

# Isolation and identification of enterococcus faecium biology essay



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Enterococci are widely distributed in the environment; within the human body, they are normal commensals of the oral cavity, gastrointestinal tract and vagina. *Enterococcus faecium* is a gram-positive bacterium group D, alpha hemolytic or nonhemolytic in the genus *Enterococcus* (Chingwaru W., 2003). It can be a commensal (non-harmful coexisting organism), in the human intestine, but it may also be a pathogen — causing diseases like neonatal meningitis.

Ordinary lactobacillus medium including: MRS medium, Luria-Bertani (LB) medium, high-sterilized stored at 4 °C refrigerator spare. In the sterile console, the yogurt containing lactic acid bacteria was diluted with sterile saline to a serial dilution (10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>) of 50 µl suspension and coated them on the plate containing MRS choice medium and marked. The plate was placed at 37 °C warm box without oxygen for 36 ~ 48 hours. Single colony was picked and repeatedly crossed to obtain the pure strains.

Bacterial identification methods include the common (tradition) classified identification, numerical value identification and automation identification. The common (tradition) classified identification needed to detect many items. It is so tedious. But numerical value identification is according to the identification target. It uses the test card. The result is expressed by the way of figure and compared with the data of database to get an identification result. Automation identification can reach whole procedure automation in the step of inoculation, cultivation, reading and report. It can supply a powerful instrument in rapid clinical microorganism diagnosis. No matter what kind of identification methods, it all needs to identify bacteria according

to the morphology, physiological characteristics and biochemical characteristics.

*Enterococcus faecium* is a kind of oval cell. Its diameter is 0.5 ~ 1.0  $\mu$ m. It can show a short-chain arrangement frequently in pairs or in liquid medium. It has slight movement. In the blood plate it can form a milky white plate or white, marginal integrity, central protuberance, large and smooth colony. Its diameter is 1-2mm, and has rare colors, mostly around the colonies without hemolysis. After the Gram stain, bacteria shows purple. It is Gram-positive coccobacteria. The chemistry character of *Enterococcus faecium* is contact-negative, and it can grow in 6.5% broth, bile Esculin agar plate and 0.1%... methylene blue. It also can grow in 10% f or 45% f, pH 9.6 broth medium, and can tolerate 65% f 30min condition. It can ferment lactose, sorbitol and mannitol and can use arginine, but can't use ferment arabinose.

### **2.3.2 Biochemical identification**

Identification by lactic acid bacteria and biochemical identification tests, respectively taking the exercise-induced, contiguity enzyme, anaerobic growth, 10% f and 45% f, 60% f 30 min, 6.5%...NaCl salt-tolerant growth, pH 9.6 alkaline environment growth, methyl red, benzopyrrole, sugar alcohol acid fermentation (glucose, galactose, maltose, lactose, raffinose, manicol and so on). Comparing the experiment result with "Berger's Manual of Determinative Bacteriology" (the ninth edition) to determine comprehensively.

### **2. 3. 3 The growth curve of Enterococcus faecium**

The growth of bacteria includes increasing volume, quantity and the improvement of metabolic function. Different bacteria have their own different growth pattern. Under the unchanged culture conditions, calculate the number of bacteria oriented to time, time as abscissa, the number of bacteria as vertical axis we can get a curve. The growth curve includes the stagnation period, logarithmic phase, stationary phase and decline phase. The length of each period is different according to the medium composition and culture conditions due to bacteria's own characteristics. In a comparative study of different bacteria, growth curve can help to choose bacterium with the same growth state and this can make a reliable experiment result. Detect the bacteria-growth curve mainly use two methods, such as turbidity method and viable organism dilution count. Turbidity method is to place the different stage and different concentration bacterial culture to the spectrophotometer, and count the bacteria by turbidity method. We can use the turbidity to represent the yield of bacterium and measure total bacterial count (including the dead bacterium). It is simple, facility comparing with the turbidity method. But it only can indicate the bacterial relative growth information. Viable organism dilution method of counting is to dilute the bacterial liquid to different dilution of  $10^{-1}$  to  $10^{-2}$  and so on. Every dilution can be cultured in the plate and colony is counted with colony events-per-unit-time meter. The total quantity of viable organism by using this method can represent the true growth information of bacterium.

### **3 Application of Enterococcus faecium**

#### **3.1 Supplementation of food with enterococcus faecium (SF68) stimulates immune functions**

The intestinal microflora play a crucial role in host defense as demonstrated by their ability to modulate both innate and acquired immunity at the local as well as systemic levels. Due to these immunological properties, specific strains of lactic acid bacteria (LAB), 3 defined as probiotics, have raised considerable interest in recent years. When ingested as a feed supplement in sufficient numbers, probiotics are live microorganisms that beneficially affect the gastrointestinal balance, going far beyond the conventional nutritional effect. The mechanisms underlying the immune modulating properties of probiotics are not fully understood. However, they may be due indirectly to the ability of probiotics to balance the intestinal microflora and/or be a consequence of a direct adjuvant effect on the production of immune factors, such as cytokines. In fact, several strains of LAB were shown to enhance nonspecific immunity in vitro as well as in vivo, including the release of tumor necrosis factor- $\alpha$  and interleukin 6 (4), increased phagocytosis in mice and humans (Perdigon, G. et al, 1988) (Schiffrin, E. J. et al, 1995) and stimulated natural killer cell activity (Matsuzaki, T., 1998, aller, D. et al, 2000). Their ability to specifically modulate the host's immune responses to pathogens was also demonstrated (Meydani, S. N. & Ha, W. K. , 2000). An increase in rotavirus-specific antibodies was detected in children with acute rotavirus diarrhea who received *Lactobacillus rhamnosus* (Kaila, M. et al, 1992). Moreover, it was shown that administration of *L. johnsonii* to human volunteers boosted the specific immunoglobulin IgA response to the *Salmonella typhi* vaccine Ty21a (Link-Amster, H. et al, 1994).

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### **3. 2 Application in treatment**

Enterococcus faecium can ferment a variety of sugar, and product acid. It has high chemical resistance, and is widely distributed in nature, but also human and animal gut microbial. It has highly detected in animal feces (Archimbaud, C. et al., 2003). Utilizing non-toxic, facultative anaerobic Enterococcus, in conjunction with other animal probiotics (Animal Microecological Agent, AMEA) can prevent and treat many digestive tract diseases of animals by replacing antibiotics.

Intestinal flora can be regarded as a normal human body organs, these normal floras directly involved in the activities of the host metabolism is normal physiological activities of the host indispensable component (Baldassarri, L. et al., 2001). Endogenous Enterococcus as one of the normal floras of the human body, mainly the site of colonization in the ileum and colon, and some strains may be directly involved in the metabolism of the host material. Enterococcus lactic acid feces as intestinal flora, with a bio-barrier function, from healthy people or animals that have separate physiological functions of bacteria to re-giving taking it right people are harmless and safe, but such micro-organisms in the body and settled and reproduction Ecology is a very complex issue. Made viable by the agent is likely to play a biological activity within a certain range, or part of participation in host metabolic activity, resulting in a certain physiological role and therefore have the disease, adjusting the significance of the ecological balance (Chow, J. W. et al., 1993).

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Currently, the application of enterococci is mainly in Enterococcus faecium. The earliest example of the use of Enterococcus faecium is out of Japan. It was first developed for the treatment of various intestinal diseases. Yogurt products on the market are containing Enterococcus faecium in Japan or pharmaceutical drugs, such as Lascpan, Lacton, Ruosu, Lebenin, Biosmin. “Mummy Love” that product in South Korea contains 10<sup>8</sup> / g feces enterococci and it can treat gastro-intestinal diseases in infants probiotic preparations (Coque, T. M. et al., 1995).

### **3. 3 Safety of Enterococcus faecium**

Endogenous intestinal bacteria, especially the colonization of lactic acid bacteria in the gut healthy people, the people are safe, because a large number of endogenous enterococci colonization in the human intestinal tract under the bit, which may affect the host intestinal digestion and absorption, <https://assignbuster.com/isolation-and-identification-of-enterococcus-faecium-biology-essay/>

and other function. It has been reported (Dicuonzo, G. et al., 2001) that yogurt containing *Enterococcus faecium* can prevent diarrhea. By double-blind test, the incidence of antibiotic group was 27.2%, while taking the probiotic group was only 3.7%. So it is not only proved to be effective, but did not find any side effects. In 1989 Food and Drug Administration (FDA) and the American Association of Feed Public (AAFCO) has also introduced the safety 42 probiotic bacteria, including *Enterococcus faecium*.

### **3.4 Oral application of *Enterococcus faecium* strain EE3**

Probiotic administration is being used increasingly in human and veterinary medicine. A variety of microorganisms, especially lactic acid bacteria (LAB), such as lactobacilli, bifidobacteria, and enterococci nonpathogenic LAB are associated with a wide variety of sources, such as plant material and various foods. Based on the definition of probiotics, adequate numbers of viable organisms must reach the intestinal tract to have a beneficial effect. For this to happen, probiotic organisms must be able to survive transit through the acidic environment of the stomach and persist in the presence of bile. They have to multiply at the site of destination in the intestine. In addition, they must be able to produce the antagonistic metabolites against dominating saprophytic microflora resulting in competitive growth. Antimicrobial activity of LAB is due to the production of organic acids (lactic and acetic acids in particular), carbon dioxide, ethanol, hydrogen peroxide, biacetyl and bacteriocins. These abilities are often found among lactobacilli and enterococci, which are most frequently used as animal feed supplements or directly as probiotic preparations. Among enterococci, only the species of *Enterococcus faecium* of human origin was used as a commercial probiotic.



*E. faecium* used as probiotics efficiently protect animals from diseases caused by *E. coli*, salmonellae or clostridia; it is also reported (Eaton, T. J. & Gasson, M. J., 2001) that the best preventive effectivity of *E. faecim* from commercial probiotic preparation Vitacanis against *Salmonella enterica* serovar Typhimurium in the gastrointestinal tract of gnotobiotic mice.

*E. faecium* EE3 strain was able to survive transit through the canine intestinal tract, leading to enrichment of the colonic microflora and local and systematic effects. This strain also persisted in the digestive tract for almost 3 months after cessation of its administration. The new canine probiotic strain of *Lactobacillus fermentum* AD1 persisted in the feces of dogs even for 5 months after cessation, however, it reached a lower final concentration (10<sup>3</sup>~10<sup>5</sup> CFU/g) than the EE3 strain (10<sup>5</sup>~10<sup>8</sup> CFU/g). On the other hand, the well-known probiotic strain *Lactobacillus fermentum* GG survived in the healthy dogs only 8d after cessation of its administration. From the clinical point of view, persistence during administration should be less important than colonization. However, the potential probiotic microorganism should be able to colonize the digestive tract at least temporarily. Decrease of total enterococci can be explained probably by competitive interactions of the EE3 strain with other enterococci. Although enterococci normally produced lactic acid, its higher amounts (as the other enterococci) could be achieved by the concentration of inoculated EE3 strain; in addition, differences in lactic acid production were found among enterococci isolated from different sources. Local effects of the EE3 strain application included an increase of LAB and a significant decrease of Pseudomonas-like bacteria and staphylococci with prolonged effect up to the end of the experiment It is reported that a

significant increase in fecal LAB during administration of food supplemented with probiotic strain *L. acidophilus* DSM 13241. However, the action of the probiotic, in terms of its effect towards the number of lactobacilli, was lost after cessation of food supplemented with the same probiotic strain (*L. acidophilus* DSM 13241.). *E. faecium* EE3 strain survived the transit through the digestive tract of dogs. Our results suggested that bacteria and staphylococci. Moreover, the levels of total lipids, proteins and cholesterol in blood of healthy dogs were also influenced to reach physiological limits.

### **3. 5 Application of *Enterococcus faecium* strain M-74**

The value of managing atherosclerosis risk factors has been confirmed in the past few decades. Hypercholesterolemia, hypertension and smoking are still considered to be the “ classical” risk factors. Intervention trials have generally shown that lowering “ risk factors” reduces the subsequent rate of coronary heart disease, stroke, and other cardiovascular diseases. The Framingham Study was the major study, which verified that cholesterol increases the risk of developing heart disease. Data output was clear: the risk of clinically apparent coronary heart disease is a continuous nonlinear function of blood cholesterol levels. Each increase in the serum cholesterol concentration by 1% results in 2~3% increase in the risk of CHD. The discovery that hypercholesterolemia plays a major role in the formation and development of atherosclerosis has led to a number of pharmacological and non-pharmacological (including dietary) approaches resulted in its elimination. Fact that the serum cholesterol concentration is influenced by food has been known for a long time ago. Later, the first evidence of affecting cholesterol level by influencing intestinal microflora was found. One

way to do this is by using probiotics. Up to now, the question of supposed hypocholesterolemic effect of probiotics has not been definitely established. While some studies documented it well, others bring contradictory findings or even doubt it. Another unresolved issue is an existing previously mentioned effect from the long term point of view. In available sources, we have not found any report where probiotics were administered for one year. Some experimental and clinical studies have documented the hypocholesterolemic effect of probiotic cultures. Not all of the probiotics seem to have the same properties and same effect on serum cholesterol levels. The aim of our study was to investigate the impact of long-term orally administered probiotic strain *Enterococcus faecium* M-74 enriched with selenium on lipid profile (total cholesterol, LDL, HDL and triglycerides) in humans.

Originally, the term “ probiotics” was used for a phenomenon, when each of both together cultivated organisms had produced substances stimulating the growth of the another one. The meaning of the term “ probiotic” has been evolving since. Many definitions have been suggested. The most commonly used, scientifically valid and therefore acceptable one is that postulated by Fuller. According to him, probiotics are living microorganisms that favourably influence the health of the host by improving composition of its intestinal microflora. The FAO (Food and Agriculture Organisation for the United Nations) and WHO (World Health Organisation) refer to probiotics as living microorganisms which when ingested in sufficient amount, beneficially influence the health of the host. Before a certain bacterial strain can be classified probiotic it must meet some criteria. The most important one is

its beneficial health effect. The knowledge of probiotic intervention in lipid metabolism is not new. In the 1970s, researchers observed hypocholesterolemic effect of yoghurt eaten daily in large amount in humans. Similar observations have been seen in animal studies. Many trials aimed to proving the hypocholesterolemic effect of milk and fermented milk products come from early observation. It is found that a low incidence of clinical coronary heart disease in some African tribes with low cholesterol levels in spite of their diet rich in saturated fats, meat and cholesterol but abundant in milk and fermented milk products along. In some studies it is observed that a gradual decrease of serum cholesterol concentration in subjects receiving capsules with *Enterococcus faecium* M-74 from baseline up to week 23 when the decrease exceeded significant level ( $P < 0.001$ ). This reduction remained important also one month after the follow-up, i. e. 4 weeks after cessation of capsules application ( $-0.81 \text{ mmol/l}$ , i. e. by 13.6%). The nonpharmacological approaches to the management of coronary artery disease risk factors still have not been exhausted, even in the modern pharmacological and interventional treatment era. In our study, the administration of the *E. faecium* M-74 probiotic strain was associated with the reduction of serum cholesterol concentration by 12% after 56 weeks. This change was mainly due to the decrease in LDL cholesterol, as no significant alterations in HDL and triglycerides were noted. With crescent amount of facts it is reasonable to hypothesize that probiotics will find a place as a therapeutic alternative. Modification of the interstine microflora (namely by probiotics) may represents a new challenge in the treatment of various disease, i. e. cardiovascular disorders.