NMR & ICP Assay Report

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NMR Analysis & ICP Report on Geranti Bio-Ge Yeast *(Ver. 0.2)*

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Introduction

<Geranti Bio-Ge Yeast> is a newly invented product, which contains organic germanium by biosynthesis of yeast and fortified germanium. Such type of organic Bio-Germanium Yeast is a substance that is made by interacting of biomolecules like proteins, nucleic acid or organic acid and inorganic form of germanium during the biosynthesis technology.

The purpose of this study is to confirm that 1) <Geranti Bio-Ge yeast> be identified organic Germanium, and 2) be to examine if 'Geranti Bio-Ge yeast' be dissociated by Gastric Juices (SGF; Simulated Gastric Fluids) or not.

Materials & Methods

1) Test compound

Free Yeast & Geranti Bio-Ge Yeast

2) Preparation of SGF (Simulated Gastric fluids, pH 1.2)

SGF (Simulated Gastric fluids, pH 1.2) was made by mixing with 2.0g NaCl, 8.2ml of 35-37% HCl, and fixed to 1,000ml of distilled water in total volume. To test the stability of Geranti Bio-Ge yeast in gastric fluid condition, two samples were prepared by dissolving 300 mg of Geranti Bio Germanium in 20 mL of distillated water and in 20 mL of stimulated gastric fluid (SGF). Added 2ml of SGF in Geranti Bio-Ge Yeast was treated in water bath during 2 hour at 37±0.5°C.

3) NMR assay (Spectroscopic analysis of Geranti Bio-Ge Yeast)

All NMR experiments were performed at 298 K for solutions of 10 mg of both samples ["Free-Yeast (does not exist germanium)" and "Geranti Bio-Ge Yeast Extract"] dissolved in 1 ml of D_2O on a Bruker Avance 600 Spectrometer equipped with a 5mm TXI PFG inverse probe head. The temperature was maintained constant within ±0.1 °C by means of the BVT-2000 unit.

1H NMR spectra were recorded at a proton frequency of 600.05 MHz with a spectral width of 7.2kHz and 64K data points. The chemical shifts were referenced to solvent signals. 2D-COSY NMR experiments were performed using the standard Bruker pulse program, "cosygpqf", with z-gradient. The 2D-spectra were acquired with spectral width of 7.2kHz for both dimensions (F_1 and F_2), with 2K data points and 320 increments. The acquisition times were 0.14 s and the recycle delays were 1.7 s. Water suppression (to reduce residual water signals in both samples, ~4.7 ppm) was archived using pre-saturation during pulse recycle delay.

FT-IR and FT-Raman assay was experimented on the supernatants and remnants of the above 3 kinds of sample materials after being dissolved in simulated gastric juices respectively and compared the differences among 3 samples ["Free-Yeast (does not exist germanium)" and "Geranti Bio-Ge Yeast Extract" and "Geranti Bio-Ge Yeast Extract treated SGF"] by ATR FT-IR and FT-Raman assay.

Results

1. NMR assay (Spectroscopic analysis of Geranti Bio-Ge Yeast)

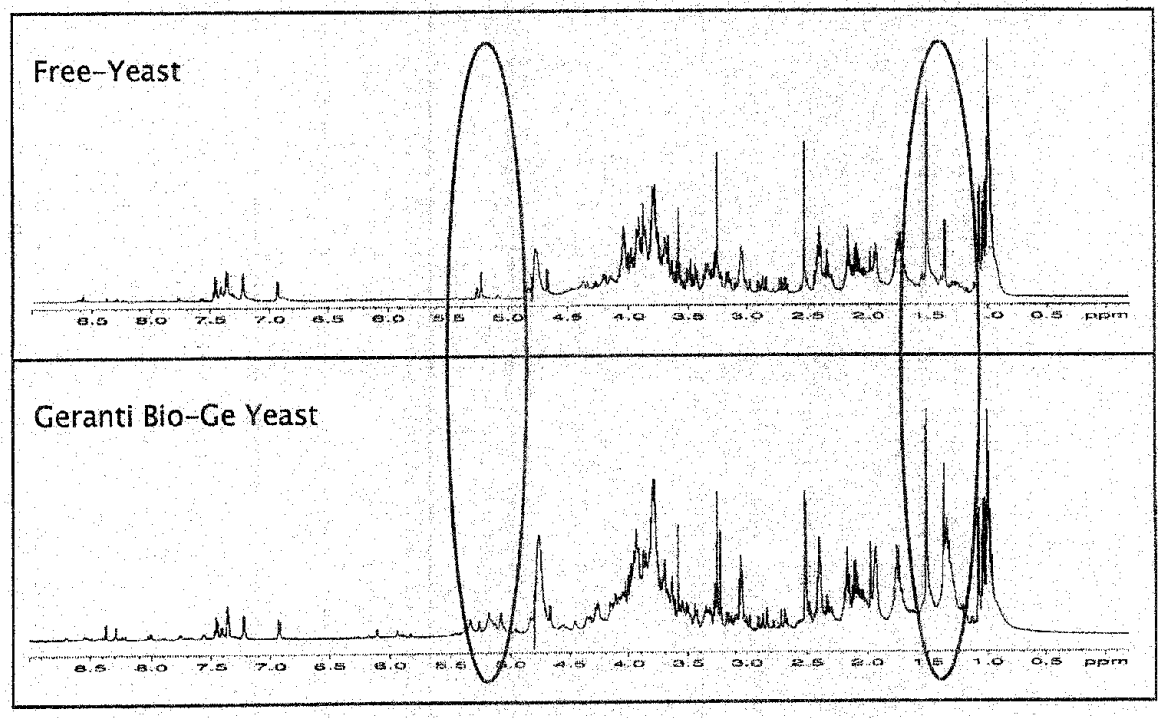

Figure 1. 1H NMR spectra of germanium free yeast (top) and Geranti Bio-Ge yeast (bottom) samples

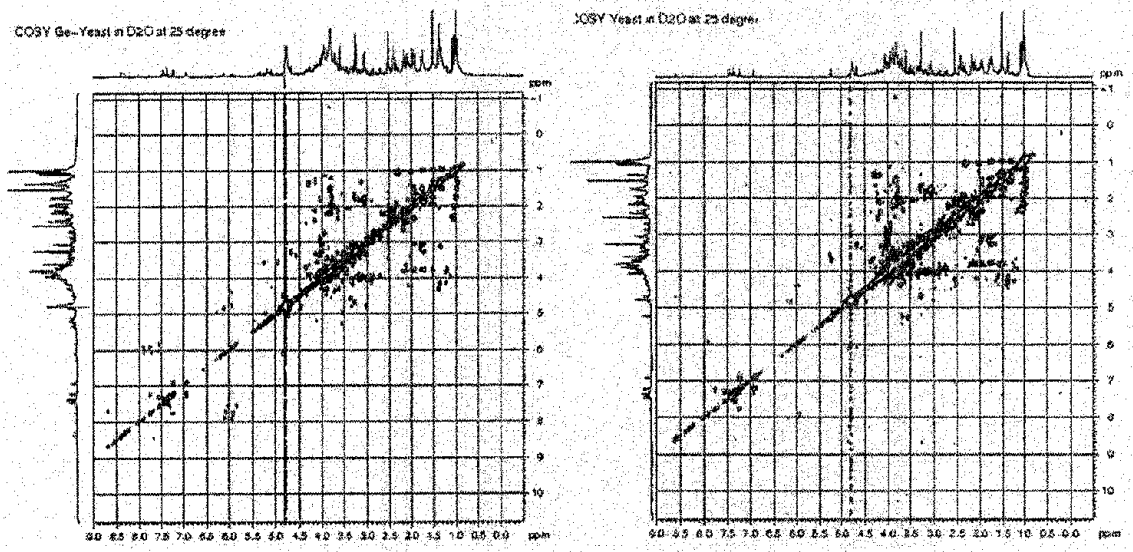

Figure 2. 2D-COSY NMR spectra of germanium free yeast (right) and Geranti Bio-Ge yeast (left) samples

The 1D and 2D-NMR spectra of both samples are similar to each other except several positions (see circles in Fig. 1). This result may imply that the structures of some proteins (or peptides) have been modified by binding with germanium.

The FT-IR and Raman spectra for free yeast and Geranti Bio-Ge yeast powders also show small differences in ~1400 cm^{-1} and ~1600 cm^{-1} regions, respectively (see Figs. 3 and 4).

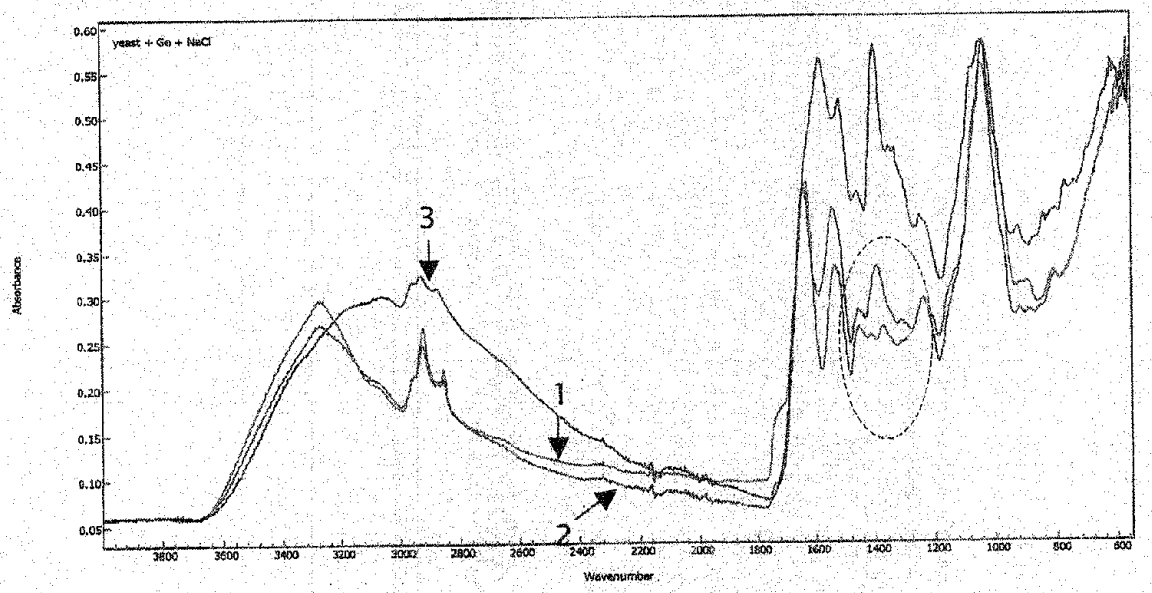

Figure 3. FT-IR Spectra for (1) Free-yeast powder, (2) Geranti Bio-Ge yeast powder and (3) solid part of Geranti Bio-Ge yeast in Gastric Juices

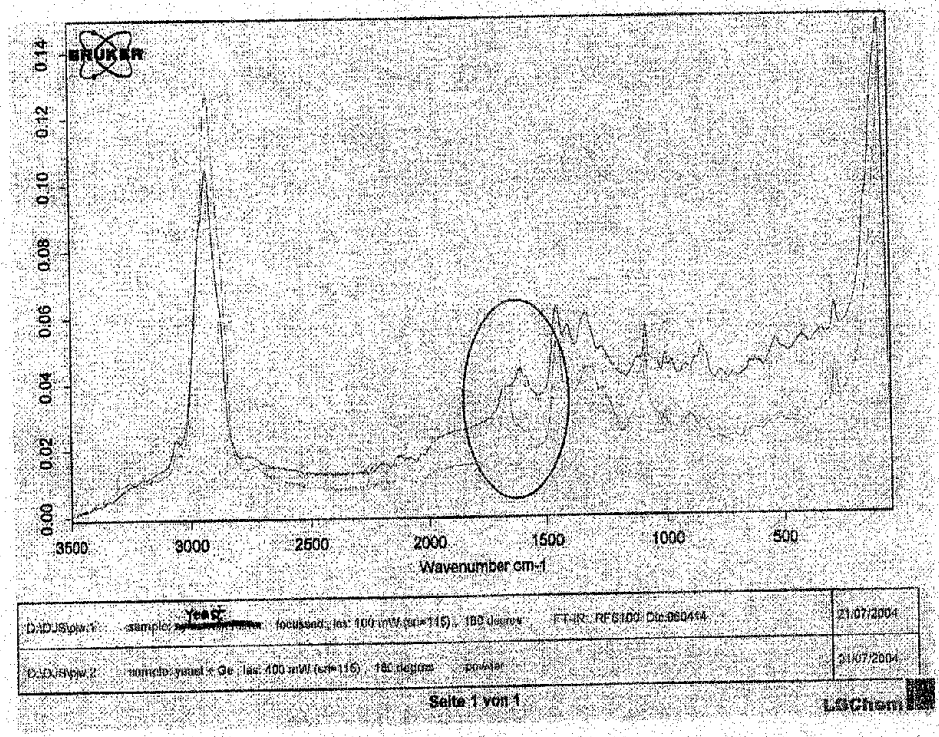

Figure 4. Raman spectra of free yeast and Geranti Bio-Ge yeast samples

The XRD patterns were analyzed for the samples of free yeast, Geranti Bio-Ge yeast, and GeO_2 powder (inorganic powder). As shown in Figure 5, inorganic GeO_2 has clear crystalline patterns. No crystalline XRD patterns for Geranti Bio-Ge yeast may imply that the Ge in yeast should not be GeO_2.

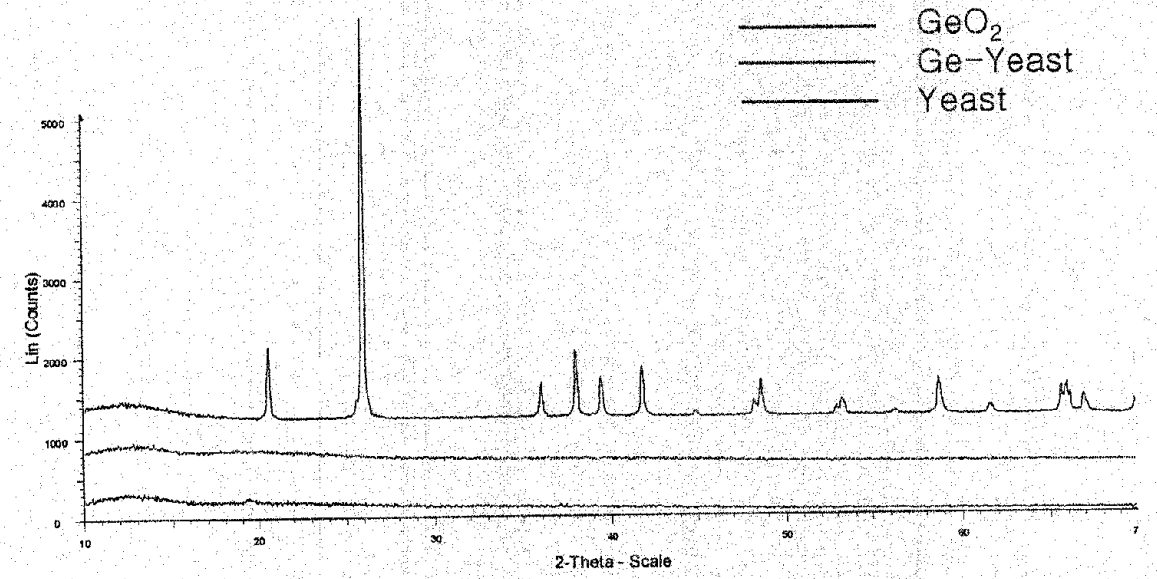

Figure 5. XRD patterns of the GeO2, Geranti Bio-Ge yeast (Ge-yeast), and free yeast samples

2. Dialysis and ICP experiments of Geranti Bio-Ge yeast to determine free germanium content in SGF (Simulated Gastric Fluid) solution

To test the stability of Geranti Bio-Ge yeast in gastric fluid condition, two samples were prepared by dissolving 300 mg of Geranti Bio Germanium in 20 mL of distillated water and in 20 mL of stimulated gastric fluid (SGF).

For the dialysis experiments, Sigma dialysis membrane tubing with a molecular weight cut-off (MWCO) of 1200 Da was used. The tubes were filled with the Geranti Bio Ge yeast solutions. In the outer solution a dialysis tube filled with germanium free water was immersed. After two hour, samples were taken from both the inside and outside solutions and analyzed for metals to allow calculation of the free (or small organic binding) and bound (by protein or peptide) germanium.

The elemental analysis of these dialysis samples was undertaken by Inductively Coupled Plasma Atomic Emission Spectroscopy(Ultima2, Horiba Jovin Yvon Co.). Each sample was measured three times with three replicates for each measurement. The

results are presented in Table 1 with concentrations of germanium given in mg/l (ppm).

Sample	Ge concentration (ppm)	
	Before dialysis	After dialysis
In water	36.3 (± 0.2)	33.4 (± 0.2)
In SGF	38.9 (± 0.3)	32.8 (± 0.2)

The results, no big difference in germanium content after dialysis, show that the germanium taken-up yeast is to be organically bound germanium and is not dissociated into free (inorganic) germanium even in the gastric fluid condition.

Conclusions

In conclusion, Ge in 'Geranti Bio-Ge yeast' is not dissociated in water or gastric juices, because Ge is bound with proteins in 'Geranti Bio-Ge yeast' or properties of 'Geranti Bio-Ge yeast' was changed by strong bond with Ge. And, it shows that the germanium taken-up yeast is to be organically bound germanium and is not dissociated into inorganic germanium even in the gastric fluid condition.