



**University of Zabol  
Graduate school  
Faculty of Agriculture  
Department of Animal Science, Animal Nutrition (Ruminant)**

**The Thesis Submitted for the Degree of *Ph.D* (in the field of Animal Science)**

**Isolation, Identification and Selection of Lactic Acid Bacteria  
from Sistani Cattel for Probiotic production and effect of its  
feeding in holestein dairy calves**

**Supervisors:**

**Dr. Mostefa Yosef Elahi  
Dr. Mohammadreza Sanjabi**

**Co-supervisor**

**Dr. Mehdi Vafaye Valleh  
Dr. Abdelfattah Z.M. Salem  
Dr. Nahid Mojjani**

**Ph.D. Candidate**

**Afsaneh Ahrari**

**Autum2022**

## **Abstract**

The cow's rumen is home to many bacteria that are responsible for converting food into energy sources. In recent years, many rumen microbes have been identified and isolated using 16sRNA gene sequencing. Some microbes are suggested as food additives to improve the growth and production of animals. The use of probiotics as a food additive has caused it to induce its effect by stimulating positive effects on the host by creating a favorable balance of intestinal microflora. Therefore, the purpose of this research is to isolate, identify and select lactic acid bacteria for probiotic production from the rumen and feces of Sistani cows. In this experiment, in order to isolate lactic acid bacteria, samples were taken from the rumen of 12 and the feces of 4 Sistani cows in the slaughterhouse of Zabol city. The collected samples were transferred to test tubes containing liquid MRS culture medium and kept in a greenhouse at 39 degrees Celsius for 48 hours. After growing from the mentioned medium, linear culture was performed on the pellets containing the MRS agar medium, and the pellets were kept in a greenhouse at 39 degrees Celsius for 48 hours under aerobic conditions. The emerged clones were examined in terms of growth characteristics and morphology as well as purity. Gram-positive, spore-free, spherical and rod-shaped catalase-negative bacteria that did not cause beta hemolysis were examined for antimicrobial, acid, bile salt and antibiotic sensitivity tests. From the initial number of 158 isolates of lactic acid bacteria, 84 isolates were investigated for antimicrobial activity against four types of Salmonella bacteria and one type of EColi bacteria. Then, the isolates obtained from the antimicrobial test were examined to perform the pH resistance test. A number of 13 selected samples from this experiment were studied to conduct the bile resistance test, of which 5 isolates were examined to conduct the antibiotic sensitivity test and entered the final stage for genetic testing and molecular testing. What was obtained from the molecular test and genetic sequence of 16sRNA were only 2 isolates that had high genetic affinity with two bacteria Streptococcus infantarius and Entrococcus Faecium after sequencing and using NCBI software. These two isolates were observed as potential probiotics in Sistani cows. In order to investigate performance indicators such as growth rate and body weight, food consumption, food conversion ratio, losses and health indicators, these bacteria were fed to dairy calves along with two other treatments. The treatments were as follows: experimental treatments: CO; Without receiving probiotic supplement, CP group: receiving imported protoxin product at the rate of 2 grams per day per calf, L1 group: receiving product number 1 at the rate of 2 grams per day per calf, and group L2 receiving probiotic product number 2 at Amount of 2 grams per day per head of calf. The research results showed that L1 and L2 isolates have suitable characteristics for probiotic use. These features are high survival rate in low pH conditions and 3% concentration of bile and preventing the growth of pathogenic bacteria such as E. coli and Salmonella as well as antibiotic resistance. Also, despite the fact that there was no statistically significant difference in many of the calf performance evaluation tests, in general, treatments with probiotics had a higher value.

**Key words:** lactic acid bacteria, probiotics, dairy calves, health, immunity