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ISOLATION AND IDENTIFICATION OF CELLULASE PRODUCING BACTERIUM FROM THE DECAYING PLANT MATERIAL AND INVESTIGATION OF ITS POTENTIAL AS A PROBIOTIC IN BROILER

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ABSTRACT

Cellulose is most abundant organic compound on earth. They are non-digestible polysaccharides which are present in soy, decaying plant material, grass, hemp etc and required a cellulase enzyme for hydrolysis. By using cellulase producing bacterium in a feed industry, soy products can then be utilized as an animal feed to fulfil the animal feed requirements. The goal of present examination is to disengage and recognize cellulytic microscopic organisms from rotting plant materials and research its potential as probiotic in broiler for improving development. For this reason, Bacterial strain was secluded from rotting natural plants and afterward distinguished as *Bacillus licheniformis* dependent on morphological, biochemical and molecular attributes. Three (3) broiler groups were constructed with each group containing 5 chicks with 3 replicates and then fed with selected bacterial strain cell suspension containing (2.2-6.5x 10^9cfu/ml/1ml drinking water (T1) and (2.2-6.5x 10^9cfu/ml/2ml drinking water (T2). Control group was fed with normal diet without any probiotic. The trial was continued for 42 days from day 1 to day 42. The chicks were observed for the body weight gain (BWG), Feed intake (FI), Feed conversion ratio (FCR), Average daily gain and Mortality (MC). On day 42, five broilers were haphazardly chosen from each gathering and blood samples that drawn and a few biochemical tests were performed to guarantee the safety of bacterial strain. Information was exposed to single direction investigation of fluctuation utilizing the general straight models (GLM) gave in SPSS 19.0.0 (2016). P value <0.05 was viewed as significant. Under the states of present examination, probiotic supplementation in broiler feed was viable in improving BWG and FCR. The mean values of Glucose, ALT, AST, ALP, GGT, TP, ALB, Globulin, T. Bilirubin, Iron, Uric acid, CK, CK-MB, LDH, BUN, Creatinine, Na, K, Cl, HCO3, Ca, Mg, PO4, Chol, TG, HDL, LDL, VLDL, Amylase and Lipase were non-symbolically different (P > 0.05) from each treated group and control group as well. The outcomes of current study revealed that probiotic treatments had no toxic effects on kidneys, heart, liver and pancreas, exhibiting its safety for broiler and food applications. So, we can say that cellulase producing bacteria do has a potential as probiotic.

KEYWORDS: Effect of Probiotic, Cellulase Producing Bacterium, Amylase and Lipase Level in Broiler, Probiotic Effect on Liver, Kidney, Heart, Electrolytes and Minerals, *Bacillus licheniformis*, FCR & BWG (Body weight gain)

1. INTRODUCTION

Poultry is one of the quickest developing segments of farming and animal cultivation segment. Feed is probably the biggest thing of expenditure in poultry creation and it alone records to 70% of all -poultry generation. The consistent increment in the expense of poultry feed fixings and intensified feed is making less benefit to poultry ranchers. To limit the expenses of feeding, a few feed added substances (as development promoters) like engineered hormone and anti-infection agents have been widely utilized for improving poultry creation as of late. To evade the wellbeing perils of antimicrobials to human

just as poultry, as of late, a probiotic as feed added substances for better and safe generation in poultry was utilized. Probiotics are live microorganisms advanced with claims that they give medical advantages when expended, for the most part by improving or re establishing the gut vegetation. Probiotics are viewed as commonly safe to expend however may cause undesirable reactions in uncommon cases.

2. OBJECTIVE

Cellulose is most abundant organic compound on earth. They are non-digestible polysaccharides that are present in soy, decaying plant material, grass, hemp etc and required a cellulase enzyme for hydrolysis. By using cellulase producing bacterium in feed industry, soy products can then be utilized as animal feed to fulfil the animal feed requirements. The goal of present examination is to disengage and recognize cellulytic microscopic organisms from rotting plant materials and research its potential like as probiotic in broiler for improving development.

3. MATERIALS AND METHODS

Bacterial strains were disconnected from rotting natural plants gathered from University of Veterinary and Animal Sciences, Lahore, Pakistan. Rotting natural plant tests (1.0 g) were blended in 100 ml typical saline, later sequentially weakened from 10−1 to 10−6 ratio with ordinary saline. 100 µl of each weakened example was vaccinated on cellulose agar medium and hatched at 37 °C for 24 h. The segregated colonies were chosen to acquire unadulterated wanted cellulytic bacterial strain. The recognizable proof of a chosen bacterial strain was done based on morphological, biochemical and molecular attributes. Gram stain, MR VP test, Citrate use test, Starch hydrolysis test, Gelatin hydrolysis test, Nitrate reduction test, Catalase test, Oxidase test, Glucose and lactose fermentation test, Indole test, Urea hydrolysis test, H2S creation test were utilized for biochemical portrayal. Genomic DNA extraction, PCR enhancement and sequencing of the 16S rRNA quality of biochemically distinguished bacterial strain were completed and strain was recognized as *Bacillus Licheniformis.*

4. STUDY DESIGN

Three (3) bunches were built with each gathering contains 5 chicks and 3 reproduces. Chosen bacterial strain was refined at 37℃ with a shake pace of 200 rpm for 1 day in cellulase broth. The cells were gathered with the centrifugation at 12,000 rpm for 20 mins, and the cell arrangement containing colony-forming units per ml (2.2-6.5x 109cfu/ml/1ml drinking water (T1) and (2.2-6.5x 109cfu/ml/2ml drinking water (T2) were utilized to feed broiler chicks. Control bunch was nourished with typical eating routine with no probiotic. The experiment was continued for 42 days from day 1 to day 42. The chicks were checked for the body weight gain (BWG), Feed intake (FI), Feed conversion ratio (FCR), Average daily gain and Mortality (MC). On day 42, five broilers were haphazardly chosen from each gathering and blood samples were drawned and biochemical tests such as Liver function tests, Renal function tests, Cardiac enzymes, Lipid profiles, Pancreatic function tests were likewise performed to guarantee the safety of bacterial strain.

5. STATISTICAL ANALYSIS

Information was exposed to single direction investigation of fluctuation utilizing the general straight models (GLM) gave in SPSS 19.0.0 (2016). P value <0.05 was viewed as significant.

6. RESULTS

Gram stain indicated secluded bacterial was gram positive thick rod which additionally recognized as *Bacillus Licheniformis* based on biochemical and molecular attributes. The strain was motile by peritrichous flagella, structures spore in non-suitable condition. Spores were heat resistant and developed in 50°C; state of spores is integral to Para focal and ellipsoidal to round and hollow. The size of spore was 0.6-10µ long. The biochemical results of selected bacillus strain are presented below in Table 1.

Table 1: Growth and Biochemical Characteristics of *B. licheniformis*

Biochemical Tests	*Bacillus licheniformis*
Gram reaction	Positive
Catalase production	Positive
Motility	Positive
Anaerobic growth	Positive
Spore type	Ellipsoidal, central, no swelling of sporangium
ONPG	Positive
ADH	Positive
LDC	Negative
ODC	Negative
Urease	Negative
TDA	Negative
Citrate utilization	Positive
H2S Production	Negative
Indole production	Negative
Voges-Proskauer	Positive
Gelatin decomposition	Positive
Nitrate reduction	Positive
Glucose fermentation	Positive
Sucrose fermentation	Positive
Maltose fermentation	Positive
Mannose fermentation	Positive
Lactose fermentation	Negative
Inositol fermentation	Positive
Sorbitol fermentation	Positive
Rhamnose fermentation	Negative
Melibiose fermentation	Positive
Amygdalin fermentation	Negative
L-arabinose fermentation	Negative
Growth at 50°C	Positive
Growth at 60°C	Negative

*ONPG, o-nitrophenyl-b-D-galactopyranoside; ADH, Arginine dehydrolase; LDC, Lysine decarboxylase; ODC, Ornithine decarboxylase; TDA, Tryptophan deaminase.

6.1. Body Weight

Table 2 represents the productive performance of broiler. In respect to initial body weight, there was non appreciable difference midst the dietary groups. At the end of 42 days of age, the highest live weight (2075.26 ± 231.30) were found in broilers of treatment 1 group. This was followed by broilers (2032.53 ± 176.71) belonging to treatment 2 group and control group (1901.73 ± 163.92). It is stated that broiler of group treatment 1and 2 weighed expressively higher than that of control ($P<0.01$).

Table 2: Mean Body Weight of Control and Treatment Gatherings of Broilers

Parameters	Normal Group	Treatment 1 Group	Treatment 2 Group
ILW (g/b)	45.72±0.12	45.64±0.34	45.70±0.400
7 day old (g/b)	205 ± 11.53	230.6 ± 13.57	224.92 ± 9.41
14 day old(g/b)	495 ± 39.77	529 ± 25.05	513.3 ± 45.65
21 day old (g/b)	804.3 ± 41.62	902.87 ± 52.89	865.66 ± 92.9
28 day old (g/b)	1141.84 ± 53.87	1307 ± 102.8	1257.66 ± 69.63
35 day old (g/b)	1501.06 ± 83.52	1754.8 ± 128.47	1679.73 ± 118.26
42 day old (g/b)	1901.73 ± 163.92	2075.26 ± 231.30	2032.53 ± 176.71

6.2. Feed Conversion Ratio

As a rule of thumb, animals that have a low FCR are considered efficient users of feed. Contrasts in feed conversion ratio (FCR) of the broiler of various dietary gatherings contrasted altogether ($P<0.01$). The lowermost value was obtained for birds that belong to treatment 1 group. Both treatment 1 and treatment 2 group broilers showed almost similar but improved efficiency which differed from control group ($P<0.01$). The results presented in Table 3 clearly exhibits an impression that the broiler receiving treatment 1 and treatment 2 were the best converters of feed into live weight and the effect was more prominent after 21days and onwards.

Table 3: Feed Conversion Ratio in Broiler from Day 7th to 42 Day

SR #	Control Group	Treatment 1	Treatment 2
week1	0.68	0.6	0.62
week2	0.78	0.73	0.75
week3	0.78	0.7	0.73
week4	0.77	0.67	0.7
week5	0.75	0.64	0.67
week6	0.72	0.65	0.67

6.3 Feed Intakes

Feed was given at the same amount to every one of the gatherings. There was no any adjustment in feed to any gathering i.e. 2100g till 7thday, 5775g afterward 7thday till 14th day, 9450g afterward 14thday till 21th day, 13125g afterward 21thday till 28th day, 16800g afterward 28th day till 35th day, 20475g afterward 35th day till 42nd day.

6.4 Average Daily Body Weight Gain in Broiler

Results for average daily body weight gain of 3 groups of broilers were displayed in Table 4. The increased daily weight gain was projected in treatment 1 gathering and least in control gathering and results were factually huge ($P < 0.01$).

Table 4: Average Daily Weight Gain in Broiler from Day 7th to 42nd Days

SR #	Control Group	Treatment 1	Treatment 2
week1	22.66g	26.28g	25.46g
week2	32.05g	34.48g	33.33g
week3	36.09g	40.77g	39.0g
week4	39.12g	45.04g	43.25g
week5	41.56g	48.8g	46.65g
week6	44.17g	48.3g	47.28g

6.5 Mortality in Broiler from 7th Day to 42nd Day

Treatment 1 and Treatment 2 receiving groups had no mortality while the survivability of the control group was 97.33%. However, it is clear that the control group suffered more compared to remaining groups.

In the present study we concluded that cellulase fabricating bacteria may act as probiotic to advance digestion which presumably fallouts in weight gain in broiler. We further tested the effect of probiotic strain on certain biochemical parameters.

6.6 Effect of Probiotic on LFTs

The results of LFTs are summarized in Table 5. The mean of Glucose, ALT, AST, ALP, GGT, TP, ALB, Globulin, T. Bilirubin, Iron, Uric acid do not differ significantly. However, the mean of Glucose was higher (205.2 ±35.92 mg/dL) in treatment 1 group and coincide with treatment group 2 (178.6 ±28.34 mg/dL) and was least in control group (159 ± 26 mg/dL). This impact could be clarified by a higher absorptive limit of the intestinal mucosa due to histo-morphological changes (Awad*et al.* 2009, Aliakbarpour*et al.* 2012) as well as a progressively successful assimilation of the digestive nutrients due to higher intestinal enzyme action (Jin*et al.* 2000; Mountzouris*et al.* 2007 Wang and Gu 2010), in this way expanding the supplements accessible to the broilers. The mean of uric acid was higher in T1 (8.19 ± 1.20 mg/dL) and followed by T2 (6.85 ± 1.52 mg/dL) and was least in control group (6.61 ± 2.16 mg/dL). The results do not differ statistically ($P > 0.05$) but these linear increase in uric acid level presumably showing better utilization of amino acids and provides an antioxidant defense in broiler against radical oxygen causing damage to body tissues. In short, we can say that probiotic strain does not have harmful effect on liver.

Table 5: LFTs in Broiler of Control and Treatment Groups

Parameter	Control Group	Treatment Group 1 (T1)	Treatment Group 2 (T2)	Level of Significance
Glucose mg/dl	159 ± 26	205.2 ± 35.92	178.6 ± 28.34	NS*
ALT (U/L)	2.2 ± 1.3	5.6 ± 1.14	1.8 ± 0.84	NS*
AST (U/L)	295.6 ± 41.6	310.4 ± 38.72	304.6 ± 30	NS*
ALP (U/L)	1396.2 ± 145.26	1027.2 ± 171.65	1144 ± 116.7	NS*
GGT (U/L)	18.4 ± 4.72	19.2 ± 2.77	16.8 ± 3.7	NS*
TP (g/dl)	4.1 ± 0.65	3.334 ± 0.36	3.178 ± 0.25	NS*
Albumin (g/dl)	1.61 ± 0.24	1.514 ± 0.24	1.328 ± 0.08	NS*
Globulin (g/dl)	2.47 ± 0.44	1.82 ± 0.15	1.85 ± 0.22	NS*
T-bill (mg/dl)	0.06 ± 0.03	0.082 ± 0.01	0.038 ± 0.02	NS*
Iron (µg/dL)	123 ± 32.89	147.2 ± 17.12	118.8 ± 15.1	NS*
Uric acid (mg/dl)	6.61 ± 2.16	8.186 ± 1.20	6.85 ± 1.52	NS*

*NS =Non-significant i.e. $P > 0.05$, ALT=Alkaline Aminotransferase AST= Aspartate Aminotransferase, ALP = Alkaline Phosphatase, GGT=Gamma Glutamyl Transferase, TP = Total Protein, T-bill = Total bilirubin. NS =Non-significant i.e. $P > 0.05$.

6.7 Effect of Probiotic Strain on Cardiac Enzymes

The result of cardiac enzymes are summarized in Table 6. Cardiovascular enzymes including LDH, CPK and isoenzyme CK-MB were non-fundamentally different between each treatment group and control bunch. So, it can be conferred that *Bacillus Licheniformis* strain did not have detrimental effect on cardiac enzymes.

Table 6: Serum Biochemical Values of Cardiac Enzymes (Mean ±SD) in Broilers of Different Groups.

Parameter	Control Group	Treatment Group 1 (T1)	Treatment Group 2 (T2)	Level of Significance
CK (U/L)	2673.8 ± 139.6	2594.96 ± 146.8	2817 ± 161.9	NS*
CK-MB (U/L)	16.2 ± 0.5	15.6 ± 0.4	16.1 ± 0.5	NS*
LDH (U/L)	2237.4 ± 156.9	2399 ± 170.7	2152.8 ± 157.2	NS*

NS =Non-significant i.e. $P > 0.05$. CPK = Creatine Phosphokinase, CK-MB = Creatine kinase Muscle-Brain, LDH = Lactate dehydrogenase.

6.8 Effect of Probiotic Strain on RFTs and Electrolytes

The results of RFTs and electrolytes are summarized in Table 7. Blood urea nitrogen, creatinine and electrolytes including sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus were non-essentially various (P > 0.05) from one another and control bunch too. The different parameters identified with kidneys and blood electrolytes were inside typical reaches indicating the wellbeing of probiotic strain.

Table 7: Various Metabolites and Electrolytes in Broiler of Different Groups

Parameter	Control Group	Treatment Group 1 (T1)	Treatment Group 2 (T2)	Level of Significance
BUN (mg/dl)	2.68 ± 0.65	3.58 ± 1.16	3.74 ± 0.66	NS*
Creatinine(mg/dl)	0.044 ± 0.01	0.06 ± 0.02	0.09 ± 0.02	NS*
Na(mmol/L)	153.4 ± 2.07	153 ± 2.5	152.4 ± 2.07	NS*
K(mmol/L)	5.27 ± 0.60	4.78 ± 0.7	4.6 ± 0.6	NS*
Cl(mmol/L)	104.8 ± 2.49	108.4 ± 3.73	109.8 ± 2.68	NS*
HCO3-(mmol/L)	19.66 ± 2.22	20.14 ± 3.5	19.98 ± 2.86	NS*
Ca (mg/dL)	11.92 ± 0.55	11.45 ± 0.89	11.5 ± 0.66	NS*
Mg (mg/dL)	2.39 ± 0.06	2.45 ± 0.08	2.16 ± 0.13	NS*
Po4 (mg/dL)	8.4 ± 0.9	7.0 ± 1.05	7.46 ± 0.78	NS*

*BUN = Blood urea nitrogen, Na = Sodium, K = Potassium, Cl = Chloride, HCO3=Bicarbonate, Ca = Calcium, Mg = Magnesium, P = Phosphorus, NS =Non-significant i.e. P > 0.05.

6.9 Effect of Probiotic on Lipid Profile

The results of lipid profile are summarized in Table 8. The results of lipid profile do not differ expressively (P > 0.05) from each other and control group. However, the mean level of cholesterol is lower in treatment T2 group (122.4 ± 12.5 mg/dL) and followed by treatment T1 group (127.8 ± 11.9 mg/dL) and was higher in control group (138.6 ± 13.9 mg/dL. The decrease in cholesterol level could be due to the cholesterol assimilation by *Bacillus Licheniformis* which in turn reduce cholesterol absorption in the system.

Table 8: Biochemical Parameters of Lipid Profile in Different Broiler Groups

Parameter	Control Group	Treatment Group 1 (T1)	Treatment Group 2 (T2)	Level of Significance
Chol (mg/dl)	138.6 ± 13.9	127.8 ± 11.9	122.4 ± 12.5	NS*
TG (mg/dl)	91.8 ± 3.8	83.6 ± 3.96	96.6 ± 4.5	NS*
LDL (mg/dl)	44.8 ± 3.16	41.6 ± 3.6	38.8 ± 3.18	NS*
HDL (mg/dl)	69.18 ± 13.14	72.2 ± 16.3	66.4 ± 3.1	NS*
VLDL (mg/dl)	14.2 ± 376	16.74 ± 1.79	19.4 ± 2.27	NS*

Chol = Cholesterol, TG = Triglyceride, LDL = Low density lipids, HDL = High density lipids, VLDL = Very low-density lipids, NS* = Non significant.

6.10 Effect of Probiotic on Pancreatic Enzymes

The impact of probiotic strain on pancreatic catalysts are condensed in Table 9. The upshots of pancreatic enzymes don't vary fundamentally (P > 0.05) from one another and control gathering. The different parameters identified with pancreas were inside ordinary extents demonstrating the wellbeing of probiotic strain.

Table 9: Biochemical Values of Pancreatic Enzymes in Different Broiler Groups

Parameter	Control Group	Treatment Group 1 (T1)	Treatment Group 2 (T2)	Level of Significance
Amylase (U/L)	532.6 ± 26.4	542.5 ± 20.2	535.4 ± 19.1	NS*
Lipase (U/L)	7 ± 0.58	7.35 ± 0.43	7.14 ± 0.61	NS*

NS=Non-significant i.e. P > 0.05.

5. CONCLUSION

Cellulase producing bacterium i.e. *Bacillus Licheniformis* (in our study) supplementation in broiler feed was effective in enlightening BWG and FCR. Some biochemical parameters were also performed to ensure the safety of probiotic. The liver, kidneys, pancreas and cardiovascular catalysts, serum minerals and lipid profiles were not altogether extraordinary in every single regarded bunch when contrasted with control. The consequences of present investigation uncovered that probiotic medicines had no poisonous impacts on kidneys, heart, liver and pancreas, showing its wellbeing for broiler and nourishment applications. In this way, we can say that cellulase producing microorganisms has an potential as probiotic.

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