ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR399

Isolation of Amylolytic Lactic Acid Bacteria from Cow, Goat and Sheep Milk in Yola Town, Adamawa State, Nigeria

*Tyokusa, A. G.
Microbiology Department
Joseph Sarwuan Tarka University
(Formerly Federal University of Agriculture),
Makurdi, Nigeria.

Danke, I. C.
Microbiology Department
Modibbo Adama University
(Formerly Federal University of Technology),
Yola, Nigeria

Corresponding Author: *Tyokusa, A. G.

Abstract:- In this study, lactic acid bacteria (LAB) were isolated from fresh milk samples from cow, sheep, and goat using MRS agar as a growth medium. *Lactobacillusplantarum*, *Lactobacillusacidophilus*, *Lactobacillusfermentum*, *Enterococcusgallinarum*, and *Enterococcusfaecium* were the species of LAB that were isolated and identified using the Vitek 2 compact system. While *Lactobacillus fermentum*, *Enterococcus faecium*, and *Lactobacillus plantarum* were isolated from goat milk, *Lactobacillus acidophilus*, *Lactobacillus gallinarum*, and *Lactobacillus plantarum* were isolated from cow milk.However, from sheep milk, only *Lactobacillus plantarum* and *Lactobacillus acidophilus* were isolated. All the isolates were subjected to amylase production test using modified MRS media and the results showed that *Lactobacillus plantarum*, *Lactobacillusacidophilus* and *Lactobacillusfermentum* produced amylase while *Enterococcus gallinarum* and *Enterococcus faecium* did not produce amylase.

Keywords:- *Milk, LAB, Vitek 2,Amylase and Modified MRS.*

I. INRODUCTION

One of the most important types of bacteria in the food industry are the lactic acid bacteria (LAB).People all over the world have long consumed them in dairy products, and the majority of them are categorized as "generally recognized as safe" (GRAS) microorganisms because they are non-pathogenic, appropriate for industrial and technological processes, tolerant of bile and acid, and capable of producing antimicrobial substances (Shehata *et al*., 2016).*Pediococcus*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and other bacteria are among the cocci and bacilli in the LAB group (Mazzoli *et al*., 2014).Application areas for lactic acid bacteria (LAB) include food, agriculture, and medicine (Bintsis, 2018).Furthermore, lactic acid bacteria produce a variety of enzymes, including lipases, ureases, peptidases, amylases, and proteases.

Amylase producing lactic acid bacteria known as amylolytic lactic acid bacteria (ALAB) secretes amylases, which make it easier to hydrolyze and ferment starch into lactic acid in a single step (Reddy *etal*., 2008).The manufacture of food additives like organic acids (like lactic acid) and enzymes (like alpha amylase) is done in the food processing industries using ALAB enzymes (Panda and Ray, 2016). The ALAB produce amylases, which are starch-modifying enzymes with a wider range of uses than chemical starch hydrolysis in the food processing sectors (Smerilli *et al*. 2015).Among these essential enzymes in the realm of biotechnology is amylase.From a fungal base, amylase appears to have been the first enzyme manufactured industrially in 1894.In the treatment of digestive diseases, it was utilized as a medicinal acid(Shanmugasundaram *etal*., 2015). According to Sachdev *et al* (2016), amylase is one of the leading enzymes used in industry from decade and that microorganisms such as *Bacillus* species are the major source of production of amylase because of the ease of availability, manipulation and operation.In contrast to chemical starch hydrolysis in the starch processing sector, a wide range of commercially available microbial amylases now hold potential applications.Among other bacteria that produce amylase, the most recently found ALAB strain *Lactobacillusparacasei* B41 was the first amylolytic representative of the *L. casei* group(Bhanwar and Gangul, 2014).

Milk is theprimary source of nutrition for young mammals (including breastfed human infants) before they are able to digest solid food (Van Winckel *etal*., 2011). The primary players in milk fermentation are lactic acid bacteria, which work with amylase and other produced enzymes to convert lactose to lactic acid. This increased acidity makes the growth conditions of microorganisms other than LAB increasingly unfavourable (Fernandez *et al*., 2015).

Considering cattle, sheep and goats rearing as major occupationinAdamawa state of Nigeria, fresh milk is always in abundant supply. Therefore, isolating amylase producing lactic acid bacteria from cow, sheep and goat milk in the state will create an avenue for exploring and harnessing the available resources within the state. Consequently, the aim

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR399

of this research work was to isolate and identify the amylase producing lactic acid bacteria from cow, goat and sheep milk from Yola, Adamawa state of Nigeria.

II. METHODOLOGY

A. *Sample Collection, Isolation and Identification of Bacteria Isolates*

Five samples of fresh cow, goat, and sheep milk each were collected in sterile containers, transported to the laboratory, and left for a 24-hour fermentation period. In total, fifteen samples were obtained.Serial dilutions were made for all the samples and appropriate dilutions (10^{-5} to 10^{-9}) inoculated on MRS agar (Dextrose-20, Agar 12., Proteose peptone-10, Beef extract-10, Yeast extract-5, Sodium acetate-5, Ammonium citrate-2, Dipotassium phosphate-2, Tween 80-1, Magnesium sulphate-0.10 and Manganese sulphate-0.05 all g/L/, pH-6.5 at 25^0C) (TITAN BIOTECH LTD India) for 24 to 48 hours at room temperature (30-37^0C). Pure isolates were obtained by sub-culturing a representative sample of distinct colonies spotted on MRS agar.The bacteria isolates were identified using the Vitek 2 compact system (BIOMERIEUX, France) in addition to the catalase test and microscopic identification.

B. *Detection of Amylase Activity*

In order to detect amylase activity, the isolated lactic acid bacteria were grown on modified MRS media containing 0.5% peptone, 0.7% yeast extract, 0.2% NaCl, 2% starch, and 1.5% agar. Following a 24-48-hour incubation period at 37°C, Gram's iodine solution (iodine: 0.2%, KI: 0.4%, distilled water: 100 mL) was added and the results were monitored (Madhav*et al*., 2011).

III. RESULTS

➢ *Results of Bacteria Isolates from Cow, Goat and Sheep Milk*

Table 1: Catalase Test and Microscopic Identification of the Isolates

S/N	ISOLATE CODE	CATALASE TEST	GRAM REACTION	SHAPE
1	C1	_	+	Rods
2	C2	_	+	Rods
3	C3	_	+	Cocci
4	G1	_	+	Rods
5	G2	_	+	Rods
6	G3	_	+	Cocci
7	S1	_	+	Rods
8	S2	_	+	Rods

KEY: C=Cow Milk Isolate, S= Sheep Milk Isolate, G= Goat Milk Isolate + = Positive, - = Negative

Table 2: Lactic Acid Bacteria Identified using the Vitek 2 Compact System from the Milk Samples

S/No	Source Lactic Acid Bacteria	
1	C1	*Lactobacillus fermentum*
2	C2	*Lactobacillus plantarum*
3	C3	*Enterococcus gallinarum*
4	G1	*Lactobacillus plantarum*
5	G2	*Lactobacillus fermentum*
6	G3	*Enterococcus faecium*
7	S1	*Lactobacillus acidophilus*
8	S2	*Lactobacillus plantarum*

KEY: C = Cow Milk, G = Goat Milk and S = Sheep Milk

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR399

➢ *Results of Lactic Acid Bacteria That Produce Amylase*

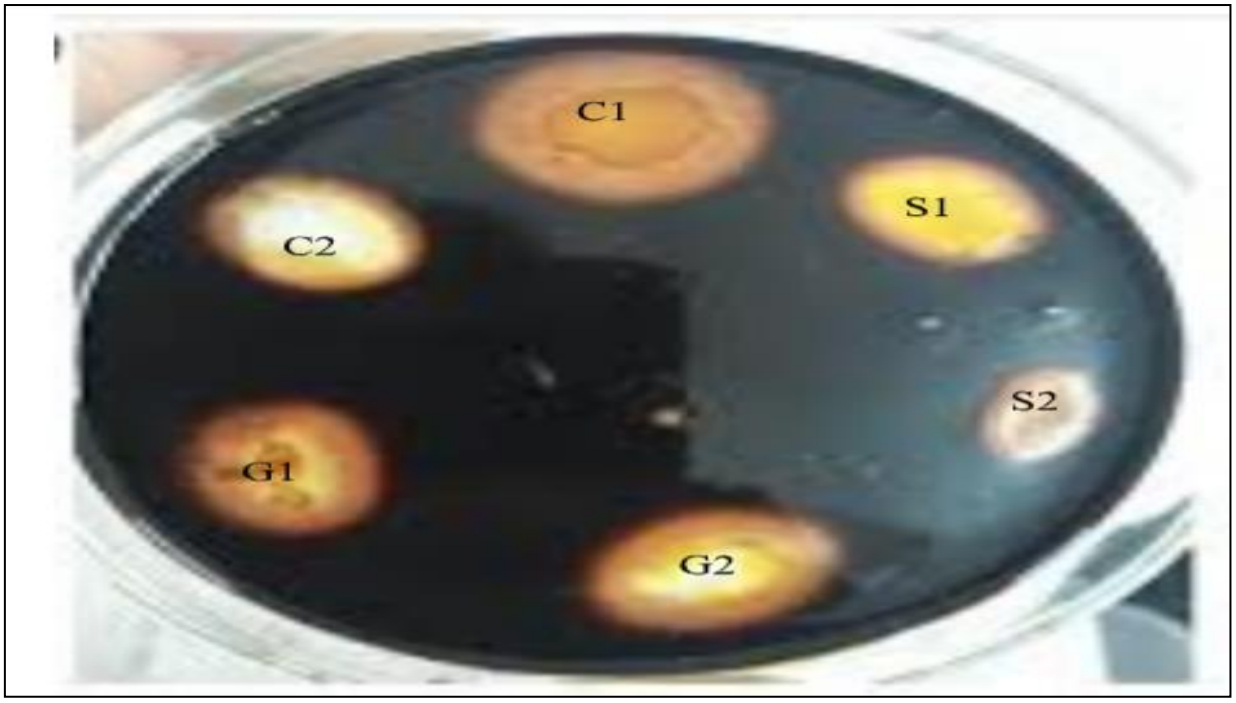

Plate 1: Detection of Lactic Acid Bacteria's Amylase Activity on a Modified MRS Agar Plate

Table 3: Amylase Producing Lactic Acid Bacteria

S/N	ISOLATE CODE	ISOLATE NAME	AMYLASE PRODUCTION
1	C1	*Lactobacillus fermentum* +	
2	C2	*Lactobacillus pantarum* +	
3	C3	*Enterococcus gallinarum* -	
4	G1	*Lactobacillus plantarum*+	
5	G2	*Lactobacillus fermentum*+	
6	G3	*Enterococcus faecium*-	
7	S1	*Lactobacillus acidophilus*+	
8	S2	*Lactobacillus plantarum*+	

KEY: C = isolate from cow milk, G = Isolate from goat milk. S = Isolate from sheep milk,
(+) = produce amylase and (-) = do not produce amylase

IV. DISCUSSION

Based on this finding, the bacteria isolated were Gram positive and catalase negative.Altogether,a total of six rods and two cocci bacteria were isolated (Table 1).The Vitek 2 compact system was used to identify the bacteria isolates (Table 2).The isolates identified were *Lactobacillusfermentum, Lactobacillusplantarum, Lactobacillusacidophilus, Enterococcusgallinarum* and *Enterococcusfaecium.L. plantarum* was found in all the three milk samples while *L. fermentum* were found in goat and sheep milk. *L. acidophilus* was however, found only in cow milk sample.Similarly, *Enterococcusgallinarum* was present in cow milk only while *Enterococcusfaecium* was isolated only from goat milk.All the isolates are members of the lactic acid bacteria (LAB). This agrees with Agagunduz *etal* (2021), who reported that, a variety of live lactic acid bacteria species can be found in fermented dairy products.Tyokusa and Owuama (2023), also isolated lactic acid bacteria(*Enterococcusgallinarum* and *Enterococcusfaecium*) and additional bacteria from cow and goat milk that are Gram positive. Raw cow milk wasused to isolate and identify LAB genera, including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Bifidobacterium* species(Fesseha *etal*., 2021). Similarly, the raw cow milk was also used to isolate the LAB namely;*Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Lactococcus*, and *Pediococcus* species (Wassie and Wassie, 2016).Guessas and Kihal (2004), also isolated LAB namely *Lactococcus* sp, *Streptococcusthermophilus*, *Leuconostoc* sp, *Lactobacilluscurvatus*, *L. helviticus*, *L. plantarum*,*L. reuteri*, *L. casei*, *L. brevis*, *L. bulgaricus*, *L. paracasei* and *L. caidophilus* from goat milk.

The *Lactobacilli* genera; *Lactobacilli plantarum*, *L. pentosus*, *L. delbrueckii*, *L. helveticus*, and *L. paracasei*-associated with a fermented goat milk product from Tajikistan were isolated byCho *et al*, (2018).In contrast, *Lactobacillus plantarum* YN.1.3, a lacticacidbacterium, was isolated by Yelnetty *et al* (2020), from goat milk.According to Chen *etal*(2020), Hu sheep milk was used to isolate

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR399

Lactococcuslactis, *Leuconostoc lactis*, and *Sphingomonas.Lactobacillusplantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bacillusshackletonii* are the four species of lactic acid bacteria (LAB) that were isolated from sheep milk and identified using physiological, biochemical, and 16S rRNA sequencing study (Patil *et al*., 2019).

All the *Lactobacillus* species isolated produced amylase while *Enterococcus* species did not produce amylase (Table 3).Amylolytic *Lactobacillus* species have been isolated from milk and other sources. Lee *et al* (2006), isolated *L. acidophilus* and *L. fermentum* with high amylase activity from intestinal contents of pigs. Also, South African barley was used to isolate strains of *Lactobacillus plantarum* that producedα-amylase (Hattingh *et al*., 2015).Likewise, from traditional fermented Dadih, Elida *etal* (2022), isolated the lactic acid bacteria that produce amylase: *Lactobacillusparacasei* subsp *paracasei* mL3, *Lactobacillusplantarum* mH4, *Lactococcuslactis* subsp *lactis* Mh6, *Lactobacillusplantarum* Mh8, and *Lactobacillusparacasei* subsp *paracasei* Ml14.

The amylase activities of the lactic acid bacteria isolates,*Lactobacillusfermentum*, *Lactobacillusplantarum* and *Lactobacillusacidophilu*s is shown on plate 1.Amylases are a class of enzymes that break down starch into simple sugars, and a wide range of species produced these proteins (Gomez-Villegas *et al*., 2021).Amylases' main function is to hydrolysed the glycosidic bonds that hold starch molecules together, converting complicated carbohydrates into simpler sugars.

Alpha, beta, and gamma amylases are the three primary classes of amylases, and they all target different parts of the carbohydrate molecule(Akinfemiwa *etal*., 2023).Thus, the degradation of the starch present in the medium resulting in the clear zones (the loss of blue colour of iodine-starch complex) around the colonyof each isolate as seen on plate 1is as a result of the amylases produced by the three LAB isolates.By analyzing the halo zone surrounding the colonies, which had a diameter of 0.9 mm and 1.23 mm, Padmavathi *et al* (2018), found that *Lactobacillusfermentum* and *Lactobacillus* species G3_4_1TO2, isolated from various materials such as milk, curd, and bovine colostrum, produced amylase enzyme.

Garcia-Cano *et al* (2020),identified endo and exoamylases as among the enzymes that break down starch. The inner section of amylose and amylopectin contains α-1,4 glycosidic linkages that endoamylases cleave to release oligosaccharides and dextrin as α-anomeric derivatives.Like glucoamylase or α-glucosidase, exoamylases can cleave the α-1,4 and α-1,6 bonds of the exterior glucose residues, releasing glucose as the end product.

One important feature of LAB that contributes to the fermentation of starch into lactic acid is its amylolytic activity.The primary cause of the amylolytic activity in ALAB is the alpha-amylase (*amyA*) genes. The *amyA* gene has 1746 base pairs, a 459-amino acid catalytic domain, and a 97-amino acid starch-binding domain (SDB) (Wang *et al*., 2006). A 954 amino acid protein with an open reading frame of 2862 bp was found to be encoded by the whole nucleotide sequence of the *LactobacillusamylovorusamyA* gene(Giraud and Cuny, 1997).

V. CONCLUSION

Fresh milk from cows, sheep, and goats obtained from Adamawa State, Nigeria, like any other place,is a good source of amylase producing lactic acid bacteria such as *Lactobacillusfermentum*, *Lactobacillusplantarum*, and *Lactobacillusacidophilu*s. The importance of this enzyme in food and other industries cannot be over emphasized. It is therefore important to harness the readily available milk in Adamawa state for the commercial production of the amylase enzyme.

REFERENCES

[1]. Agagunduz, D., Yilmaz, B., Sahin, T. O., Gunesliol, B. E., Ayten, S., Russo, P., Spano, G., Rocha, J. M., Barkiene, E. and Ozogul, F. (2021). Diary Lactic Acid Bacteria and Their Potential Function in Dietetics: The Food-Gut-Health Axis. *Foods* 10(12):3099 doi:10.3390/foods10123099

[2]. Akinfemiwa, O., Zubair, M. and Muniraj, T. (2023). Amylase. In: StatPears publishing.

[3]. Bhanwar, S. and Ganguli, A. (2014). α-amylase and β-galactosidase production on potato starch waste by *Lactococcuslactis* subsp. *lactis* isolated from pickled yam. *Journal of Scientific and Industrial Research*73: 324–330.

[4]. Bintsis, T. (2018). Lactic acid bacteria: their applicationsin foods. *Journal ofBacteriology and Mycology* 6(2:89-94 DOI: 10.15406/jbmoa.2018.06.00182

[5]. Chen, T., Wang, L., Li, Q. et al (2020). Functional probiotics of lactic acid bacteria from Hu sheep milk. *BMC Microbiology*20, 228 https://doi.org/10.1186/s12866-020-01920-6

[6]. Cho, G. S., Cappello, C., Schrader, K., Fagbemigum, O., Oguntoyinbo, F. A., Csovcsics, C., Rösch, N., Kabisch, J., Neve, H., Bockelmann, W., Briviba, K., Modesto. M., Cilli, E., Mattarelli, P. and Franz, C. M. A. P. (2018). Isolation and Characterization of Lactic Acid Bacteria from Fermented Goat Milk in Tajikistan. *Journal of Microbiology and Biotechnology* 28(11)

[7]. Elida, M., Agustina, A., Ermiati, E. and Desminarti, S. (2022). Isolate Characterization and Amylolytic Properties of Lactic Acid Bacteria from Traditional Fermented Dadih. IOP *Conference Series: Earth and Environmental Science*. Doi: 10.1088/1755-1315/1097/1/012025

[8]. Fernandez, M., Hudson, J. A., Korpela, R. and de los Reyes-Gavilan, C. G. (2015). Impact on Human Health of Microorganisms Present in Fermented Diary Products: An Overview. *BioMed Research International* doi: 10.1155/2015/412714

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR399

[9]. Fesseha, H., Taye, Y., Degu, T. and Mathewos, M. (2021). Isolation and Identification of Lactic Acid Bacteria from Cow Milk and Milk Products. *The Scientific World Journal.*

[10]. Garcia-Cano, I., Rocha-Mendoza, D., Kosmerl, E., Zhang, L. and Jimenez-Flores, R. (2020). Technically relevant enzymes and proteins produced by LAB suitable for industrial and biological activity. *Applied Microbiology and Biotechnology* doi.org/10.1007/s00253- 019-10322-2

[11]. Giraud, E. and Cuny, G. (1997). Molecular characterization of the alpha-amylase genes of *Lactobacillusplantarum* A6 and *Lactobacillus amylovorus* reveals an unusual 3' end structure with direct tandem repeats and suggests a common evolutionary origin. *Gene*. doi:10.1016/s0378-1119(97)00309-0

[12]. Gomez-Villegas, P., Vigara, J., Romero,L., Gotor, C., Raposo, S., Goncalves, B. and Leon, R. (2021). Biochemical Characterization of the Amylase Activity from the New Haloarchaeal Strain *Haloarcula* sp. HS Isolated from Odiel Marshlands. *Biology* 10(4):337 doi:10.3390/biology10040337

[13]. Guessas, B. and Kihal, M. (2004). Characterization of lactic acid bacteria isolated from Algerian arid zone raw goat milk. *African Journal of Biotechnology* 3(6).

[14]. Hattingh, M., Alexander, A., Meijering, I., van Reenan, C. A. and Dicks, L. M. T. (2015). Amylolytic strains of *Lactobacillusplantarum* isolated from barley. *African Journal of Biotechnology* 14(4).

[15]. Lee, H, S., Gilliland, S. E. and Carter, S. (2006). Amylolytic Cultures of *Lactobacillusacidophilus*: Potential Probiotics to Improve Dietary Starch Utilization. *Journal of Food Science* 66(2):338-344

[16]. Madhav, K., Verma, S. and Tanta, R. (2011). Isolation of amylase producing Bacillus species from soil sample of different regions in Dehradun and to check the effect of pH and Temperature on their amylase activity. *Journal of Pharmaceutical and Biomedical Sciences* 12(12)

[17]. Mazzoli, R., Bosco, F., Mizrahi, I., Bayer, E. A. and Pessione, E. (2014). Towards lactic acid bacteria-based biorefineries. *Biotechnology Advances 32*: 1216–1236. DOI:10.1016/j.biotechadv.2014.07.005

[18]. Panda, S. H. and Ray, R. C. (2016). Amylolytic Lactic Acid Bacteria, Microbiology and Food Technological Interventions in Food Fermentation In book: Fermented foods. Doi:10.1201/b19872-9

[19]. Padmavathi, T., Bhargari, R., Rao Priyanka, P., Niranjan, N. R. and Pavitra, P. V. (2018). Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *Journal of Genetic Engineering and Biotechnology* 16(2): 357-362 doi: 10.1016/j.jgeb.2018.03.005

[20]. Patil, A.,Disouza, J. and Pawar, S. (2019).Shelf-life stability of encapsulated lactic acid bacteria isolated from sheep milk thrived in different milk as natural media. *Small Ruminant Research* vol 170:19-25

[21]. Reddy, G., Altaf, M., Naveena, B. J., Venkatteshwar, M. and Kumar, E. V. (2008). Amylolytic bacteria lactic acid fermentation-a review. *Biotechnology Adv*ances 26:22-33

[22]. Sachdev, S., Ojha, S. and Mishra, S. (2016). Bacillus Spp. Amylase: Production, Isolation, Characterization and Its Application. *International Journal of Applied Sciences and Biotechnology*. Doi.10.3126/ijasbt.v4i1.14574

[23]. Shanmugasundaram, S. Eswar, A., Mayavu, P., Surya, M. and Anbarasu, R. (2015). Screening and Identification of Amylase Producing Bacteria from Marakkanam Saltpan Environment, Tamil Nadu, India. *Asian Journal of Biomedical and Phamarceutical Sciences*

[24]. Shehata, M. G., El Sohaimy, S. A. El-Sahn, M. A. and Youssef, M. M. (2016). Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Annals* of *Agricultural Science*. 6(1):65–75.

[25]. Smerilli, M., Neureiter, M., Wurz, S., Haas, C., Fruhauf, S. and Fuchs, W. (2015). Direct fermentation of potato starch and potato residues to lactic acid by *Geobacillus stearothermophilus* under non-sterile conditions. *Journal of Chemical Technology and Biotechnology*. 90(4) 648-657

[26]. Tyokusa, A. G. and Owuama, C. I. (2023). Bacteriocins Production Potentials of Some Gram Positive Cocci Bacteria Isolated from Milk and Other Food Sources. *Current Trends on Biotechnology and Microbiology* 3(4)DOI: 10.32474/CTBM.2023.03.000172

[27]. Van Winckel, M. Velde, S. V., De Bruyne, R. and Biervliet, S. (2011). "Clinical Practice". *European Journal of Pediatrics* 170(12): 1489-1494 doi:1007/s00431-011-1547

[28]. Wang, N. Zhang, T., Wang, Q., Liu, J., Wang, H., Xue, Y. and Ma, Y. (2006). Gene cloning and characterization of a novel alpha-amylase from alkaliphilic Alkalimonas amylolytica. *Biotechnology Journal* 1(11): 1256-65. Doi:10.1002/biot.200600098

[29]. Wassie, M. and Wassie, T. (2016). Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk. *International Journal of Advanced Research in Biological Sciences* 3(8)

[30]. Yelnetty, A. Purwadi, Ekawati, T. T. (2020). Indigenous Lactic Acid Bacteria Isolated from Spontaneously Fermented Goat Milk as Potential Probiotics. *Pakistan Journal of Biological Sciences* 23(7):883-890