteria were good enough for growing at 1-7% of NaCl concentrations but at 8 and 9% of concentrations each bacterium showed moderate growth enlightened at Table 2. No expansion was found at 10% of NaCl concentration. Isolated bacteria had the competence to settle fit in mock gastric acid atmosphere at low pH (pH 2.2) and approving pH (pH 6.6) but their stamina were decreased after 24 h of incubation at 37°C. The uphill shapes in Figure 1a indicate tolerability of the bacteria at pH 2.2. All isolated species showed excellent proliferative ability at 0.1 and 0.2% of phenol and moderate ability at 0.3 and 0.4%. The lines in Figure 1b and c specify tolerability of the bacteria in 0.2 and 0.4% of crude phenol respectively. Data was expressed as average value of isolated bacteria at various concentrations after 12 and 24 h of incubation at 37ºC. The isolated bacteria were too competent to proliferate in the above mentioned concentrations of bile acid after 24 h of incubation at 37ºC. The optical density averages were diagramed in Figure 1d for symbolizing tolerance ability of the bacteria to synthetic bile salt at highest concentration (0.6%). The isolated bacteria were able to deconjugate 'taurine-conjugated' bile acid and to generate deoxycholic acid. The activity of isolated bacteria turns their colonies into intense rough white or impulsive halos signifying the bile salt hydrolase positive. Coagulation of milk was observed due to formation of lactic acid while isolated bacterial culture was supplemented with fresh skim milk. All the isolated bacteria were competent to clot milk and turned into curd which is one of the most important properties of probiotic bacteria.

DISCUSSION

To be an attractive probiotic enough to attract livestock industries, LAB should possess better biological activities as well as physicochemical attributes resistance to adverse conditions in digestive tracts, desiccation and conservation parameters (Bayane et al., 2006). The LAB used as starters play an essential role to inhibit the growth of food spoilage bacteria by producing lactic acid

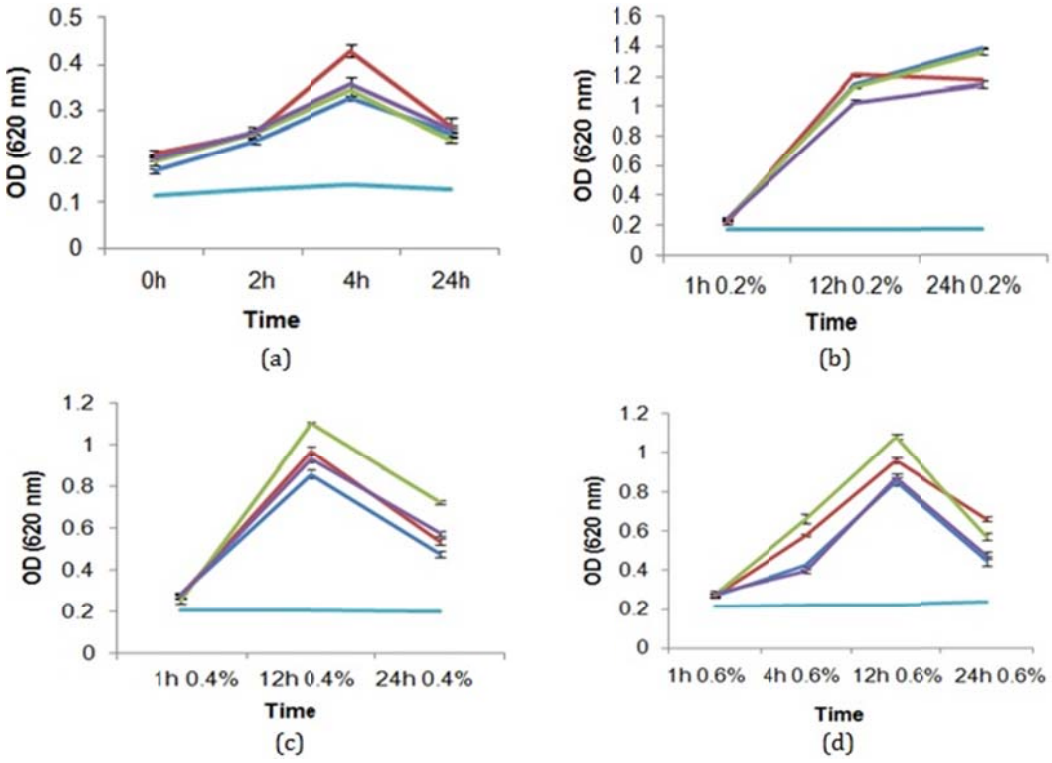

Figure 1. Survival and multiplication abilities of isolated bacterial species in **(a)** artificial gastric juice at pH 2.2; **(b)** crude phenol (0.2%); **(c)** crude phenol (0.4%), and **(d)** artificial bile salt (0.6%). Here, for *L. acidophilus*; for *L. brevis*; for *Bifidocterium spp.* (1); for *Bifidocterium spp.* (2); and for control.

and occasionally antimicrobial compounds like bacteriocins (Buckenhüskes, 1993; Gomez and Malcata, 1999). Isolation and identification of different probiotic strains from poultry feces indicate that poultry are good source of probiotic bacteria. They did exhibit good probiotic characteristics which might be considered as excellent probiotic candidate for feed producing industry that could be beneficial for poultry and animal health. There is an urgent need for development of indigenous probiotic strains for expressing optimal functionality and that reasoned the present experiment to consider isolating probiotic bacteria from regional poultry feces.

L. acidophilus, *L. brevis* and two *Bifidobacterium* spp. were isolated from two chicken fecal samples. Different types of probiotic bacteria originate in different types of chicken samples from different locations due to various reasons. The expansion of probiotic bacteria varies mainly for various ecological ambiences. Among them optimal temperature, convenience of carbohydrates, and favorable pH condition significantly vary the proliferation outline of specific bacteria. For instance *L. acidophilus* grows actively at low pH values (below pH 5.0) and an optimal growth temperature of around 37°C but *Lactobacillus delbrueckii* subspecies *bulgaricus* is very susceptible to O_2 contact with 45°C optimal growth temperature. Besides, *Lactobacillus brevis* has favorable temperature fixed between 40 and 45°C but remain active at 60°C for 30 min.

According to Food and Agricultural Organizations (FAO) and World Health Organization (WHO), one of the main properties of probiotic bacteria is salt tolerance, mainly NaCl (FAO/WHO, 2002). In this study the isolated bacteria had terrific tolerance against 1-7% NaCl. They showed stumpy altitude of growth at 8 and 9% NaCl while no expansion was found and at 10%. Hoque et al. (2010) reported to isolate *Lactobacillus* spp. from yoghurt samples that experienced different concentrations of NaCl from 1 to 10% with positive growth. The NaCl tolerance test results of the present study were found analogous with that. Another experiment of Elizete and Carlos (2005) showed that isolated *Lactobacilli* from gastrointestinal tract of swine were endurable to 4-8% NaCl. The research of Schillinger and Lucke (1987) showed expansion of *Lactobacilli* isolated from animal protein and meat products in the presence of 7.5% NaCl, and the outcome is nearly similar to our study.

FAO/WHO (2002) reported that probiotic bacteria have to proliferate at various pH because gut have to experience a fair range of acidic conditions depends on food type. In synthetic gastric fluid the isolated bacteria in this study showed good acceptance of overnight growth. After 24 h at pH 2.2, all the isolates confirmed lowest

survival capacity compared to prior hours. In addition, at pH 6.6, all the isolated bacteria showed more or less similar survival and multiplication abilities that was considered as favorable environment. This result was parallel to the findings of Maniruzzaman et al. (2010).

After 12 and 24 h of incubation, enhanced resistance and multiplication competence were observed against 0.1 and 0.2% of crude phenol. With increasing the concentration of crude phenol at 0.4%, tolerance of the bacteria was found to decrease significantly. Xanthopoulos, et al., (2000) found the same experimental result. According to Havenaar and Huis (1992), bile salt tolerability was the most common phenomenon for probiotic bacteria. Prasad, et al., (1998) showed that resistance of bacterial isolates was excellent against 0.05, 0.1, 0.15 and 0.3% of artificial bile acid after 24 h of incubation. The present research work found the same consequences. The bile salt hydrolase activity test result of the present study was analogous to Dashkevicz and Feighner (1989) who expanded an agar plate to recognize bile salt hydrolase activity in *Lactobacilli*.

Conclusion

With the findings of the present research work, it would be possible to provide preliminary information for production of probiotic feed products for poultry. It is also anticipated that the deliverables of the research work would promote establishment of community based environmentally sustainable probiotic industries by wider participation of vulnerable, poor and destitute women of the society through financial support from the Government and/ or donors.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENT

The authors acknowledge Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh for sponsoring and providing laboratory facilities.

REFERENCES

Bayane A, Roblain D, Dauphin RD, Destain J, Diawara B, Thonart P (2006). Assessment of the physiological and biochemical characterization of a Lactic acid bacterium isolated from chicken feces in Sahelian region. Afr. J. Biotechnol. 5(8): 629-634.

Buckenhüskes HJ (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiol. Rev. 12:253-272.

Cole CB, Fuller R, Newport MJ (1987). The effect of diluted yogurt on the gut microbiology and growth of piglets. Food Microb. 4: 83–85.

Dashkevicz M, Feighner SD (1989). Development of a differential medium for bile salt Hydrolase-active *Lactobacillus* spp. Appl. Environ. Microb. 55(1):11-16.

Elizete DFRP, Carlos SR (2005). Biochemical characterization and identification of probiotic *Lactobacillus* for swine. B. CEPPA. Curitiba. 4:299-310.

Erkus O (2007). Isolation, phenotypic and genotypic characterization of yoghurt starter Bacteria. Izmir Ins. of Tech. Turkey, Thesis of MS.

FAO/WHO (2002). FAO/WHO Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. FAO/WHO Working group, London, Ontario, 2002.

Fujisawa T, Benno Y, Yaeshima T, Mitsuoka T (1991). Taxonomic study of the *Lactobacillus acidophilus* group, with recognition of *Lactobacillus gallinarum* sp. nov. and *Lactobacillus johnsonii* sp. nov. and synonymy of *Lactobacillus acidophilus* group A3 (Johnson et al. 1980) with the type strain of *Lactobacillus amylovorus* (Nakamura 1981). Int. J. Syst. Bacteriol. 42:487-491.

Fujisawa T, Shirasaka S, Watabe J, Mitsuoka T (1984). *Lactobacillus aviarius* sp. nov: A new species isolated from the intestine of chicken. Syst. Appl. Microbiol. 5:414-420.

Fuller R (1989). Probiotics in man and animals: A review. J. Appl. Bacteriol. 66:365-378.

Gomez MP, FX Malcata (1999). *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci. Technol. 10:139-157.

Gong J, Forster RJ, Yu H, Chambers JR, Sabour PM, Wheatcroft R, Chen S (2002). Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol. Lett. 208:1-7.

Graciela FV, Maria PT (2001). Food Microbiology Protocols (J.F.T. Spencer and A.L.R.D. Spencer, Ed.), Totowa, New Jersey: Humana Press Inc., Chap. 21 (Probiotic properties of *Lactobacillus*): 173-181.

Gusils C, Perez CA, Gonzalez S, Oliver G (1999). *Lactobacillus* isolated from intestines: potential use as Probiotics. J. Food. Prot. 62:252-256.

Harley JP, Prescott LM (2002). Laboratory Exercises in Microbiology 4th Edition. The McGraw-Hill Companies: New York.

Havenaar R, Huis in't Veld JHJ (1992). Probiotics: A general view. In: Wood BJB: The Lactic Acid Bacteria, Vol. 1: The Lactic Acid Bacteria in Health and Disease, Chapman and Hall, New York, NY. pp. 209-224.

Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, London, UK.

Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JHJ (1998). Overview of gut flora and probiotics. Int. J. Food Microbiol. 41:85-101

Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD (2010). Isolation, Identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. World J. Dairy Food Sci. 5(1):39-46.

Jin LZ, Ho YW, Abdullah N, Jalaludin S (1997). Probiotics in poultry: modes of action. Worlds Poult. Sci. J. 53: 352–368.

Kandler O, Weiss N (1986). Regular non-sporing Gram-positive rods, Genus *Lactobacillus* (Beijerinck 1901, 22), In: PHA Sneath, NS Mair, ME Sharpe, JG Holt (Eds.), Bergey's manual of systematic bacteriology, 8th ed, vol. 2, William and Wilkins Co, Baltimore, pp. 1208-1234.

Maniruzzaman M, Khan MFR, Amin MM, Paul AK, Islam M (2010). Isolation and identification of bacterial flora from milk of apparently healthy buffalo-cows. Int. J. Bioresour. 1(3):13-16.

Nahanshon SN, Nakaue HS, Mirosh LW (1993). Effect of direct-fed microbials on nutrient retention and parameters of Single Comb White Leghorn pullets. Poult. Sci. 72(2): 87.

Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M (1999). *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Appl. Environ. Microbiol. 65: 4981-4986.

Prasad J, Gill H, Smart J, Gopal PK (1998). Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. Int. Dairy J. 8: 993-1002.

Qin ZR, Fukata T, Baba E, Arakawa A (1995). Effect of lactose and *Lactobacillus acidophilus* on the colonization of *Salmonella enteritidis* in chicks concurrently infected with *Eimeria tenella*. Avian Dis. 39:548-553.

Reller LB, Mirrett S (1975). Motility-Indole-Lysine medium for presumptive identification of enteric pathogens of *Enterobacteriaceae*. J. Clin. Microbiol. 2: 247-252.

Saccaro DM, Hirota CY, Tamime AY, Oliveira de MN (2011). Evaluation of different selective media for enumeration of probiotic micro-organisms in combination with yogurt starter cultures in fermented milk. Afr. J. Microbiol. Res. 5(23):3901-3906.

Schaeffer AB, Fulton M (1933). A simplified method of staining endospores. Science 77:194.

Schillinger U, Lucke FK (1987). Identification of lactobacilli from meat and meat products. Food Microbiol. 4:199-208.

Xanthopoulos V, Litopoulou-Tzanetaki E, Tzanetakis N (2000). Characterization of *Lactobacillus* isolates from infant feces as dietary adjuncts. Food Microbiol. 17:205-215.