Growth of *Lactobacillus acidophilus* and *Escherichia coli* when both were cultivated together in lactose-peptone medium under aerobic or anaerobic condition.

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Introduction

Lactobacillus acidophilus usually performs various beneficial actions both in several parts of the human body and in making of certain fermented milk products.

In the human gut, *L. acidophilus* is very important in certain physiological and pathophysiological processes and helps to prevent colonization by pathogens such as enteropathogenic *Escherichia coli*[1—5]. In dairy and other products, fermentation with *L. acidophilus* also plays an important role in control or growth of *E. coli* responsible for spoilage or foodborn illness[6,7].

Although there have been reported a lot of papers about such beneficial antibacterial actions of *L. acidophilus*, relatively little is known about ecological relationship between *L. acidophilus* and *E. coli*.

In the present study, the authors examined growth of *L. acidophilus* and *E. coli* when both were cultivated together under aerobic or anaerobic condition, thereby to learn the relationship of initial population of both strains with their final population.

Material and Methods

Strain used: *L. acidophilus* IFO 3205 and *E. coli* K-12 were selected in this experiment for the same purpose described previously[7]. These strains were incubated for 20 to 24 hrs at 37°C in LPY-medium consisting of lactose 1%, peptone 0.7%, yeast extract 0.5%, Na_2HPO_4 0.6% and NaH_2PO_4 0.4% and transferred at least twice before use as inocula.

Inoculation of *E. coli* and *L. acidophilus* in LPY-medium: Ten milliliters of LPY medium was dispensed in a test tube. After sterilization, 0.5 ml of cell suspension

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of *L. acidophilus* IFO 3205 and 0.5 ml of cell suspension of *E. coli* K-12 were inoculated in LPY-medium.

The cell suspension of each strain was prepared from 24 hrs' culture by dilusion with sterilized saline to give 10^7 to 10^8 cells/ml.

Cultivation of LPY-medium: LPY-medium to which *E. coli* K-12 and *L. acidophilus* IFO 3205 were inoculated was incubated at 37°C for 24 hrs.

Incubation under anaerobic condition was accomplished by the Gas Pack System manufactured by Baltimore Biological Laboratories containing the test tube described above. The oxygen present in the air within the jar was replaced by CO_2 and H_2O by the use of a cold catalyst, and a disposable foil envelop in which sodium boronhydride in one section and a mixture of citric acid and sodium dicarbonate in a second section are contained. Incubation of the test tube was carried out at 37°C for 24 hrs.

After incubation, requisite dilutions were prepared from the cultures cultivated under both aerobic and anaerobic conditions. Parts of these dilutions were plated into LPY-agar medium for the estimation of total cell counts of *E. coli* and *L. acidophilus*. Other parts of the dilutions were plated into selective medium for the enumeration of *E. coli*.

Enumeration of *E. coli*: *E. coli K*-12 was enumerated by use of deoxycholate agar (Nissan). All plates were incubated at 37°C for 24 hrs under aerobic or anaerobic condition. All experiments were carried out in duplicate.

Results and Discussion

Table 1 shows cell count of *E. coli* K-12 and *L. acidophilus* IFO 3205 before and

Table 1. Cell counts of *E. coli* K-12 and *L. acidophilus* IFO 3205 when both were cultivated together under aerobic condition.

Before incubation (cell counts/ml)		After incubation (cell counts/ml)	
E. coli K-12	*L. acidophilus* IFO 3205	*E. coli* K-12	*L. acidophilus* IFO 3205
18×10^6	99×10^7	60×10^4	98×10^8
54×10^6	77×10^7	22×10^4	10×10^9
90×10^6	55×10^7	10×10^7	13×10^9
13×10^7	33×10^7	73×10^6	14×10^9
25×10^7	22×10^7	28×10^7	86×10^8
28×10^7	11×10^7	25×10^7	74×10^8
29×10^7	55×10^6	10×10^8	90×10^8
31×10^7	11×10^6	13×10^8	10×10^9

(Incubation : 37°C, 24 hrs.)

after incubation when both were cultivated together at 35°C for 24 hrs under aerobic condition. It can be readily seen from Table 1 that *L. acidophilus* extremely exceeds *E. coli* in their cell counts after 24 hrs' incubation when incubation sizes of both strains were almost the same order ($X10^7$/ml).

Superior growth of *L. acidophilus* to *E. coli* is observed to be more significant when both strains were incubated under anaerobic condition. As shown in Table 2,

Table 2. Cell counts of *E. coli* K-12 and *L. acidophilus* IFO 3205 when both were cultivated together under anaerobic condition.

Before incubation (cell counts/ml)		After incubation (cell counts/ml)	
E. coli K-12	*L. acidophilus* IFO 3205	*E. coli* K-12	*L. acidophilus* IFO 3205
22×10^6	99×10^7	3×10^1	80×10^8
66×10^6	77×10^7	16×10^1	84×10^8
15×10^7	33×10^7	50×10^2	92×10^8
14×10^7	26×10^7	71×10^5	80×10^8
61×10^7	13×10^7	66×10^5	48×10^8
64×10^7	65×10^6	33×10^5	55×10^8
67×10^7	13×10^6	85×10^5	29×10^8

(Incubation : 37°C, 24 hrs.)

cell counts of *E. coli* are drastically reduced after incubation for 24 hrs, in all combination of inoculation sizes when inoculation sizes of both *E. coli* and *L. acidophilus* were almost the same order ($X10^7$/ml). Furthermore, it can be noted from Fig. 1 that *L. acidophilus* is able to grow against *E. coli* under anaerobic condition,

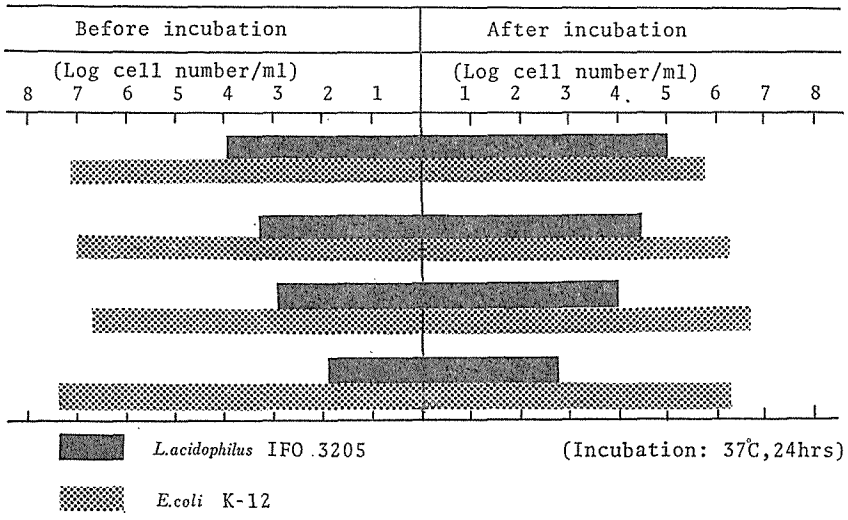

Fig. 1 Cell counts of *L. acidophilus* IFO 3205 and *E. coli* K-12 before and after incubation when both were cultivated together under anaerobic condition.

even when initial inoculation sizes of both *L. acidophilus* and *E. coli* were 10^2/ml and 10^8/ml, respectively. These results obtained indicate that *L. acidophilus* clearly depress growth of *E. coli* and that depression against *E. coli* was observed to be more drastic under anaerobic condition.

The significance of this phenomenon especially has interesting bearing on the intimate relationship of *L. a*cidophilus with the human gut. It is well known that Lactobacilli which are common in the human intestinal flora include the following; *L. acidophilus*, *Bifidobacterium bifidum* (formerly *L. bifidum*), *L. leichmannii*, *L. casei var alactosus* and *L. plantarum*[8]. Of these species, only the *first* two are present in sufficient number to be of importance in the intestinal tract and play an important role in a drastic reduction of putrefactive bacteria including *E. coli*. In this respect, the findings obtained in our present experiment strongly support such significant role of *L. acidophilus* in the intestinal tract, because the condition of intestinal that is always kept anaerobic.

FRANK et al[9]. have recently studied on behaviour of enteropathogenic and non-pathogenic strains of *E. coli* when they were grown in skimmilk with and without 0.25 or 2.0% added lactic starter bacteria. They observed that with either concentration of the starter all *E. coli* strains were completely or partially inhibited after 6 to 9 hrs of incubation at 32°C or 21°C, and concluded that combination of incubator temperature and initial concentration of lactic acid bacteria was most effective in controlling growth of *E. coli* in fermented skimmilk.

In our present experiments, when the cultures were treated exactly alike with the exception of incubation temperature, which was lowered to 30°C, trends similar to those observed at 37°C were obtained in all cases of aerobic cultivations (data not presented here). From this fact, we concluded that, contrary to the findings of FRANK et al.[10], temperature of incubation had little or no effect on the associative growth patterns in mixed strains of *E. coli* and *L. acidophilus*.

There can be considered several reasons for the fact of antibacterial action of *L. acidophilus* against *E. coli*. Acids and anti-metabolites produced by *L. acidophilus* undoubtedly constitute most important reasons for the inhibition of growth of *E. coli*.

Especially, production of anti-metabolites is one of the most interesting and peculiar properties of *L. acidophilus*, and several kinds of anti-metabolites have been isolated from *L. acidophilus* strains. VINCENT et al[11]. obtained from several strains of *L. acidophilus* an antibiotic substance, lactocidin. HAMDAN et al[12]. isolated another antibiotic substance, acidoline, from skimmilk inoculated with *L. acidophilus* (Chr. Hansens Lab. strain 2181). Recently, the authors have also reported growth inhibitory actions of the cell free extracts of several strains of lactic acid bacteria which are widely distributed in dairy products and in the human intestinal tract against *E. coli* K-12 and *Bacillus* species[13,14]. Among the strains examined, the cell

free extract of *L. acidophilus* IFO 3205, which is used in the present experiment showed relatively strong growth inhibitory action against *E. coli* K–12. Being based on this fact, the authors extracted the antibiotic substance which exhibits antimicrobial activity against *E. coli* K–12 with methanol and further concentrated and purified by Sephadex G–25. With respect to UV and IR spectra and amino acid analysis, the antibiotic substance was considered to be a peptide which consists of 11 kinds of amino acids and was suggested that its molecular weight was approximately 3,500 [8].

Although it is difficult to explain all over ecological interaction between *L. acidophilus* and *E. coli* in the human intestinal tract, the findings obtained in the the present experiments justify the importance of *L. acidophilus* in the human intestinal tract, and support a belief of long standing that *L. acidophilus* may prevent or ameliorate coliform associate diarrhoeas.

Summary

The purpose of this study was to help clarify the interaction between *Lactobacillus acidophilus* and *Escherichia coli* when both were grown together.

L. acidophilus IFO 3205 and *E. coli* K–12 were selected and incubated together for 24 hrs at 37°C in LPY–medium under both aerobic and anaerobic conditions. *L. acidophilus* extremely exceeded *E. coli* in their cell counts after 24 hrs' incubation under aerobic condition in all combination of inoculation sizes when inoculation sizes of both strains were almost the some order of 10^7 cell counts per milliliter. Superior growth of *L. acidophilus* to *E. coli* was observed to be more significant when both were incubated under anaerobic condition.

The significance of this phenomenone have been discussed with special respects to the behaviour of *L. acidophilus* in the human intestinal tract.

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乳糖ペプトン培地における *Lactobacillus acidophilus* と *Escherichia coli* の好気的ならびに嫌気的混合培養と両菌株の増殖

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要　約

Lactobacillus acidophilus の *Escherichia coli* の増殖に対する抗菌性について筆者らは主として，*L. acidophilus* の菌体内抽出液を用いて検討してきた。本報では *L. acidophilus* と *E. coli* を混合培養し，培養前後の菌数の変化から *L. acidophilus* の *E. coli* に対する増殖抑制作用を調べ，*L. acidophilus* の示す抗菌現象，特にヒト腸管内での *L. acidophilus* の優位性確立の解析に資した。

L. acidophilus IFO 3205 と *E. coli K* 12 をそれぞれ異なった菌数で乳糖ペプトン培地に接種し，37°C，24時間，好気的ならびに嫌気的条件下で培養した。培養後，選択培地により，両菌株の菌数を測定した。

両菌株をほぼ同一のオーダー（10^7/ml）で接種，培養した場合，*E. coli* に比べ，*L. acidophilus* が優性に増殖し，特に，嫌気的培養下ではその傾向が著しく，24時間の培養によって，*E. coli* の菌数が10^7/ml から10^5/ml のオーダーに激減することを認めた。更に，嫌気的条件下では *L. acidophilus* と *E. coli* の接種菌数がそれぞれ10^2/ml，10^8/ml と *E. coli* の菌数が *L. acidophilus* のそれをはるかに凌駕していても *L. acidophilus* は *E. coli* に抗して増殖し得ることを認めた。