Immunostimulating Effects of Extract from Culture of Effective Microorganisms (XCEM)

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<Background> More than two years have passed since disastrous accident at Fukushima Daiichi Nuclear Plant. Highly radio-contaminated water is still leaking from the plant, and we are left totally at a loss how to restore people's life to its normal state. Even among specialists, no appreciable general agreement has reached regarding the extent of acute radiation damage and the late-onset effect caused by low-level exposure. It is certain, however, that this accident afflicted the residents around Fukushima area with bigger amount of radioactivity than before. People are suffering also from psychological stress caused by anxiety about further radiation exposure. Especially, it should be pointed out the possibility that the radioactive exposure and the psychological stress gradually weaken the immunity force of the residents. In order to stimulate and maintain the immunity, it is necessary to take a multi–faceted measure, such as taking contamination-free food stuff, high quality vitamins, fermentation products and health foods. We have developed XCEM, an extract obtained from mixed culture consisted of safe and effective microorganisms such as lactic acid bacteria, yeast and photosynthetic bacteria. XCEM has been used as raw material for health food production, and the XCEM-containing soft drink has long been preferred by health-sensitive people. Therefore, we think it highly probable that the long standing use of XCEM-related products may reflect its immunity reinforcing capacity. Here, we report the results of our first trial to address whether XCEM exerts such an enhancing effect on natural immunity.

<Methods>

1) Evaluation of immunostimulating activity in natural immunity: XCEM is an extract prepared by filtration and heat sterilization of fermentated products from mix culture of lactic acid bacteria, yeast and photosynthetic bacteria. Macrophage (RAW264.7) was stimulated with XCEM and the production of nitric oxide (NO) and tumor necrosis factor (TNF-*α*) was assayed as a measure of macrophage activation. Macrophage (J774.1) was stimulated by XCEM and the number of beads ingested by macrophages was counted as a measure of phagocytic activity. In both cases growth medium only and *Pantea agglomerans* LPS (LPSp) were used as negative control and positive control, respectively. LPS concentration of XCEM was assayed separately and found to be 11.3ng/ml.

2) Evaluation of enhancement of heat-resistant capacity: XCEM was added to the water

containing known number of lactic acid bacteria (*Lactobacillus casei*). The cells in suspension, was heated 60C° for 10 minutes and then cultured for 48 hours with MRS growth medium. The number of viable bacteria was counted at the end of 48 hours cultivation.
3) Molecular weight distribution of XCEM was analyzed by size exclusion chromatography.

<Results>
1) Evaluation of Immunostimulating activity: XCEM at 3% showed enhancement of NO production, but no dose-dependency was observed. In TNF-*α* production test, XCEM at 0.03 – 10% showed significant dose-dependent enhancement. In a test for phagocytic activity, the phagocytic rate was 29.3% in negative control and 40.7%, in 10% XCEM treatment, respectively, showing a significant 1.4 times increase with XCEM treatment. The XCEM at 1% also showed a increase with a phagocytic rate of 41.0%, that is 1.4 times of the control. The phagocytic index was 0.51 and 0.87 in negative control and 1% XCEM group, respectively showing a significant 1.7 increase. The XCEM at 10% also increased phagocytic index by 0.81, 1.6 times higher than control, but with no statistically significant difference.
2) Evaluation of heat-resistant capacity: In control, the heat treatment reduced the number of viable bacteria from 1×10^8 to 2.3×10^2 cfu/ml. With the addition of XCEM, the survival rate was improved by 10 fold with the actual number of 3.4×10^3 cfu after heat treatment.
3) Molecular weight distribution: Eighty-three % of peptides in XCEM had a molecular weight of less than 500. Ninety-one % of sugars had a molecular weight of less than 3,000, of which 76% had a molecular weight of less than 500.

<Discussion>
Recently, it has been revealed that microbial components activate the natural immunity of plants and animals. Beta glucans of yeast, peptide glycans of lactic acid bacteria and LPS of gram negative bacteria have been reported immunostimulating effects in innate immunity. Since lactic acid bacteria, yeast and gram negative bacteria such as photosynthetic bacteria are used for XCEM production, we were prompted to see whether XCEM has immunostimulating effects. We studied XCEM with a focus on immunostimulating effects in natural immunity by analyzing various responses upon macrophage activation, such as NO or TNF-*α* production and phagocytic parameters. XCEM at 3% enhanced NO production, but without dose-dependent manner while LPS as a positive control showed dose-dependent response. Stimulation of NO production by 3% XCEM corresponded to one-tenth of that of 1ng/ml LPS. Therefore, it was concluded that XCEM has no stimulation effect or a little stimulation if any. Further studies are needed as to the existence of NO inhibiting substance in XCEM. As for TNF-*α,* XCEM treatment significantly enhanced TNF-*α* production in a dose-dependent fashion. Furthermore, in phagocytic activity test, XCEM treatment significantly increased both phagocytic rate and

its index as compared with the control. Taken altogether, it concluded that XCEM has an effect of macrophage activation.

Heat Shock Protein (HSP) is one of the molecular chaperones. HSP is induced to produce by stress such as heat and protects cells under stress. HSP also activates immune monitoring systems in living organisms and plays a role to activate the natural immunity. As a preliminary step to see the effect of XCEM on HSP production, the effect of heat treatment on lactic acid bacteria (*L. casei*) by XCEM was examined. Under the heat stress conditions, the survival was improved with XCEM 10 times higher as compared with the control. This result indicated that XCEM has an effect to make the bacteria heat resistant.

The results that XCEM activates macrophage and exerts immunostimulating effect in natural immunity and that XCEM renders lactic acid bacteria heat-resistant point to the possibility that XCEM increases HSP expression which is strongly correlated with natural immunity.

Regarding the active components in XCEM, molecular weight distribution analysis argues against the possibility of containing macromolecules such as beta glucan and peptide glycan. The molecular weight reported for the smallest LPS is 3,000. Therefore, it is unlikely that active component in XCEM corresponds to LPS. Further studies are needed for the identification in XCEM of active components of low molecular weight.

<Conclusions>

XCEM, an extract from culture of Effective Microorganisms activates macrophages which play an important role for natural immunity.

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