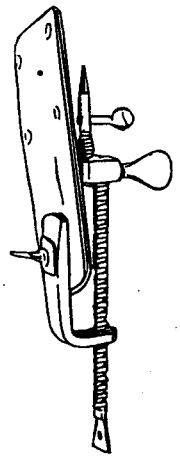
INTERACTIONS IN THE MICROBIAL WORLD

26-31 August 2001, Amsterdam, The Netherlands

9th International Symposium on Microbial Ecology

ISME-9



AMSTERDAM 2001

Amsterdam International Exhibition and Congress Centre

FINAL PROGRAMME

and abstracts

Submitted Abstract Data

Please note that after submitting your abstract data, by clicking the "submit" button below, you will not be able to make any changes.

abstract nr.: 416

Significance of dead bacteria in gastro-intestinal tracts: biological activities of heat-killed *Enterococcus faecalis* EF-2001 cells

Author: Yanagisawa, T. , Nihon Berum Co. Ltd., Tokyo, Japan
Co-author(s): Shimohashi, H. , Institute of BioQueen Co. Ltd., Tokyo, Japan
Iwasa, T. , BRM Institute of Nihon Berum, Tokyo,
Topic: Microbial ecology of the gastro-intestinal tract
Keywords: dead bacteria, *Enterococcus faecalis*, heat-killed bacteria, intestinal bacteria

Many dead bacteria are assumed to exist in human gastro-intestinal tracts in addition to more live ones, because bactericides such as gastric acid, lysozyme, bile acid, pancreatic fluid and so on are secreted there, and because numbers of culturable bacteria in human feces are only about 70% of the ones counted microscopically on the smears. Then, heat-treated whole cells of *E. feacalis* EF-2001 (EFH-2), isolated from human feces, were administered orally to mice for weeks to investigate the significance of dead bacteria or the components to the host.
The fecal flora of mice deleted by ampicillin and cefalexin were prompted to recover fast by EFH-2 compared to the control without it, especially with lactobacilli and bifidobacteria reappearing fast or at higher levels. Mice fed 10% EFH-2 mixed diet for 1 month then received an oral challenge of an *E. coli* strain resistant to streptomycin(SM). The EFH-2 possessed a promoting effect on elimination of the SM-resistant strain from feces. In addition, when mice immunocompromised with an injection of cyclophosphamide (5mg/mouse) were infected with an intravenous challenge of *E. coli* NB-101, the viable numbers of the *E. coli* in the livers at 48 and 72 hours after the infection decreased significantly in mice previously fed 10% EFH-2 for 2 weeks. These effects of EFH-2 via the intestines will be discussed.

Presentation Preference: Poster Presentation

Submit | Cancel

[P. 18. 016]

Significance of dead bacteria in gastro-intestinal tracts: biological activity of heat-killed *Enterococcus faecalis* EF-2001

Yanagisawa T[1]., Shimohashi H.[2], and Iwasa T[1]
([1]BRM Institute of Nihon Berum Ltd., Co., and [2]Instutue of BioQueen Ltd., Co.,
2-29-8 Higashikoigakubo, Kokubunji, Tokyo 185-0014, Japan)

Many dead bacteria have been assumed to exist together with more live microorganisms in the intestinal tracts of humans and animals. Then, heat-treated *E. faecalis* EF-2001 cells (EFH-2) were administered orally to mice for weeks, in order to investigate the significance of dead bacteria or the components to the host.

The faecal flora of mice was almost completely excluded by ingestion of cephalexin and ampiciline, in drinking water for 3 days, and effects of EFH-2 on normalization of the disturbed flora were observed by examining faeces of the mice which were orally administered EFH-2 (1.5mg/day/mouse) for 13 days thereafter. Lactobacilli reappeared earlier and in higher counts, bifidobacteria in higher counts, and *Enterobacteriaceae* inversely in lower counts in EFH-2-given mice than those in the control without it. Enterococci(streptococci) and *Bacteroidaceae* recovered similarly in both groups (Fig. 1). Thus, oral administration of EFH-2 was effective to improve the disturbance of intestinal flora.

Mice were fed 10 or 5% EFH-2-mixed diet for 1 month, and then received an oral challenge of *E. coli* strain resistant to streptomycin(SM-r *E. coli*). Thereafter, numbers of SM-r *E. coli* in faeces of mice were examined. EFH-2 diet was further given to the mice throughout the experimental period. The EFH-2 revealed an enhancing effect on elimination of the SM-r *E. coli* from faeces (Table 1).

To understand this phenomenon, we also had to consider an activation of the imumune system by EFH-2. Mice were fed 10% EFH-2-mixed diet for 14 days, and then injected intraperitoneally with cyclophosphamide(CY:5mg/mouse). The immunocompromized mice were then infected with an intravenous challenge of *E. coli* NB-101(1.0×10^7 cfu), and viable numbers of the *E. coli* were examined in the livers and spleens of the mice. The numbers of *E. coli* decreased significantly in the livers of EFH-2 mice at 48 and 72 hrs after the *E. coli* infection, compared with those in control ones (Table 2),

but not changed in the spleens between both the groups. Oral administration of EFH-2, therefore, could activate the weakened immune system, perhaps with working as an adjuvant stimulating non-specific immunocytes.

We compared the levels of TNF-α induced from macrophages with stimulation of the dead cells of lactic acid bacteria in vitro. The results (Fig. 2) indicated that high levels of TNF were induced with enterococci and lactobacilli dwelling in the upper intestine, and that low levels of TNF with bifidobacteria in the lower intestine.

CONCLUSION

Oral administration of dead bacteria, heat-killed *E. faecalis* cells(EFH-2) showed the following effects:

(a) EFH-2 improves or prevents the disturbance of intestinal microflora;
(b) EFH-2 activates the systemic immune system;
(c) EFH-2 induces strongly TNF-α from macrophages in vitro, as an index on enhancement of immune responses.

Dead bacteria or the components in the intestine would be also thought to play roles similar to EFH-2 in humans.

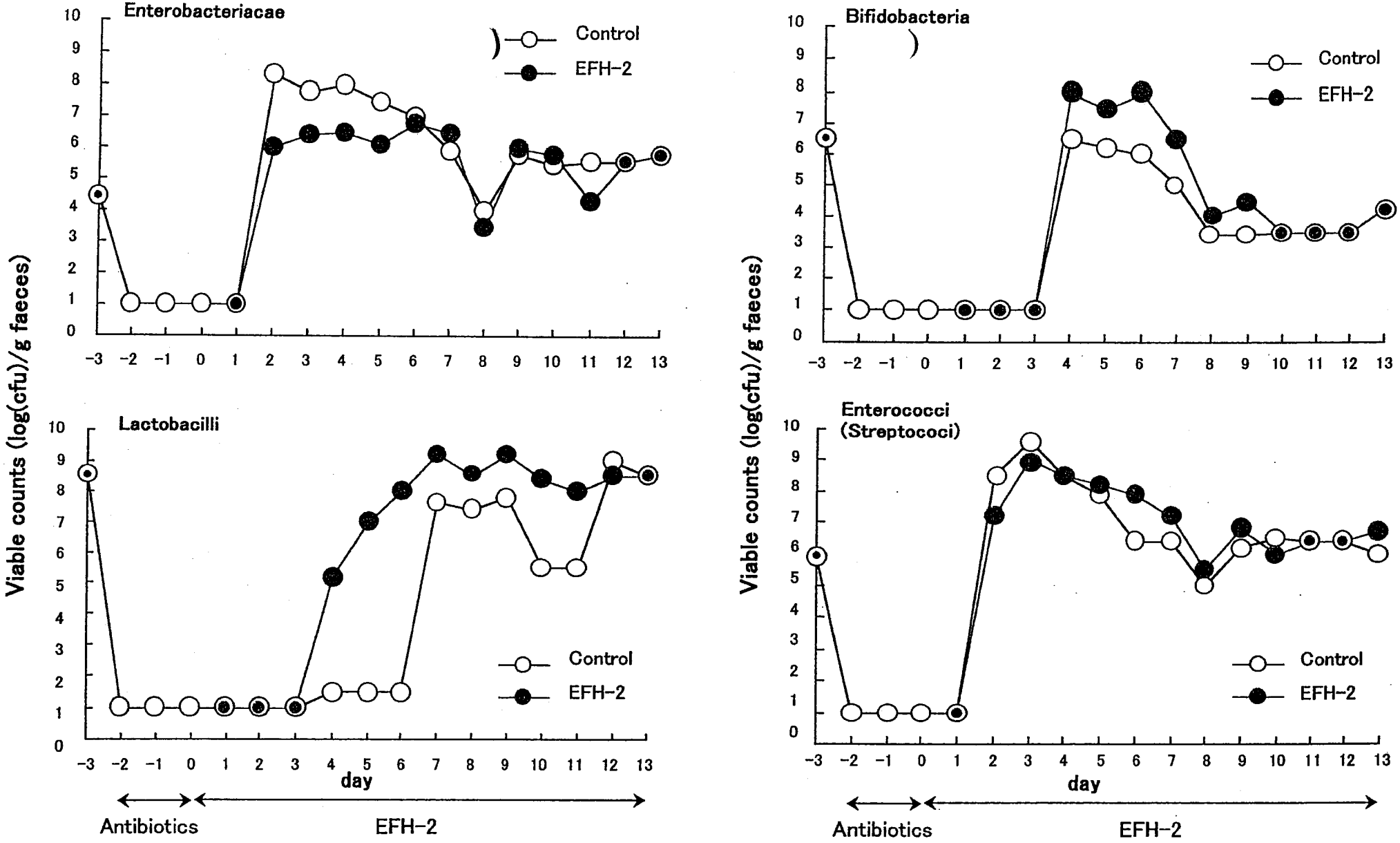


Fig. 1 Restoration-promoting effects of orally-administered EFH-2 on intestinal microflora disappeared with antibiotic treatment in mice.

Mice were given cephalexin(CEX,3mg/ml) and ampiciline(ABPC,5mg/ml) in drinking water for 3 days, and thereafter, administered EFH-2 (1.5mg/day/mouse) orally for 13 days. The faecal flora was examined through the experimental peried. n=5.

⊙ : Number of bacteria before antibiotic treatment.

Table 1 Number (log cfu/g faeces) of SM-resistent *E. coli* in faeces of mice given EFH-2 after an orall challenge of the *E. coli*

Group	Day(s) after challenge of SM-resistent *E. coli*				
	1	2	3	4	7
Control	7.5±0.2	6.3±0.3	5.3±0.2	4.8±0.4 (n=5)	4.6±0.2 (n=3)
5% EFH-2	7.4±0.3	6.1±0.4	4.9±0.4	4.5±0.5 (n=3)	4.2 (n=2)
10% EFH-2	7.2±0.3	4.8±0.2 **	4.2±0.5 *	4.3 (n=2)	3.2 (n=1)

Mice were given 5% or 10%EFH-2 mixed diet for a month, and then challenged orally with SM-resistent *E.coli* (3.5×10^9 cfu). Numbers of the *E. coli* were examined at intervals in faeces of mice.

Means±SE of 6 mice. * $P<0.05$ or ** $P<0.01$ (Student *t*-test). (n=) : number of mice detected for SM-resistent *E. coli* in the feces.

Table 2 Effect of EFH-2 ingested on number of *E. coli* NB-101 in the livers of immunocompomised mice after an intravenous challenge of *E. coli*

Group of mice	CY treatment	Number of *E. coli* in livers (log/g)		
		Time(hour) after administration of *E. coli*		
		24 hr	48 hr	72 hr
Control	+	6.3±0.7	6.9±0.6	6.2±0.5
EFH-2	+	4.8±0.8	4.5±0.5 *	4.4±0.6 *
Conventional	−	4.2±0.4 *	2.7±0.3 **	ND

Mice were fed 10% EFH-2 mixed diet for 14 days, and then injected intraperitoneally with cyclophosphamide 5 mg. The compromised mice were infected intravenously with 1.0×10^7 cfu of *E. coli* NB-101. Throughout the experiment period, 10% EFH-2 diet was fed to mice. Mean± SE of 5 mice. * $P<0.05$ and ** $P<0.01$ (Student *t*-test). ND (Not detected)

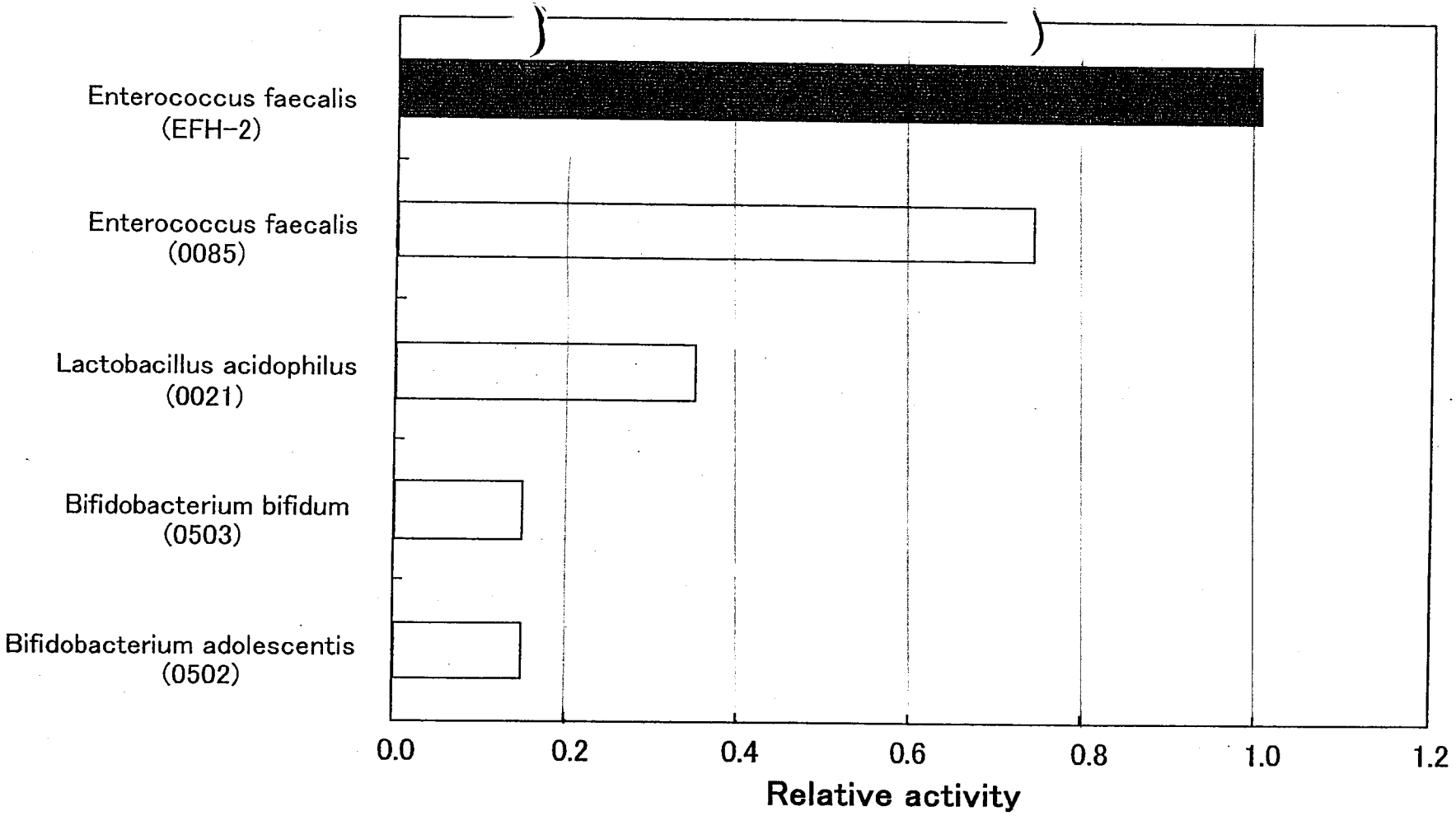


Fig. 2 **TNF-α induction of macrophages by stimulation of dead cells of lacticacid bacteria isolated from the intertinal flora (in vitro test).**

Exudate cells were collected from the aldorminal cavities of mice injected intrapentoneally with 1% glycogen, and incubated at 37°C for 1–2 hours under 5% CO_2. The macrophages adherent were collected, and then incubated with various kinds of dead bacteria for 2 hours to induce TNF-α.