NO.1

Year 2023	**Summary of Thesis**

Student No.	Last name, First name
M2220290	Yajima Takumi

(Title)

Elucidation of phytoalexin produced by broccoli with bacterial soft rot through metabolome analysis.

Broccoli shipped from JA Hokkaido's fruit-sorting facilities are sorted by workers who determine quality by directly holding the broccoli in their hands and examining each one. In addition to sorting broccoli by its size, this sorting process also involves sorting out diseased broccoli. One of those diseases is bacterial soft rot. Bacterial soft rot occurs most frequently in hot-weather crops and causes yellowish-brown soft rot on the stems and leaves, and sticky rot on the flower buds (Fig 1. left). In both cases, this disease is known for its distinctive odor, and the presence or absence of this strong putrid smell is a criterion for distinguishing it from other soil-borne diseases. This disease can also occur during storage and transportation, as it can develop after harvest and damage adjacent bulbs. Therefore, it is essential to remove the diseased ones at the fruit selection stage. However, there is no efficient way to sort out diseased plants that cannot be visually detected other than to sniff it, and it is nearly impossible to sort out the defective products of a few percent in the tens of thousands of broccoli plants that are shipped each day.

Bacterial soft rot is caused by the bacterium, Pectobacterium carotovorum (Fig. 1. right), which is a polyphagous bacterium that is found not only in Brassicaceae, such as Chinese cabbage, cabbage, broccoli, and radish, but also in the family of Brassicaceae. This bacterium is polyphagia, causing symptoms on various parts of many plants, including onions, potatoes, tomatoes, peppers, melons, carrots, and leeks, as well as cruciferous vegetables such as Chinese cabbage, cabbage, broccoli, and radishes. Bacterial soft rot is caused by a digestive enzyme called pectinase secreted by the pa-

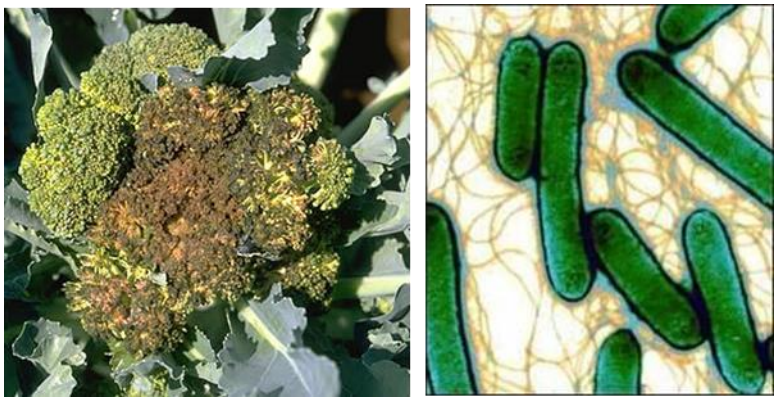

Fig. 1. Broccoli infected by bacterial soft rot (left) and its pathogen (right).[3]

Graduate School of Science and Engineering, Chitose Institute of Science and Technology

NO . 2

thogen, which hydrolyzes pectin, a cellular constituent of the crop.[1,2] In this study, we performed component identification using a metabolite database based on the results of LC/MS/MS measurements of three types of broccolis: fresh broccoli, broccoli that has been left to rot at room temperature, and broccoli that has been infected by bacterial soft rot. Based on the results, principal component analysis (PCA score plot) and factor loadings analysis (PCA loading plot) was performed to identify the protective substances produced by broccoli from among the contained multiple components when infected by bacterial soft rot.

To search for components expressed by the onset of soft rot disease, five samples of broccoli infected by bacterial soft rot as well as 10 fresh broccoli plants provided by JA Hokkaido were sampled over a 15-day period to explore the process of broccoli deterioration over time and characteristic components of broccoli infected by bacterial soft rot disease. The results of this study were as follows.

The bacterial soft rot infected broccoli samples were assigned to Lv. 1 to 5 according to the degree of disease progression (Fig. 2). Using LC/MS measurements, we performed a principal component analysis (PCA) and found in the PCA score plots that the plots were aligned according to the degree of rot progression. (Fig. 3) In the PCA loadings plot for this, we succeeded in extracting the components, Cpd: A, B, C, and D, which are characteristic only of broccoli infected with bacterial soft rot. (Fig. 4) LC/MS/MS measurements were performed on these four components, and the structures of components A, B, and D were successfully identified.

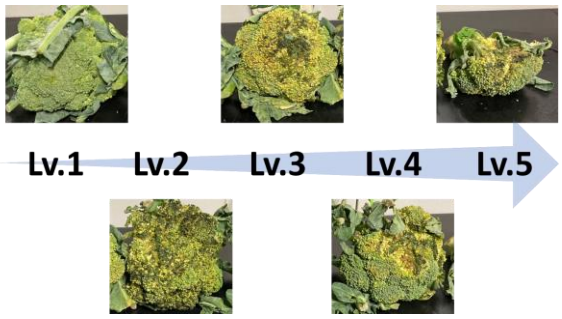

Fig. 2. Lv.1~5 broccoli infected by bacterial soft rot.

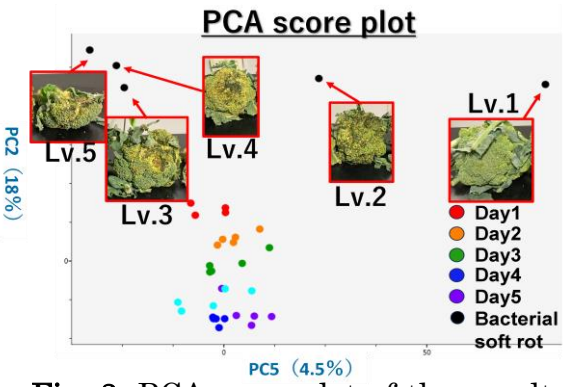

Fig. 3. PCA score plot of the results of LC/MS/MS measurements.

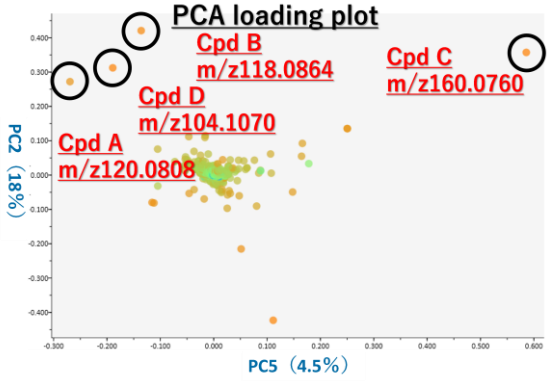

Fig. 4. PCA loading plot of the results of LC/MS/MS measurements.

References

1. Mitsuo Takasugi, *Chemistry and Biology* 31, 1 (1993)
2. Kenji Monde et al., *Journal of Pesticide Science* 20, 339-343 (1995)
3. https://alchetron.com/Pectobacterium-carotovorum

Graduate School of Science and Engineering, Chitose Institute of Science and Technology