**Note**

**Royal Jelly from *Apis cerana japonica* and *Apis mellifera***

Tetsuo **Takenaka** and **Yoko Takenaka***

Faculty of Agriculture, Tamagawa University, 6–1–1 Tamagawa-gakuen, Machida-shi, Tokyo 194, Japan

* T & T Food Institute, 555–20 Aina, Atsugi-shi, Kanagawa 243, Japan

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The chemical composition and the properties of protein in royal jellies collected from *Apis cerana japonica* and *Apis mellifera* were respectively analyzed.

* A. cerana royal jelly (CRJ) contained more protein and less carbohydrate than *A. mellifera* royal jelly (MRJ). The water-soluble proteins were analyzed by electrophoresis and immunologically. The major protein components of CRJ and MRJ had different molecular weights, isoelectric points, and immunological characteristics.

**Key words:** royal jelly; *Apis cerana japonica*; *Apis mellifera*; 10-hydroxydecanoic acid; glycoprotein

*Apis cerana japonica* Rad., a subspecies of *A. cerana* Fab., is indigenous to Japan where it coexists with *A. mellifera* which was introduced in 1877 for commercial beekeeping from Europe. Although bee products from the *A. cerana japonica* subspecies have been commercially neglected, it is regarded as an important genetic resource, because it is highly resistant to the paracapsid mite, *Varroa jacobsoni*, and to its diseases. This genetic resistance is an important factor in Asian beekeeping.

Queen rearing to maintain a colony of honeybees is another important factor in managing bees. Hoshiba et al. have reported trials with *A. cerana* queen rearing in *A. mellifera* colonies, and a trial with *A. cerana* queen rearing on royal jelly produced by *A. mellifera* in the laboratory. *A. cerana* queens could not grow sufficiently in a colony of *A. mellifera*, so they suggested a difference between the royal jelly from each species. In this report, chemical and immunological analyses of the royal jellies from both *A. mellifera* and *A. cerana japonica* are described.

*A. cerana japonica* and *A. mellifera* were kept in an apiary at Tamagawa University. Plastic queen cups were mounted on a grafting bar to accept 1-day-old larvae. A frame with such bars was introduced into a queenright colony, and royal jellies from *A. cerana japonica* (CRJ) and from *A. mellifera* (MRJ) were collected after 48 h. These collections were made during May 1993. Moisture was determined by direct drying at 105°C. Crude protein was determined by the micro-Kjeldahl method with a conversion factor of 6.25, and crude lipid was determined by Soxhlet extraction with ether for 18 h. Ash was determined by heating at 550°C for 3 h, and carbohydrate was determined by subtracting the foregoing amount from the total amount. Acidity was determined by an alkaline titration method and is expressed as ml of 1 N NaOH per 100 g of fresh RJ.

Sugars were analyzed by the HPLC method in a Shodex Ionpak KA-801 column (8 mm diam. × 300 mm long), using deionized water at a flow rate of 1.0 ml/min at 50°C, and were detected with an RI detector. HDA was analyzed in a Carapack PAC 120 column (4.6 mm diam. × 250 mm long), using methanol: H2O = 1:1 (pH 2.2) at a flow rate of 1.0 ml/min at 50°C, and were detected with a UV detector (210 nm). SDS polyacrylamide gel electrophoresis was carried out by a modification to the Weber and Osborn method using 18.0% separation gel and 3.0% stacking gel. Protein bands were separately stained with 0.1% Coomassie Brilliant Blue G-250 and with PAS reagent for glycoprotein. Phosphorylase-b (97,400 Da), bovine serum albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,400), and alpha-lactalbumin (14,400) were used as molecular-weight markers. Isoelectric focusing was carried out by the Wringley method, immunological double-diffusion followed by the Ouchterlony method, and immunoelectrophoresis followed the Grabar and Williams method. An antiserum to water-soluble protein of MRJ was prepared in rabbits.

The chemical comparison of RJ produced by the two species is shown in the Table. The relatively higher concentration of protein in CRJ seems to be characteristic of the species. Other marked differences are the content of 10-hydroxydecanoic acid (HDA) and the glucose/fructose ratio in the carbohydrate. In particular, HDA in CRJ was about one-third that in MRJ. HDA is used in Japan as a quality indicator for commercial RJ. In this respect, CRJ has a special feature which should be considered separately from MRJ. The low glucose/fructose ratio in CRJ may reflect the activity of glucose oxidase which oxidizes glucose to gluconic acid. The acidity level was almost the same in both RJs.

After electrophoresis of the protein in royal jelly, MRJ showed 21 protein bands. CRJ also showed 21 bands (Fig. 1). The range in molecular weights of the proteins containing sugar in both RJs was 14–120 kDa, and the major proteins that were heavily stained were of between 40 kDa and 80 kDa. A high-molecular-weight protein fraction which did not move (No. 1) was found in CRJ. The fourteen bands indicated in Fig. 1 were common to both RJs. Among these common bands, four (Nos. 6, 7, 12, and 16) of the six major ones (Nos. 4, 6, 7, 12, 16, and 21) in CRJ were more heavily stained than in MRJ. On the other hand, band

| Table Chemical Composition of Royal Jellies Produced by *Apis mellifera* and *Apis cerana japonica* |
|---------------------------------|---------------------|------------------|
| MRJ (n = 18) | CRJ (n = 5) | |
| Moisture | 68.3 ± 1.4% | 65.3 ± 2.5% |
| Protein | 12.7 ± 0.8 | 16.4 ± 2.5 |
| Carbohydrate | 11.9 ± 0.7 | 9.4 ± 0.6 |
| Fructose | 5.3 ± 0.4 | 4.8 ± 0.5 |
| Glucose | 5.0 ± 0.5 | 3.6 ± 0.4 |
| Others | 1.6 ± 0.4 | 1.3 ± 0.7 |
| Lipid | 6.1 ± 0.4 | 7.4 ± 0.6 |
| 10-HDA+ | 2.4 ± 0.2 | 0.9 ± 0.2 |
| Ash | 1.0 ± 0.2 | 1.5 ± 0.2 |
| pH | 3.7 | 3.8 |
| Acidity* | 42.2 ± 2.1 | 39.3 ± 3.1 |

* Abbreviations: CRJ, *A. cerana japonica* royal jelly; MRJ, *A. mellifera* royal jelly.

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**Fig. 1.** SDS PAGE Patterns of the Proteins from MRJ and CRJ.

MRJ, royal jelly from *Apis mellifera*, CRJ, royal jelly from *Apis cerana japonica.*

The circle symbol (○) indicates common bands for MRJ and CRJ.

**Fig. 2.** Isoelectric Focusing PAGE Patterns of the MRJ and CRJ Proteins.

Nos. 10 and 11 were major and specific to MRJ.

Takenaka and Echigo\textsuperscript{100} have reported that the major MRJ proteins contained sugar moieties. Although the protein patterns were quite different between MRJ and CRJ, the major proteins in MRJ and CRJ were glycoproteins, when stained with PAS.

Both RJs were analyzed by isoelectric focusing (Fig. 2). The proteins of CRJ were separated into 10 bands with isoelectric points (pI) from 3.0-9.7, and of MRJ were separated into 10 bands with pI's ranging from 3.0-8.2. Only two bands were common: No. 2 in CRJ (No. 1 in MRJ, pI 8.2) and No. 8 (No. 9 in MRJ, pI 4.0). Alkaline pI 9.7 protein, No. 1, was found only in CRJ. These results indicate a big difference between CRJ and MRJ.

The immunological difference between the RJ proteins was examined in two ways. Figure 3 shows the result of a double-diffusion analysis, using antibodies to MRJ water-soluble proteins. Several heavy-precipitating lines were found, and some formed a spur between CRJ and MRJ. These indicate that the proteins in both RJs contained the same or a partially common structure.

The MRJ proteins produced three cathode- and two anode-precipitating bands (Nos. 4 and 5 shown) by using immunoelectrophoresis against the MRJ antiserum (Fig. 4). Under the same conditions, CRJ showed two cathode bands (Nos. 6 and 7). The No. 11 band of MRJ and the No. 12 band of CRJ in Fig. 1 were isolated by preparative electrophoresis, and their immunological characteristics were examined. The former was in accordance with the No. 1 precipitation band, and the latter was the same as No. 6. Both proteins were clearly immunologically different, although they were adjacent (similar molecular weight) by electrophoresis (Fig. 1).

Chemical analyses and electrophoreses of the proteins revealed differences between CRJ and MRJ. Such differences have not been noted before, although it is not strange, since these RJs are...
produced by different species. Morphometric and mitochondrial DNA analyses have recently been carried out\textsuperscript{11,12} to distinguish between inter- and intra-
*Apis* species. The biochemical approach of the present study can contribute to such distinction.

The differences between CRJ and MRJ may explain why *A. cerana* queens cannot be successfully reared on MRJ in the laboratory.\textsuperscript{9} Shue and Dixon\textsuperscript{13} have reared *A. mellifera* queens on an artificial diet, and found that the protein in the RJ was vital. The importance of proteins in determining queen development in both species will be established through such rearing experiments.

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References