Isolation and Identification of Industrial Enzyme (Amylase) Producing Bacteria from Salaiva Sample of Gallus Gallus Domesticus

Archana shree K P [1],Vinitha P[2], Umabharathi S[3],Vaikeswari R[4], Viswanarmatha S[5], Vinothini R[6], Vinisha M[7], Uma maheswari U[8], Yadhavi K[9],Vijitha K[10]

[1] *Assistant Professor, Department of Microbiology, Velumanoharan Arts and Science College forWomen, Ramanathapuram, Tamil Nadu, India.*

[2345678910] *Student, bachelor of Science, Department of Microbiology, Velumanoharan Arts and Science College for women's, Ramanathapuram, Tamil Nadu, India.*

Abstract: **Characterization of the Gallus gallus a-amylase gene family revealed that the chicken genome contains two distinct amy loci. One of the two loci is expressed in the chicken pancreas while cDNA clones for the second locus were detected in a library constructed from liver mRNA. Fluorescent in situ hybridization to chromosome spreads showed that the two loci are both located on chromosome 8 within the chicken genome.**

Moreover, each locus contains both an intact, expressed gene copy as well as a pseudogene. The expressed gene and the pseudogene are arranged in a divergent configuration in the pancreatic amy locus, while in the hepatic locus the intact gene and the pseudogene are arranged in tandem. The data suggest a complex pattern of evolution for the chicken amylase gene family which includes multiple gene duplication events, insertion/deletion events, as well as changes in spatial expression patterns.

1. INTRODUCTION

Kingdom *Animalia*, Family *Phasianidae*, Genus *Gallus*, Species *G. gallus*, Subspecies *G. g. domesticus.* The domestic chicken, *Gallus gallus domesticus*, is the world's most ubiquitous bird. Chickens being a cheap and easily available animal protein source, are important to human and also play a significant role in religion, entertainment (e.g. cockfighting), ornamental breeding and in biomedical research (e.g. embryogenesis). Domestication has led to an impressive diversification of chicken breeds with more than one thousand local chicken breeds across the world (Mariadassou *et al*., 2021).

Domestic chickens are believed to have originated in Southeast Asia, with Thai native chickens being regarded as the original domesticated chickens. Because local populations' distinctive genotypes and features are in danger of disappearing and posing a threat to a reliable food supply, the decline in the genetic diversity of native chicken populations documented in recent genetic research has aroused concerns. This shows that a higher attention should be paid to managing the genetic resources of native chickens. (Dorji *et al*., 2012).

Industrial enzymes as industrial biocatalysts pose numerous advantages over the traditional chemical process in term of sustainability and process efficiency. Much scientific research has proven that marine bacteria were able to produce a wide range of industrial enzymes. The industrial enzymes derived from marine bacteria include α-amylase, α-glucosidase, agarose, α-galactosidase, cellulases, chitinase, lipase, protease. Some of these marine bacterial producing industrial enzymes were *Arthrobacter sp*, *Chromo bacterium sp, Clostridium sp, Enterobacter sp, Flavobacterium sp, Klebsiella sp, Pseudoalteromonas sp, Pseudomonas sp, Psychrobacter sp, Streptomyces sp, Vibrio sp, Marinobacter sp, Bacillus sp*., Etc (Cheng *et al*., 2020).

Recent years have seen the emergence of marine microbes as a prolific resource for the separation of industrial enzymes. Enzymes secreted by marine bacteria provide a number of benefits for industrial use. Since marine bacterial enzymes function best at high salinities, they can be used in a variety of abrasive industrial processes. The majority of marine bacterial enzymes are extremely thermotolerant and can last for a long time at room temp. (Mohapatra *et al*., 2003).

Accumulation of microbes called lactic acid bacteria is becoming more and more significant in biotechnology. They are extensively employed in the preparation of milk.
Traditionally, the dairy, fermentation, fruit and vegetable, and livestock feed sectors were the primary users of LAB. Because lactic acid bacteria are thought to be harmless for both people and animals, their significance is constantly expanding (Kieliszek *et al*., 2021).

Industrial enzymes producing bacteria from fish. The gut microbiota in production of digestive enzymes, a brief introduction regarding endogenous enzyme activities in fish seems pertinent. It has only been during the last decade that there has been an improved understanding of the importance of commensal intestinal microbiota in fish intestine. The first studies on enzyme production by the fish gut bacteria, were reported in 1979. Microbial amylase activity in the fish gut has been documented in several studies. Bacteria belonging to Bacillus sp. are by far the most important source of several commercial microbial enzymes. Some information is available regarding production of proteases by fish gut bacteria. The first studies on protease-producing bacteria isolated from the digestive tract of fish, Gray mullet and Grass carp (Ray *et al*., 2012).

Enzyme production bacteria from silkworm larval gut. The insect gut is inhabited by a wide diversity of microorganisms as a result of its constituting intestinal microbial ecosystem. It has been reported that many silkworms intestinal bacteria produce digestive enzymes like amylase and cellulose. Silkworm intestinal bacteria produce digestive enzymes like amylase and cellulose (Liand *et al*., 2015).

In the studies described here, we examined the impact of the signals in the wasp's display using domestic chicks (*Gallus gallus domesticus*) in a simulated prey-learning exercise. The experiment's primary goal was to see if the display's use of music had any multimodal impacts. Due to its distinctive buzzing noise and the exhibition of two well- studied aposematic cues, the yellow colour and the black stripes, we discovered the wasp to be the perfect model organism for our goals. The three signs were merged to produce synthetic prey that displayed one, two, three, or none of the wasp-like signs. To enable the study of the impacts of the signals on the chicks, three stages of testing were conducted. (Hauglund *et al*., 2006).

The use of the developing chicken egg (*Gallus gallus domesticus*) in scientific research is discussed in detail in the present study, with an emphasis on cardiac development. A schedule of significant cardiac growth milestones, useful experimental guidelines, and illustrations of commonly used experimental methods in chicks are all included (specifically tissue chimeras, genetic manipulations, and live imaging). Additionally, this paper discusses how investigating developmental processes can improve our comprehension of disease conditions. (Vilches 2019). The domestic chicken (*Gallus gallus domesticus*), which is especially suitable for such research, was used in this investigation. Young chicks in controlled settings are readily observed to have a fear of food and objects. Immuno histochemical techniques for examining c-fos expression as well as a brain map are well known. (Perez *et al*., 2020).

Food fermentation has been aided by microorganisms since the dawn of time, and many food products are still prepared using fermentation techniques. Because they are more durable than enzymes from plants or animals, microbial enzymes have a significant impact on the agricultural industry. Because of their high uniformity, process change and optimization can be carried out very simply. They can be made through fermentation methods in an economical way with less time and space requirement. Many of these enzymes have numerous uses in a variety of commercial fields, for example, amylolytic enzymes have uses in the culinary, detergent, paper, and cloth industries. (Raveendran *et al*., 2018).

Animal and plant sources cannot be explored commercially due to their little enzyme yield. Therefore, microbial sources like filamentous fungi *(Aspergillus* sp, *Penicillium* sp and *Rhizoctonia solani*), yeasts (*Kluyveromyces* sp, *Pichia* sp and *Cryptococcus* sp) and bacteria (*Streptomyces* sp and *Xanthomonas* sp) has become an eminent choice for inulinase production. Microbial sources have advantages of easy handling, cultivation and genetic manipulation. Amongst microorganisms, fungal and yeast inulinases have been studied extensively. In comparison, a very few studies have been reported on inulinases from bacterial sources. Due to their simple molecular organization and high thermostable ability, bacterial inulinase producers are gaining immense

momentum. Inulinases are versatile class of industrial enzymes which are mainly used for the production of carbohydrate-derived products like high fructose syrup, fructooligosaccharides, bioethanol, biofuels, citric acid, lactic acid etc (Singh *et al*., 2017).

It has been demonstrated that mangrove habitats are a possible source for discovering a variety of bacterial species that make enzymes, proteins, antibiotics, and have genes for salt tolerance. All of these bacterial species have the potential to be used in the future. Due to their high capacity for production, cheap cost of use, and ease of genetic modification, bacteria are crucial for the production of enzymes. Many industries, including food manufacturing, detergents, textiles, agribusiness, medicine, medical treatment, and molecular biology, currently use microbial enzymes (Mamangkey *et al*., 2021).

Most commercial enzymes presently in use work hydrolytically to degrade a variety of natural substances. Due to the prevalence of proteases in the dairy and detergent sectors, they continue to be the most common form of enzyme. The second-largest group consists of various carbohydrases, mostly amylases and cellulases, which are utilised in sectors like the flour, cloth, detergent, and pastry industries. (Kirk *et al*., 2002). Proteases with unique characteristics can be produced by genetically modifying proteases from isolates of Bacillus sp. Different Bacillus species have also been investigated for their ability to produce proteases from other microbes heterologous. A wide variety of pH as well as temperature action, stable, alkaline and toxic chemical tolerance, including oxidants and surfactants, are just a few of the exceptional properties of bacillus proteases that make them ideal for many commercial uses. (Danilova and Sharipova 2020).

Initially created as straight forward fermentation broths of naturally occurring microbes, commercial enzyme products. Complex combinations of secreted enzymes generated at comparatively low yields of less than 10 g/l were frequently the end products. Currently, recombinant technology accounts for over 90% of commercial enzyme production in order to optimise product purity and production efficiency. Expression is carried out in fungal or bacterial hosts that have been altered to minimise the expression of heterologous genes and eliminate undesirable side effects, sometimes to levels considerably above 40 g/l. Proteases, Amylases, Phytases, Laccases, Cellulases and hemicellulases are also available in industrial enzymes production (Cherry and Fidantsef 2003).

The success of enzymes in industry is not as high as expected. This is because they have a biological origin; they have been selected by nature over millions of years of evolution to fulfill some physiological requirements. Most enzymes are water soluble, which means that the recovery and reuse of enzymes may not be simple. The target enzyme will be produced, along with many other enzymes; this may reduce the final volumetric activity or if some contaminant enzyme recognizes the substrate or product, it may decrease the selectivity or specificity of the process (Rueda *et al*., 2016). The use of enzymes as industrial biocatalystsmay offer many advantages when compared to traditional chemical processes. On the other hand, the high selectivity of the enzymes allows producing a unique product among several possible ones, reducing the protection-deprotection steps and the number of by- products. It should be also considered that, due to the mild experimental conditions and enzyme selectivity, a process catalyzed by enzymes could hardly produce any toxic side-product (Volpato *et al*., 2010).

Fermentation techniques involved in industrial enzyme production. They are Strain development, Solid state fermentation, Submerged fermentation and Solid liquid separation etc. Because the enzyme industry is highly competitive, reliable estimates of the extent to different fermentation techniques are used are not easy to establish. Although specific procedures adopted by different manufacturers will vary to a degree, there remain only two principal methods of cultivation, i.e., solid state and submerged fermentation. The bulk of microbial enzymes are produced in aerobic submerged culture, which allows greater environmental control of growth factors than in solid state methods (Lambert and Meers 1983).
The generation of enzymes has a great deal of promise with solid state fermentation (SSF). The methods where the raw fermented result may be used directly as an enzyme source can be of particular interest. Microbial enzymes have important roles in bio transformations involving organic solvent media, primarily for bioactive substances, in addition to their

traditional uses in the food and fermentation sectors (Pandey *et al*., 1999).

The biotechnology industry is expanding worldwide. In this context, the production of enzymes is crucial and has enabled large economic benefits. On a global scale, the market for enzymes has raised billions of dollars annually, and research work in the area has benefited from an increasing number of patents and published scientific articles. The biotechnological use of bacteria to obtain products of economic interest, in particular enzymes, has been explored for a long time. Among different bacterial species, the genus *Bacillus* has been extensively prospected in industrial bioprocess. Other bacterial genera such as *Streptomyces* and *Pseudomonas* are also much employed as enzyme production sources. Apart from the application of bacterial species in bioprocessing, fungal species are also explored in enzyme technology (Da Silva and Ronivaldo 2017).

The disadvantage of the valve homogenizers when applied to extraction of heat sensitive materials is the need for external cooling which can only be applied after the disruption with the concomitant temperature increase has taken place. which decreases the separation efficiency of the centrifugation. In addition, the viscosity of the fluid containing the cells can decrease somewhat after multiple passes, as the DNA long chain is broken up (Geciova *et al*., 2002).

The particular role that amylases play in the commercial starch conversion process makes them important enzymes. These enzymes have proven useful in the medicinal, paper, food, sugar, and starch liquefaction sectors. Amylases are used by the bakery industry to prevent bread and other cooked goods from staleness, and by the paper industry to reduce the viscosity of starch and produce the proper covering for paper. The amylase enzyme is used as a digestive help in the medicinal business and for warp sizing of cloth strands in the textile industry. Proteases have demonstrated their capacity to function in conditions of high pH, high temperature, and the presence of inhibitory substances. Proteases from other microorganisms and the alkaline protease generated by Bacilli have generally found more uses in bio-industries like: medicines, tanneries, food processing, leather processing, washing powders, and studies in molecular biology and peptide synthesis (Nigam 2013).

2. AIM AND OBJECTIVE

Aim

Isolation of Industrial enzyme producing bacteria from *Gallus gallus domesticus*

Objectives

1. Collection of buccal swab from *Gallus gallus domesticus*.
2. Enumeration of bacteria from the buccal swab.
3. Isolation of Industrial enzyme producing bacteria using selection agar media.
4. Physical identification of bacteria by Gram staining and motility test.
5. Biochemical identification of bacteria by IMViC test.
6. Bulk production of Industrial enzyme producing organism.
7. Identification of intracellular and extracellular enzyme producing by well diffusion method.
8. Statistical analysis of result by GraphPad Prism.

3. MATERIALS AND METHODS

Collection of buccal swab from *Gallus gallus domesticus (Chicken)*

The sample was collected in the district of Trichy. The sample were stored under sterile conditions in refrigerator.

Figure.1 Sample collection

Preparation of sample

The test sample (*Gallus gallusdomesticus*) was taken into spread plate method was used to microbial isolation.

LB Agar medium

The medium was prepared by dissolving Tryptone-0.5g, NaCl-0.5g, Yeast extract- 0.25g and agar powder- 1.75 gm of the commercially available LB Agar Medium in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten. LB agar medium by spread plate method and the plate was incubated at 27° C for 48 hrs. After incubation, bacterial colonies were isolated and plated in to a fresh plate.

CFU (Colony forming unit)

The given sample (GGD-100 µl) was taken using spread plate method of test samples by spread plate method using LB Agar cultured overnight at 37 °C in a bacteriological incubator.

$$\text{CFU/ml} = \frac{\text{(No. of colonies} \times \text{Total dilution factor)}}{\text{Volume of culture plated in ml Gram}}$$

Staining

Procedure

A loop full of bacterial culture was spread in the glass slide. The slide was smeared in front of the flame. The slides were stained with crystal violet dye and kept it for 1 min and washed the slide in a distilled water. Gram's iodine was added and incubated for 1 minute, then rinsed with distilled water. The decolorizing agent was added and kept for 1 min and then safranin strain was added, after a minute it was washed using distilled water. The slides were observed under the Trinocular microscope the purple colors indicated gram positive bacteria and the pink color indicated gram negative organism.

Motility test - Hanging Drop Method

Procedure

The motility test was performed by hanging drop method. The cover slip was taken where its edge was coated with Vaseline. The test samples were transferred to the cover slip which was placed over the cavity slide. The slide was viewed under 100X magnification and the organisms' characteristics being motile or non-motile were noted down.

IMVIC TEST (Physicochemical characterization)

Indole test

Procedure

Inoculate the bacterium to be tested in tryptone broth. To allow incubated at 37ºC for 24hrs. After Incubation add few drops of the Kovac's reagent. Becomes the result formation of a red colour ring top of positive reaction (or) pink colour ring at the top positive reaction and yellow colour ring at the top negative reaction.

Methyl red test

Procedure

Inoculate the bacterium to be tested in MRVP broth. To allow test tube inoculate at 37ºCfor 24hrs. After incubation add 5 drops of the methyl red reagent. Formation of red colour ring top of the positive reaction, formation of yellow colour ring at top negative reaction.

Voges Proskauer test

Procedure

Inoculate the bacterium to be tested in MRVP broth. To allow Incubate at 37ºCfor 48hrs.After incubation add 0.6ml of alpha naphthol add 0.2ml of 40% KOH to the broth. Allow tube to stand for 15 minutes. Formation of red colour ring positive reaction and formation of yellow colour ring negative reaction.

Simmons Citrate Utilization test

Procedure

Pick up bacterial colonies from straight wire, inoculate into slant of Simmons citrate agar. To allow Incubate at 37ºC for 24 hrs. Finally, Medium colour change from green to blue positive reaction. Medium colour changes green to yellow negative reaction. Enzyme producing bacteria using selective media Milk agar medium

The medium was prepared by dissolving Peptone-0.5g, Nacl-0.5g, Yeast extract- 0.2g, Beef extract-0.1g, Milk powder- 0.1g and agar powder- 1.75 gm of the commercially available Milk Agar Medium in 100ml of distilled water. The dissolved medium was

autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten. Milk agar medium by spread plate method and the plate was incubated at 27° C for 48 hrs. After incubation, bacterial colonies were isolated and plated in to a fresh plate.

Starch agar medium

The medium was prepared by dissolving Peptone-0.5g, Nacl-0.5g, Yeast extract- 0.2g, Beef extract-0.1g, Starch powder- 0.1g and agar powder- 1.75 gm of the commercially available Milk Agar Medium in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.
Starch agar medium by spread plate method and the plate was incubated at 37° C for 48 hrs.
After incubation, bacterial colonies were isolated and plated in to a fresh plate.

Enzymatic activity of bacteria

Enzymes that are present inside the cell membrane are called intracellular enzymes and extracellular enzymes are those which are present outside the cell. Intracellular enzymes may reside in the cytoplasmic fluid or they may be bound to cellular organelles. The synthesis of enzymes takes place within the cytoplasm.

The bacterial culture was inoculated in the 100 ml nutrient broth and incubated for 24hrs at 37°C. The OD value of bacterial culture was measured using a colorimeter at 600 nm. The bacterial culture was further centrifuged at 3500 rpm for 10 mins. The supernatant was collected and screened for the presence of extracellular enzyme production. The bacterial pellet was collected and lysed using 0.1 % of TRITON X – 100 at 4°C for 1 hr. The bacterial lysate was centrifuged at 5000 rpm for 10 min and screened for intracellular enzymatic activity using the substrate-based agar well diffusion method. The zone of clearance made by the test samples was assessed for the presence of intracellular and extracellular activity

4. RESULT AND DISCUSSION

Enumeration of bacteria from the buccal swab of GGD
We have taken 100µl of test sample. Bacteria were enumerated by spread plate method. The CFU/ml was found to be 2.3 x10^2.

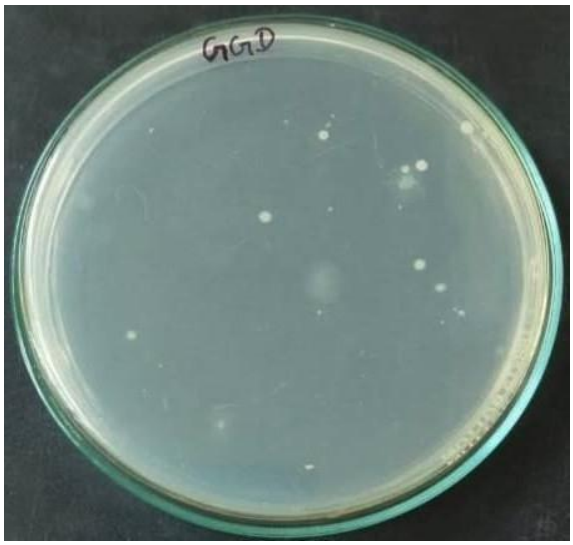


Figure.2 Enumeration of microorganism from test sample-Buccal swab

Table.1 CFU values of test sample

S. No	Name of the test samples	No. of bacterial colonies-24 hrs	Total volume of test sample	CFU/ml/gm-24 hrs
		LB Agar	100 µl	LB Agar
1.	GGD	23		2.3×10^2

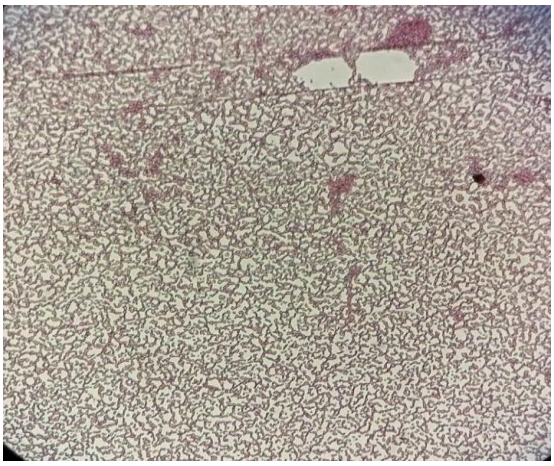
Isolation of Industrial enzyme producing bacteria using selective agar media Bacteria were isolated from group of colonies.

Figure.3 Isolation and selection of Industrial enzyme producing bacteria

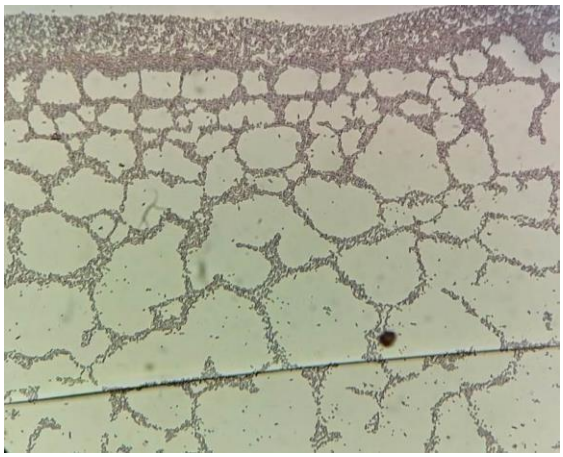
Physical identification of bacteria by Gram staining method

The isolated bacteria were observed by compound microscope. We have taken loop full of bacterial culture for to do the gram staining procedure. The organisms were identified based on color and shape. Gram-positive organisms are either purple in color, while gram- negative organisms are either pink or red in color. Bacilli are rod shaped, while cocci are spherical. In our findings we identified different types of microbial colonies from test sample.

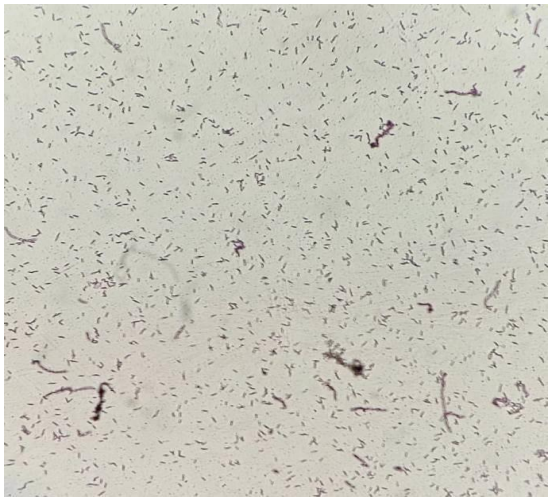
They are: 1) Gram negative – Rod chain 2) Gram negative- Cocci chain 3) Gram negative- Rod 4) Gram negative- Cocci chain 5) Gram negative- Rod chain



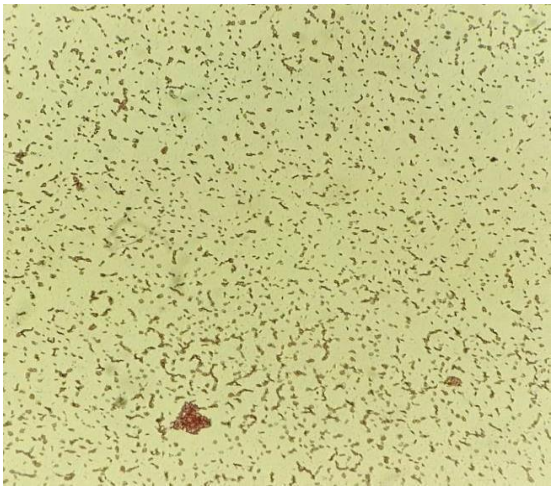
1- Gram negative- Rod chain



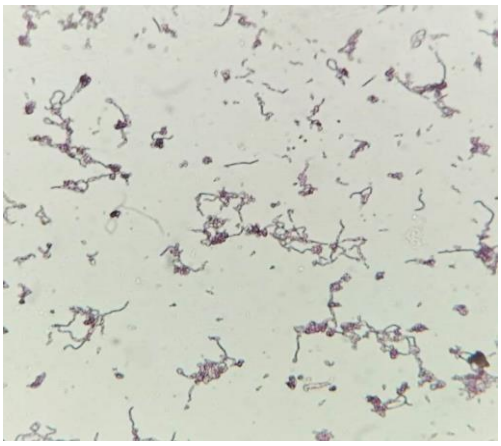
2- Gram negative- Cocci chain



3- Gram negative- Rod



4- Gram negative- Cocci chain



5 -Gram negative- Rod chain

Figure.4 Different microbial colonies using gram staining

Table.2 Result of Gram staining

S. No	Name of the sample	Result of gram staining
1.	GGD	1- Gram negative- Rod chain
2.		2- Gram negative- Cocci chain
3.		3- Gram negative- Rod
4.		4- Gram negative- Cocci chain
5.		5- Gram negative- Rod chain

Physical characterization of bacterial isolate by motility test

The motility test was performed by hanging drop method. Hanging drop method showed that the isolated bacteria were motile.

Table.3 Motility test - Hanging drop method

S. No	Name of the sample	No. of colonies	Result of Hanging Drop method
1.	GGD	2	Motile

Biochemical characterization of probiotic bacteria by IMViC test

IMViC test results showed

1) Indole test: Negative 2) Methyl-Red test: Positive
3) Voges- Proskauer test: Negative
4) Citrate test: Negative

Figure.5 IMViC test

Table.4 Results of IMViC test

S.NO	Name of the test samples	Name of the Physico chemical characterization test	Results
1.	GGD	Indole test	-
2.		Methyl red test	+
3.		Voges Proskauer test	-
4.		Simmons Citrate Utilization test	-

Bulk production of Industrial enzyme producing organism

Industrial enzyme producing bacteria were produced by bulk production manner using Milk agar medium

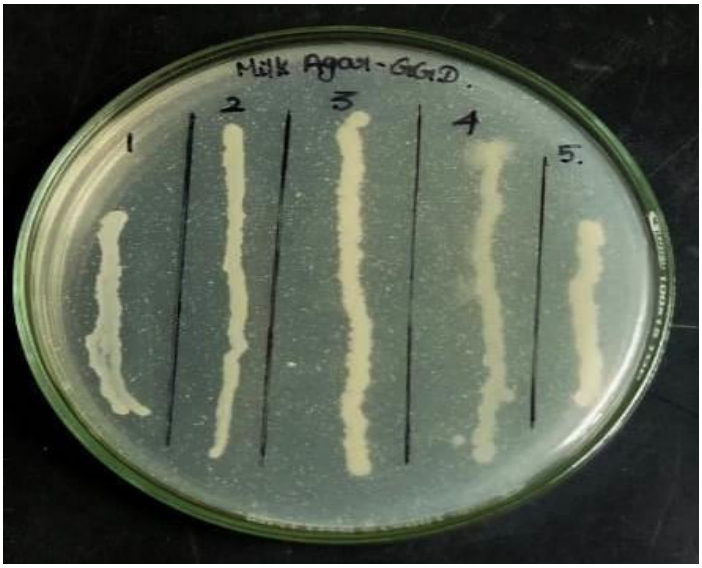


Figure.6 Enzyme producing bacteria using selective Media (Milk agar)

Industrial enzyme producing bacteria were produced by bulk production manner using starch agar medium.

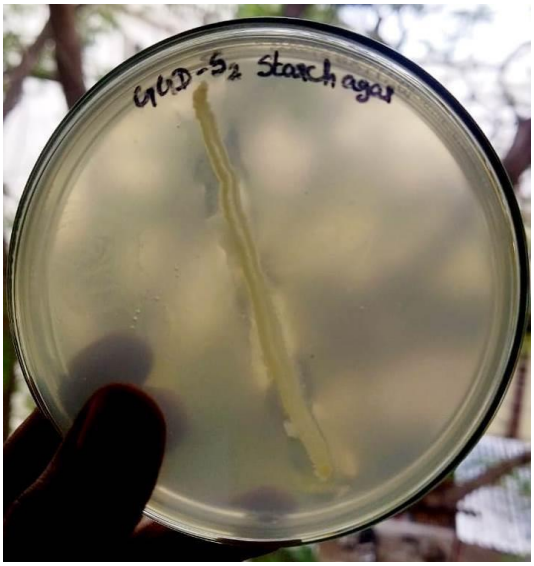
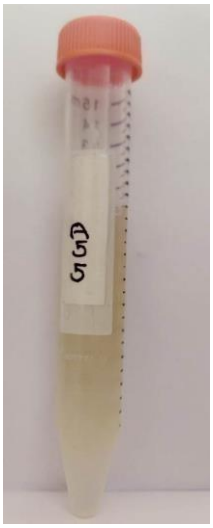


Figure.7 Enzyme producing bacteria being selective media (starch agar)

Enzymatic activity of bacteria

The zone of clearance made by the test samples was assessed for the presence of intracellular and extracellular activity. Test sample bacterial lysate and supernatant from milk agar medium did not show any zone of inhibition. Test sample bacterial lysate from starch agar medium showed 9.5±0.7 zone of inhibition and the supernatant did not show any zone of inhibition.



Supernatant

Lysate

Figure.8 Enzymatic activity of bacteria

Table.5 Zone of inhibition

S.NO	Name of medium	Lysate	Supernatant
1.	Milk agar medium	0	0
2.	Starch agar medium	9.5±0.7	0

Zone of inhibition

Zone of inhibition have showed in this graph.

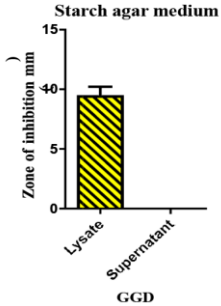


Figure.9 Zone of inhibition

5. CONCLUSION

Bacteria were isolated from *Gallus gallus domesticus* buccal sample contains Industrial enzyme producing bacteria. The zone of clearance made by the test samples was assessed for the presence of intracellular and extracellular activity. Enzymes which are present in our test sample shows enzymatic activity. Industrial enzymes are important for various biotechnological application.

6. REFERENCES

[1] Abdollahzadeh, R., Pazhang, M., Najavand, S., Fallahzadeh-Mamaghani, V., & Amani-Ghadim, A. R. (2020). Screening of pectinase-producing bacteria from farmlands and optimization of enzyme production from selected strain by RSM. *Folia microbiologica*, *65*, 705-719 doi:10.1007/s12223-020-00776-7

[2] Anwar, A., &Saleemuddin, M. (1998). Alkaline proteases: a review. *Bioresource technology*, *64*(3), 175-183 doi:10.1016/s0960-8524(97)00182-x

[3] Baltaci, M. O., Genc, B., Arslan, S., Adiguzel, G., &Adiguzel, A. (2017). Isolation and characterization of thermophilic bacteria from geothermal areas in Turkey and preliminary research on biotechnologically important enzyme production. *Geomicrobiology*

[4] Banerjee, G., Ray, A. K., Askarian, F., &Ringø, E. (2013). Characterisation and identification of enzyme-producing autochthonous bacteria from the gastrointestinal tract of two Indian air-breathing fish. *Beneficial microbes*, *4*(3), 277–284 https://doi.org/10.3920/BM2012 hu.0051

[5] Bhatia, S. K., Vivek, N., Kumar, V., Chandel, N., Thakur, M., Kumar, D., ...& Kumar, G. (2021). Molecular biology interventions for activity improvement and production of industrial enzymes. *Bioresource technology*, *324*,124596 doi:10.1016/j.biortech.2020.124596

[6] Cabarles, J., Lambio, A., Vega, S., Capitan, S., &Mendioro, M. (2012). Distinct morphological features of traditional chickens (*Gallus gallus domesticus L.*) in Western Visayas, Philippines. *Animal Genetic Resources/Resources Génétiques Animales/Recursos Genéticos Animales,* *51*, 73-87. doi:10.1017/S2078633612000410

[7] Chakraborty, S., Khopade, A., Kokare, C., Mahadik, K., &Chopade, B. (2009). Isolationand characterization of novel α-amylase from marine Streptomyces sp. D1. *Journal of Molecular Catalysis B: Enzymatic*, *58*(1-4), 17-23 doi:10.1016/j.molcatb.2008.10.011

[8] Cheng, T. H., Ismail, N., Kamaruding, N., Saidin, J., & Danish-Daniel, M. J. B. R. (2020). Industrial enzymes-producing marine bacteria from marine resources. *Biotechnology Reports*, *27*, e00482.https://doi.org/10.1016/j.btre.2020.e00482

[9] Cherry, J. R., &Fidantsef, A. L. (2003). Directed evolution of industrial enzymes: an update. *Current opinion in biotechnology*, *14*(4), 438-443 doi:10.1016/s0958-1669(03)00099-5

[10] Costa, W. L. G. D., Barbosa, I. M. F. N., Prado, D. P. G. D., Domann, N., &Rezende, H. .... &Pardosi, L. (2021). Isolation and enzyme bioprospection of bacteria associated to Bruguiera cylindrica, a mangrove plant of North Sumatra, Indonesia. *Biotechnology reports*, *30*, e00617 https://doi.org/10.1016/j.btre 2021.e00617

[11] Craigen, B., Dashiff, A., &Kadouri, D. E. (2011). The use of commercially available alpha-amylase compounds to inhibit and remove Staphylococcus aureus biofilms. *The openmicrobiology journal*, *5*, 21 doi:10.2174/1874285801105010021

[12] Da Silva, R. R. (2017). Bacterial and fungal proteolytic enzymes: production, catalysis and potential applications. *Applied Biochemistry and Biotechnology*, *183*, 1-

[13] Danilova, I., &Sharipova, M. (2020). The Practical Potential of Bacilli and Their Enzymes for Industrial Production. *Frontiers in Microbiology, Diseases* doi:10.1111/tbed.14221

[14] Dorji, Nedup; Duangjinda, Monchai; Phasuk, Yupin (2012). Genetic characterization of Bhutanese native chickens based on an analysis of Red Junglefowl (*Gallus gallus gallus* and *Gallus gallus spadecieus*), domestic Southeast Asian and commercial chicken lines (*Gallus gallus domesticus*). *Genetics and Molecular Biology,* 35(3), 603–609. doi:10.1590/S1415-47572012005000039

[15] Fernandes, P., &Carvalho, F. (2017). Microbial enzymes for the food industry. In *Biotechnology of microbial enzymes* (pp. 513-544). Academic Press http://dx.doi.org/10.1016/B978-0-12-803725-6.00019-4

[16] Ferreira Júnior, Á., Santos, J. P. D., Sousa, I. D. O., Martin, I., Alves, E. G. L., & Rosado (2021). Characteristics of theproteolytic enzymes produced by lactic acid bacteria. *Molecules*, *26*(7), 1858 doi:10.3390/molecules26071858

[17] Ferreira, V. H. B., Reiter, L., Germain, K., Calandreau, L., &Guesdon, V. (2020).

Uninhibited chickens: ranging behaviour impacts motor self-regulation in free-range broiler chickens (*Gallus gallus domesticus*). *Biology letters*, *16*(1), 20190721. https://doi.org/10.1098/rsbl.2019.0721

[18] Garg, G., Singh, A., Kaur, A., Singh, R., Kaur, J., & Mahajan, R. (2016). Microbial pectinases: an eco-friendly tool of nature for industries. *3 Biotech*, *6*, 1-13 doi:10.1007/s13205-016-0371-4

[19] Geciova, J., Bury, D., &Jelen, P. (2002). Methods for disruption of microbial cells for potential use in the dairy industry—a review. *International Dairy Journal*, *12*(6), 541-553 doi:10.1016/s0958-6946(02)00038-9

[20] Gupta, R., Beg, Q., & Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Applied microbiology and biotechnology*, *59*, 15-32 doi:10.1007/s00253-002-0975-y

[21] H. A. (2022). A systematic review of Toxoplasma gondii genotypes in *Gallus gallus domesticus* worldwide: The focus is Brazil. *Transboundary and Emerging Diseases*, *69*(5), 2440-2450 doi:10.1111/tbed.14221

[22] Hauglund, K., Hagen, S. B., & Lampe, H. M. (2006). Responses of domestic chicks (*Gallus gallus domesticus*) to multimodal aposematic signals. *Behavioral Ecology*, *17*(3), 392-398. doi:10.1093/beheco/arj038

[23] Hood, E. E. (2002). From green plants to industrial enzymes. *Enzyme and microbial technology*, *30*(3), 279-283 doi:10.1016/s0141-0229(01)00502-6

[24] R. (2018). *Gallus gallus domesticus*: immune system and its potential for generation ofimmune biologics. *Ciência Rural*, *48*doi:10.1590/0103-8478cr20180250 *journal*, *34*(1), 53-62 doi:10.1080/01490451.2015.1137662

[25] Kieliszek, M., Pobiega, K., Piwowarek, K., &Kot, A. M. (2021). Characteristics of the proteolytic enzymes produced by lactic acid bacteria. *Molecules*, *26*(7), 1858 doi:10.3390/molecules26071858

[26] Kirk, O., Borchert, T. V., &Fuglsang, C. C. (2002). Industrial enzyme applications. *Current opinion in biotechnology*, *13*(4), 345-351 doi:10.1016/s0958- 1669(02)00328-2

[27] Kumar, A., Dhar, K., Kanwar, S. S., & Arora, P. K. (2016). Lipase catalysis in organic solvents: advantages and applications. *Biological procedures online*, *18*, 1-11 doi:10.1186/s12575-016-0033-2

[28] Lambert, P. W., &Meers, J. L. (1983). The production of industrial ... &Pardosi, L. (2021). Isolation and enzyme bioprospection of bacteria associated toBruguiera cylindrica, a mangrove plant of North Sumatra, Indonesia. *Biotechnology reports*, *30*, e00617 https://doi.org/10.1016/j.btre.2021.e00617

[29] Liang, X., Fu, Y., & Liu, H. (2015). Isolation and characterization of enzyme-producing bacteria of the silkworm larval gut in bioregenerative life support system. *Acta Astronautica*, *116*, 247-253 doi:10.1016/j.actaastro.2015.07.010

[30] Mariadassou, M., Suez, M., Sathyakumar, S., Vignal, A., Arca, M., Nicolas, P., Faraut, T., Esquerré, D., Nishibori, M., Vieaud, A., Chen, C. F., Manh Pham, H., Roman, Y., Hospital, F., Zerjal, T., Rognon, X., &Tixier-Boichard, M. (2021). Unraveling the history of the genus Gallus through whole genome sequencing. *Molecular phylogenetics and evolution*, *158*, 107044. https://doi.org/10.1016/j.ympev.2020.107044

[31] Mohapatra, B. R., Bapuji, M., &Sree, A. J. A. B. (2003). Production of industrial enzymes (amylase, carboxymethylcellulase and protease) by bacteria isolated from marine sedentary organisms. *Acta Biotechnologica*, *23*(1), 75-84 doi:10.1002/abio.200390011

[32] Nigam P. S. (2013). Microbial enzymes with special characteristics for biotechnological applications. *Biomolecules*, *3*(3), 597–611. https://doi.org/10.3390/biom3030597

[33] Pandey, A., Selvakumar, P., Soccol, C. R., & Nigam, P. (1999). Solid state fermentation for the production of industrial enzymes. *Current science*, 149-162 https://www.jstor.org/stable/24102923

[34] Perez, E. C., Meurisse, M., Hervé, L., Georgelin, M., Constantin, P., Cornilleau, F., &Bertin, A. (2020). Object and food novelty induce distinct patterns of c-fos immunoreactivity in amygdala and striatum in domestic male chicks (*Gallus gallus domesticus*). *Behavioural brain research*, *381*, 112453. perspectives. *Current medicinal chemistry*, *17*(32), 3855-3873 doi:10.2174/092986710793205435

[35] Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A.

K., Madhavan, A., Rebello, S., & Pandey, A. (2018). Applications of Microbial Enzymes in Food Industry. *Food technology and biotechnology*, *56*(1), 16–30. https://doi.org/10.17113/ftb.56.01.18.5491

[36] Ray, A. K., Ghosh, K., &Ringø, E. J. A. N. (2012). Enzyme□producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, *18*(5), 465-492 doi:10.1111/j.1365-2095.2012.00943.x

[37] Rueda, N., Dos Santos, J. C., Ortiz, C., Torres, R., Barbosa, O., Rodrigues, R. C., ... & Fernandez-Lafuente, R. (2016). Chemical modification in the design of immobilized enzyme biocatalysts: Drawbacks and opportunities. *The Chemical Record*, *16*(3), 1436-1455 doi:10.1002/tcr.201600007

[38] Shini, S., Kaiser, P., Shini, A., &Bryden, W. L. (2008). Biological response of chickens (*Gallus gallus domesticus*) induced by corticosterone and a bacterial endotoxin. *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, *149*(2), 324–333. https://doi.org/10.1016/j.cbpb.2007.10.003

[39] Singh, R. S., Chauhan, K., & Kennedy, J. F. (2017). A panorama of bacterial inulinases: production, purification, characterization and industrial applications. *International journal of biological macromolecules*, *96*, 312-322 doi: 10.1016/j.ijbiomac.2016.12.004

[40] Singh, R., Kumar, M., Mittal, A., & Mehta, P. K. (2016). Microbial enzymes: industrial progress in 21st century. *3 Biotech*, *6*(2), 174 https://doi.org/10.1007/s13205-016-0485-8

[41] Singhania, R. R., Patel, A. K., Thomas, L., Goswami, M., Giri, B. S., & Pandey, A. (2015). Industrial enzymes. In *Industrial biorefineries& white biotechnology* (pp. 473-497). Elsevier https://doi.org/10.1016/B978-0-444-63453-5.00015-X

[42] Singhania, R. R., Sukumaran, R. K., Patel, A. K., Larroche, C., & Pandey, A. (2010). Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. *Enzyme and Microbial Technology*, *46*(7), 541-549 doi:10.1016/j.enzmictec.2010.03.010

[43] Toledo Fonseca, E., De Oliveira Silva, F. M., Alcântara, D., Carvalho Cardoso, R., Luís Franciolli, A., Sarmento, C. A., Fratini, P., José Piantino Ferreira, A., &Miglino, M. A. (2013). Embryonic development of chicken (*Gallus Gallus Domesticus*) from 1st to 19th day-ectodermal structures. *Microscopy research and technique*, *76*(12), 1217–1225. https://doi.org/10.1002/jemt.22288

[44] Tu, Y. G., Sun, Y. Z., Tian, Y. G., Xie, M. Y., & Chen, J. (2009). Physicochemical characterisation and antioxidant activity of melanin from the muscles of Taihe Black-bone silky fowl *(Gallus gallus domesticus Brisson*). *Food Chemistry*, *114*(4), 1345-1350 doi:10.1016/j.foodchem.2008.11.015

[45] van Beilen, J. B., & Li, Z. (2002). Enzyme technology: an overview. *Current Opinion in biotechnology*, *13*(4), 338-344 doi:10.1016/s0958-1669(02)00334-8

[46] Vilches-Moure J. G. (2019). Embryonic Chicken (*Gallus gallus domesticus*) as a Model of Cardiac Biology and Development. *Comparative medicine*, *69*(3), 184–203. https://doi.org/10.30802/AALAS-CM-18-000061

[47] Vilches-Moure, J. G. (2019). Embryonic chicken (*Gallus gallus domesticus*) as a model of cardiac biology and development. *Comparative medicine*, *69*(3), 184-203 doi:10.30802/AALAS-CM-18-000061

[48] Volpato, G., C Rodrigues, R., & Fernandez-Lafuente, R. (2010). Use of enzymes in theproduction of semi-synthetic penicillins and cephalosporins: drawbacks and enzymes. *Philosophical Transactions of the Royal Society of London. B, BiologicalSciences*, *300*(1100), 263-282 doi:10.1098/rstb.1983.0004

[49] Waegeman, H., &Soetaert, W. (2011). Increasing recombinant protein production in Escherichia coli through metabolic and genetic engineering. *Journal of Industrial Microbiology and Biotechnology*, *38*(12), 1891-1910 doi:10.1007/s10295-011-1034-4

[50] Waegeman, H., &Soetaert, W. (2011). Increasing recombinant protein production in Escherichia coli through metabolic and genetic engineering. *Journal of Industrial Microbiology and Biotechnology*, *38*(12), 1891-1910 doi:10.1007/s10295-011-1034-4

[51] Webster Leonardo Guimarães da Costa; Isa Marianny Ferreira Nascimento Barbosa; Débora Pereira Gomes do Prado; Natália Domann; Hanstter Hallison Alves Rezende; (2021). *A systematic review of Toxoplasma*

gondii genotypes in Gallus gallus domesticus worldwide the focus is Brazil. Transboundary and Emerging

[52] Woldekiros, H. S., &D'Andrea, A. C. (2017). Early evidence for domestic chickens (*Gallus gallus domesticus*) in the Horn of Africa. *International Journal of Osteoarchaeology*, *27*(3), 329-341 doi:10.1002/oa.2540

[53] Yang, W., Meng, F., Peng, J., Han, P., Fang, F., Ma, L., & Cao, B. (2014). Isolation and identification of a cellulolytic bacterium from the Tibetan pig's intestine and investigation of its cellulase production. *Electronic Journal of Biotechnology*, *17*(6), 262-

[54] Zamost, B. L., Nielsen, H. K., & Starnes, R. L. (1991). Thermostable enzymes for industrial applications. *Journal of Industrial Microbiology*, *8*(2), 71-81doi:10.1007/bf01578757