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Anti-cancer effect, Radioprotection effect and Anti-allergy for Enterococcus Faecalis (EF 2001)
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Key word: Enterococcus / Fekaris 2001 (BeRMKAIN), Math/A fibrosarcome, analgesic, anti-neoplasm effects

ABSTRACT
The preparation reduced to powder, which removed fungi mainly from Enterococcus / Fekaris 2001 (BeRMKAIN), which was Enterococcus / Fekaris 2001, was used in this study. Because L/P activity was recognized from Enterococcus / Fekaris (BeRMKAIN), it was examined about the anti-neoplasm effects that it faces Math/A fibrosarcome in the mice, and the analgesic action by the preceding research. They are inoculated 1 x 10^6 cells and 2 x 10^3 directly in the right and the left low abdomen region the intratumorals as for the anti-neoplasm simulation by the way of the administration meth A fibrosarcome to each of the BALB/c mouse. The dimension of the tumor enhancement was measured more than the implantation posticus the 3rd day from a total 10 times, the top of the cutaneous even in the 2nd de dic in dicn the every other day, and the total was examined, and gradation was seen at the time as the sutra. It was dissolved with the fraction of 1mg/0.1ml/mouse in the isotonic sodium chloride solution, and specimen substantial administered neoplasm vicinal rear 3, 4, the 5th-day total 3 times for the right intratumorale. And, pain relief simulation was done with the acetic acid lasing method. The L/P activity had statistical significant difference with the 200ug/mouse dose rate (p<0.05). Catastaltic determination was judged by the antitumor action the tumor enhancement in both low abdominal regions. Then, catalatlic determination was recognized with 1000mg/kg by the analgesic action to the control group.

INTRODUCTION
Enterococcus / Fekaris 2001 likes a rich place in food and the nutrient such as an intraintestinal, and lives. Therefore, it is the typical Bacterium group, which coexists with the relations, which are close to our dietary life and the life circle kept. Anciently, humans have used Enterococcus / Fekaris 2001 skillfully. Bean paste, a traditionally fermented food like the pickles and yogurt, a fermented milk preparation like a cheese are the typical examples. Enterococcus / Fekaris 2001 as a food is taken together with
the fermented food. Recently, it was together wide with the verification of the functionality, and it was used for the food processing as well, and relations with the well being of the human were argued large. It has the medicament reason effects of the Enterococcus / Fekaris 2001 reported the antitumor action, the cancerous prophylactics function, the immunization activity function, the interferon enhancement, the blood pressure descent function, the mutagenicity material ablation function, and so on (1,2,3,4). Lactic acid Coccaceae (Enterococcus facialis 2001 stock; The following EF2001 abbreviation) was cultivated, and Enterococcus / Fekaris 2001 preparation mixed the EF2001, which contains Enterococcus / Fekaris 2001 production material, and constituents. After that, the grain movement panicles granulator vessel manufactured it, and it got preparation Enterococcus / Fekaris 2001(BeRMKAIN) (5,6). Freeze drying made it do the water-soluble component of the Enterococcus / Fekaris 2001 preparation Enterococcus / Fekaris 2001(BeRMKAIN), and got the fraction which contained water bacteriolysis constituent in this study. This reduced to powder was used as material, and antitumor action against the leucocyte (lymphocyte) increase function of the mice; the Math-A fibrosarcoma cancerous of the mice was examined. And, it was examined about the analgesic action by the way of the acetic acid Writhing of the mice.

MATERIAL AND METHODS
Enterococcus / Fekaris 2001(BeRMKAIN) 1000 (Enterococcus / Fekaris Bacterium 2001 stock) was used in this study. After aqua distillate 200 ml was added to Enterococcus / Fekaris 2001(BeRMKAIN) 20g and stirred, it was put for 2 hours by the room temperature as a preparation of the water extracts Enterococcus / Fekaris 2001(BeRMKAIN). After that, 30min centrifugal separation was done with 1000rpm, and freeze-drying did clear supernatant liquid. Water extracts Enterococcus / Fekaris 2001(BeRMKAIN) was made the material of the physiology activity of investigation. We used the new live birth of the brood of the Swiss-Webster mouse within from 12 to 24 hours old for the simulation. The simulation procedure of the leucocyte (lymphocyte) increase function went by using the procedure that Hand's made Metcalf's procedure strange (7,8). Isotonic sodium chloride solution was administered in the control group, and intraperitoneal administration did water extracts Enterococcus / Fekaris 2001(BeRMKAIN) (200ug/mouse) in the specimen group. Blood was drawn from the caudal vein before the administration in the administration posticus 6 days, 10 days, the 14th day, and determination made gracilis panicles blood film. It dyed in the Wright staining technique, and lymphocyte and leucocyte were counted by using the microscope (magnification 10x40 times) with mechanical stage. The ratio (L/P ratio to the leukocyte) of the number of the lymphocyte was calculated. The decision of the effect went by the t-test. The BALB/c maleness mouse was used age of six weeks as an animal used for experiment of the antitumor action. Simulation was done in accordance with Ebinas' "double-grafted tumor system" (9). 1x 10^6 and 2x 10^7 Math-A fibrosarcoma cellularis were transplanted to each of the right of the normalize mice and the left abdomen region. The fifth-day total administered isotonic sodium chloride solution for three days and for four days three times in the control group the neoplastic cell implantation posticos Water extracts Enterococcus / Fekaris 2001(BeRMKAIN) (1 mg/0.1ml/mouse) was administered directly in the specimen group for the intratumorale of the right low abdomen region. As for the determination, the head sutra
RESULTS
Fructification against the increase function of the leucocyte (lymphocyte) ratio is shown to Table1. The L/P ratio of the administration group became the administration post the 6th day in 0.65 +/- 0.112, the 10th day in 0.94 +/- 2.051 and the 14th day in 3.69 +/- 0.086. It had 1% and the statistical significant difference 5% by the critical rate for 6 days in the 10th day in the 14th day to the control. The fructification of the antitumor action simulation against the cancerous of Meth/A-fibrosarcome is shown to Table2, Table3, and Table4. As for the anatomic fructification, which went after the neoplasm implantation in the 25th day, the neoplasm ponderis of the right hypogastric region was by the control group in 1.468 +/- 0.521. Well the neoplasm elimination of 1 example was seen to this specimen administration group in 0.640 +/- 0.211. But, catalastic wasn't recognized as the neoplasm ponderis of the left abdomen region. Next, the neoplasm of the right abdomen region became with the specimen administration group by the target gradation from the 3rd day at the time as the suture of the 25th-day tumor enhancement in the 2nd day in 137.3 +/- 29.2 mm², the 23rd day in 131.3 +/- 25.3 mm², the 25th day in 127.9 +/- 33.0 mm². Therefore, obviously it had statistical significant difference to the control group. But, some catalastic wasn't recognized as the neoplasm of the left abdomen region between the specimen administration group and the control group. Analgesic action is shown in the following. The number of Writhing of the control group was 33.0 +/- 4.0. It was 8.0 +/- 3.8 by the number of Writhing of the specimen administration group to this. It was 15.9 +/- 4.6 by the number of Writhing of the Aspirin administration group. Therefore, an Aspirin administration group recognized some catalastic 1% of the critical rate with the administration group 5% critical rate.

CONCLUSION
The L/P ratio of the administration group was compared with the control group after the administration by the 14th day, and statistical significant difference was clear as a result
of the increase function to the leucocyte (lymphocyte) (P<0.01).
Lactate is released in large quantity from sites of sepsis and inflammation (11).
Haji-Michals asked whether the increased lactate production found in sepsis could be
explained by the augmented glycolysis of inflammatory cells. The glycolytic
metabolism of rat peritoneal leucocytes was measured following cecal ligation and
perforation (CLP) or sham laparotomy. CLP augmented glucose uptake, the pentose
phosphate pathway, and glucose oxidation. Lactate output increased from 1.03 +/- 0.05
to 1.20 +/- 0.05 mmol x cell (-1) x min (-1) (P < .001). Total lactate output of peritoneal
lavage fluid increased from 7.94 +/- 2.59 to 28.12 +/- 5.60 nmol L x min (-1) (P < .005).
The effect of lipopolysaccharide (LPS) on the lactate output of whole blood from 31
critically ill patients was measured. Leukocyte lactate production was calculated by
multiple linear regression analysis.
This rate of production is so high that it suggests that their different cell populations and
state of activity may affect the lactate output of different tissue beds in sepsis.
This study supports the hypothesis that lactate may be more a product of inflammation
than a marker of tissue hypoxia in sepsis (12).
In Simoes study, HGThe equilibrium point between blood lactate production and
removal (La- (min)) and the individual anaerobic threshold (IAT) protocols have been
used to evaluate exercise. Then, capillary blood (25 micro litter) was collected for [La-]
b and [Glc]b measurements.
The IAT and IGT were determined by [La-]b and [Glc]b kinetics during the second test.
The La-(min) and Glc(min) were determined considering the lowest [La-] and [Glc]b
during the third test. No differences were observed (P < 0.05) and high correlations
were obtained between the velocities at IAT. Therefore, Simoes concluded that for
these subjects it was possible to evaluate the aerobic capacity by IGT and Glc (min) as
well as by IAT and La- (min) (13).
As a result of the antitumor action against the cancerous of Meth/A-fibrosarcoma, The
neoplasm ponderis of the right abdomen region was compared with the control group
after the neoplasm implantation in the 25th day, and the neoplasm elimination example
of one example was seen with the specimen administration group. But, statistical
catastaltic wasn't recognized as the neoplasm ponderis of the left abdomen region. The
gradation was compared with the control group in the neoplasm of the right abdomen
region from the 3rd day at the time as the sutra of the 25th-day tumor enhancement, and
statistical significant difference was clear next with the administration group from the
2nd de die in diem (P<0.05).
In Battelinos reports, tumor necrosis factor-alpha (TNF-alpha), an important mediator
of endotoxic shock, induces hypoglycemia and shock in adult animals. Therefore, indomethacin ameliorates TNF-alpha-induced hypoglycemia in the adult. However, effects of TNF-alpha on glucose metabolism in the newborn have not been well
documented (14).
The present study showed that in 10-day-old rats injected with TNF-alpha (4.5 x 10(7) U/kg, intraperitoneal) the plasma glucose concentration increased from 4.1 +/- 0.3
mmol/L to 6.9 +/- 0.5 mmol/L (P < .05) at 2 hours and subsequently decreased to 1.4
+/- 0.5 mmol/L (P < .05) at 6 hours, although plasma lactate concentration increased
from 1.1 +/- 0.1 mmol/L to 5.5 +/- 0.3 mmol/L (P < .05) at 6 hours. Plasma insulin
concentration remained unchanged throughout the experiment. Glucose uptake
increased in association with the increase of GLUT1 mRNA abundance. TNF-alpha
decreased mRNA abundance of GLUT 2 and phosphoenolpyruvate carboxykinase (PEPCK) in liver, suggesting decreased gluconeogenesis. Battelinos concluded that TNF-alpha induced hypoglycemia, increasing GLUT1 mRNA abundance and glucose uptake and decreasing PEPCK mRNA abundance in 10-day-old rats (15). Then, Battelinos concluded that TNF-alpha induced hypoglycemia, increasing GLUT1 mRNA abundance and glucose uptake and decreasing PEPCK mRNA abundance in 10-day-old rats (15).

In Arkalis study, the survival time of the tumor-bearing mice was prolonged in the CDDP-MC group, but not in the group treated with cisplatin solution. By using this modified formulation of cisplatin, the toxicity of the drug can be reduced, and effective concentrations of the drug can be maintained locally for prolonged periods of time (16). Tokudas recognized the innidation catalytic of peritoneal metastasis by using poly-D, L-lactic acid and polyethylene glycol acid. Therefore, the survival of rats with peritoneal metastasis was better in the CDDP-PPMS group than in the CDDP-SOL group (17).

But, statistical catalytic wasn’t recognized as the neoplasm of the left abdomen region between the specimen administration group and the control group in this research. It thinks with the thing that water extracts Enterococcus / Fekaris 2001(BeRMKAIN) functioned directly in the neoplasm of the right abdomen region from this fructification for the hyperplastic catalytic of the neoplastic cell by some function institution. And, there was no difference to the neoplasm of the left abdomen region. But, because catalytic determination was judged, Enterococcus / Fekaris 2001(BeRMKAIN) of the water exhaustion is thought T- cellularis macrophage potentiating involve. And, recipient interstitial is thought about because it is the fructification which immunization activity was (18).

Cell survival, cellular damage, and antagonistic activity were investigated after spray drying of four bacteriocin-producing strains of lactic acid bacteria in Mauriellos study. Trials were performed with bacteria suspended in skimmed milk or directly grown in whey.

Three air temperatures at the inlet of the drier (160, 180, and 200 degrees C) and three flow rates (10, 13, and 17 ml/min) were assayed. During storage for 2 months at 4 degrees C, all samples, but mainly the lactococcal strains, displayed a gradual decrease in cell survival.

Bacteriocin activity remained at the same level, allowing powders to be considered as effective biopreservatives (19).

Statistical significant difference was clear with the Enterococcus / Fekaris 2001(BeRMKAIN) administration group of the water exhaustion in comparison with the control group as a result of the analgesic action simulation (P<0.01). Aspirin is thought acid non-steroid anti-inflammatory and analgesic action synthesis inhibition Prostaglandin show. Enterococcus / Fekaris 2001(BeRMKAIN) of the water exhaustion may be probably based on the obliteration of the production of the algesic substance, disengagement, catalytic or the path of pain and others.

REFERENCES
bacteriocin-producing lactic acid bacteria.
Anti-allergy, Anti-cancer and Radioprotection Effect for Enterococcus Faecalis (EF 2001)

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Material & Methods

- Anti-cancer effects
  - Seven-weeks-old male ICR (Crj) mice
- Cancer cells: sarcoma 180 (2 x 10⁶)
- EF 2001 5mg/kg of heat-killed EF2001 (EF2001) were injected interpretational (endoceliac) each for 2 weeks every other day
- Statistical methods: t-test
Enterococcus Faecalis under microscope (left) × 20, (right) × 12900
1. Radiation protection effects

Seven-weeks-old male C3H mice
12mg/Kg 24mg/Kg of heat-killed EF2001 (EF2001) were injected interpretational each for 2 weeks every other day

8Gy of whole body irradiation (Philips co. 200kV)

Change of body weight Survival after irradiation

Sections of the large and small intestines with a microscope
2. Assay of NK cell activity by $^{51}\text{Cr}$ label YAC-1 cells

Injection of the EF2001, in the same condition using the examination of radiation protection effect.

The mice was scarified and the spleen was extracted.

The spleen was smashed with stainless steel mesh, and then mixed PBS and the suspect ion was centrifuged three times.

The concentration of the spleen cells was adjusted to be $2 \times 10^7$ cells/ml. Then, $1.25 \times 10^6$, $2.5 \times 10^6$, $5.0 \times 10^6$, $10.0 \times 10^6$, $20.0 \times 10^6$ of the spleen cells were added to $1 \times 10^4$ of YAC-1 cell which labeled $^{51}\text{Cr}$ of 1mCi and incubated 96 hole plate for 6 hours.

Only liquid component in each hole measured with liquid scintillation.
Change of body weight

The body weight loss was inhibited after injection of EF2001.
Survival after irradiation
Surviving fraction was increased after injection of EF2001.
Large intestines with a microscope

normal

control

12mg/kg

24mg/kg
Small intestines with a microscope

normal

control

12mg/kg

24mg/kg
NK cells activity
Activities of NK cells are enhanced 1.46 and 1.94 times in EF200112mg and EF200124mg groups respectively.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/Kg po)</th>
<th>n</th>
<th>No. of Writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Water)</td>
<td>0</td>
<td>6</td>
<td></td>
<td>( - )</td>
</tr>
<tr>
<td>BRM</td>
<td>1000</td>
<td>5</td>
<td></td>
<td>(75.3)</td>
</tr>
<tr>
<td>Aspirin aluminum</td>
<td>500</td>
<td>6</td>
<td></td>
<td>(51.9)</td>
</tr>
</tbody>
</table>

**Effects of BeRMkain (= BRM) and Aspirin aluminum on the Acetic acid Induced Writhing in Mice**

a) : $P < 0.05$ VS. Control
b) : $P < 0.01$ VS. Control
Table 1

Time Course of Increase of Lymphocyte/Polymorph Ratio in mice

a) : Pr<0.05
b) : Pr<0.01
Anti-tumor effect of BRM on Meth A fibrosarcoma (solid type) in BALB/c mice

* p<0.05 vs Control
Anti-tumor effect of BRM on Meth A fibrosarcoma (solid type) in BALB/c mice
Anti-tumor effect of EF-2001 on Meth A fibrosarcoma (solid type) in BALB/c mice

**P<0.01  *P<0.05 vs Control group
Anti-tumor effect of EF-2001 on S-180 in ICR mice

**P<0.01  *P<0.05 vs Control group**
BLB/C mice of IgE in the blood. Each histogram represents the mean value ±SE for 10 mice IgE (M). Significantly different *p<0.05 Control vs. EF 2001.
Fig. 2. C3H mice of IgM in the blood. Each histogram represents the mean value ±SE for 10 mice IgM (M) Significantly different *p<0.05 Control vs. EF 2001.
Fig. 3. C3H mice of IgG in the blood. Each histogram represents the mean value ±SE for 10 mice IgG (M). Significantly different *p<0.05 Control vs. EF 2001.
Conclusion 1

- Anti-cancer effects: EF 2001 administration group: positive
- EF 2001 to the radiation protection effect: precision
- Immune activity effect: EF 2001 administration group: positive
- Anti-aging effect: EF 2001 administration group: positive
- Long life effect: EF 2001 administration group: positive
- It put the *Enterococcus Faecalis* dosage, and the level of total IgE in serum glutamic-oxaloacetic fell.
- The level of total IgM in serum glutamic-oxaloacetic increased the *Enterococcus Faecalis* dosage group. However, the level of total IgG in serum glutamic-oxaloacetic rather fell slightly.
Conclusion 2

1. It let cell-mediated immunity such as a macrophage and natural killer T cell activate, and the immunization activation action that Enterococcus Faecalis has does not become it, and promotion of humeral immunity anti-action is thought about.

2. In the dosage of Enterococcus Faecalis, an IL-2 level in blood rises, and it think that cytokine of the spleen changed from Th2 type into Th1 type. Therefore, it decrease IgG and an IgE level, and it is suggested that it showed an allergic restraint effect.