博士（農学）　兰　巍巍

学位論文題名

# Studies on soft rot of chicory and its causal bacteria

## （チコリー軟腐症状とその病原細菌に関する研究）

# 学位論文内容の要旨

***Backgrounds***

Chicory (*Cichorium intybus* L. var. *sativum* Bisch.) includes both a horticultural crop grown for direct consumption as a cooked food and an industrial crop whose purpose is the extraction of polysaccharide. Root chicory is an alternative sugar crop, which accumulates inulin in roots. Inulin is a polymer that is widely used in several industrial applications. At present, inulin is mostly supplied by root chicory, which is mainly grown and processed in European countries. Due to the future use of domestic raw materials for more sustainable and economical production of products such as oligosaccharide, the expansion of root chicory cultivation to Hokkaido in northern Japan is an attractive option. Since the sowing and harvesting techniques of sugar beet and potato can be used for root chicory, no investment into new machinery is expected to be necessary, except for some minor modifications.

***Symptomatology***

Bacterial soft rot was found on root chicory in fields at the Ornamental Plants and Vegetables Research Center, Hokkaido Research Organization, Takikawa, and Institute of Nippon Beet Sugar Manufacturing, Obihiro, in August 2010 and 2011. Severely infected plants in fields show discoloration, wilting of foliage, and black necrosis of petioles near the crown. Wilted leaves subsequently collapse and die, forming a dry, brown or black rosette. The root and crown become partially or wholly soft-rotted. On the infected root area, the slimy mass turns dark brown or black.

***Pathogenicity***

Pathogenicity assay for the root chicory using all chicory isolates by artificial inoculation was carried out, and the similar symptoms were observed within one week. At first, water-soaked lesion enlarged to slimy soft rot associated with discoloration for the most of isolates, or associated with a ripe odor for MT-5 and MT-7, while B-P2 did not cause soft rot, but formed a scab-like lesion on the inoculation site. Disease severity of thirteen chicory isolates was significantly different; the most aggressive isolates were A-R2, A-R4, A-R5, A-R6, B-R3, B-R4 and B-R5.

***Isolation and identification of causal bacteria***

Causal bacteria were straight rods (0.5-1.0 × 1.0-3.0 μm) with rounded ends that occurred singly or in pairs. The cells were motile and four to six flagella were discernible. The causal bacteria isolated from diseased chicory were gram-negative, positive for anaerobic growth, growth at 28°C and 37°C, and catalase, but negative for oxidase, and pigment production. In addition, they were positive for indole production, phosphatase activity, and gas production from glucose, and sensitive to erythromycin. Acid was produced not from trehalose. Sodium

malonate and L-tartrate were utilized, and neither utilized α-methyl-D-glucoside. These biochemical and physiological chacteristics indicated that A-R1-6 and B-R1-6 are *Dickeya* sp. While the B-P2 was similar to *P. carotovorum* subsp. *carotovorum*, the isolates MT-5 and MT-7 were closely related to *Pectobacterium carotovorum* subsp. *odoriferum*. The *Dickeya* sp. strains (A-R1-6 and B-R1-6) were classified into previous *E. chrysanthemi* biovars 1 or 7, which coincide with *D. dianthicola*.

***Host range***

A-R1–6 and B-R1–6 were almost pathogenic to potato, eggplant, Welsh onion, sweet potato, chrysanthemum, and carnation. MT-5 and MT-7 were pathogenic to potato (other plants were not tested for both the isolates in this study). The common symptom began as water-soaked accompanied with discoloration, when disease conditions were optimum even totally soft rot. No symptoms were observed in sweet potato inoculated with A-R5 and B-R3, or on potatoes inoculated with B-R3 and B-R4. However, the chicory isolates (except for B-P2) were not pathogenic to tomato. After inoculated with B-P2, leaves of tomato collapsed and dried, subsequent seedling wilt occurred within 2 days. B-P2 not only showed the broadest host range, but also showed the aggressive virulence for the host plants potato, eggplant and Welsh onion.

***Phylogenetic analyses***

Molecular identification was performed using specific primer sets. The expected 420-bp PCR-amplified fragments corresponding to the conserved regions of *Dickeya* species' pectate lyases-encoding gene cluster (*pel*ADE) were obtained for all the *Dickeya* spp. isolates. PCR performed with the oligonucleotide primer set (EXPCCF/EXPCCR) yielded a 550-bp amplified fragment, indicating that isolates B-P2, MT-5, and MT-7 are from *P. carotovorum* subsp. The results of the phylogenetic analyses showed that all of A-R1–6, B-R1–6 were phylogenetically close to *D. dianthicola* CFBP1200$^T$ (GQ891984). Similarly, B-P2 was phylogenetically close to *P. carotovorum* subsp. *carotovorum* ATCC15713$^T$ (FJ895851), while MT-5 and MT-7 were closely related to the strain CFBP1878$^T$ (JF926793.1) belonging to *P. carotovorum* subsp. *odoriferum*. Phylogenetic analysis based on the 16S rRNA gene showed that MT-5 and MT-7 were grouped together with the type strain *P. carotovorum* subsp. *odoriferum* CFBP1878$^T$ (AF373191) and were distinct from other *Pectobacterium* spp. B-P2 was phylogenetically close to *P. carotovorum* subsp. *carotovorum* DSM30168$^T$ (AJ233411).

***Influence of soil saturation and temperature on bacterial soft rot of root chicory***

Severity was significantly greater under the soil temperature at 30°C than at 25°C and 20°C inoculated with the both isolates, A-R6 and B-R2 when the soil was saturated. This result suggested that root rot of chicory has occurred remarkably in 2010 when the temperature and humidity during June to September were higher than usual.

***Conclusion***

Causal bacteria were exclusively isolated from the rotted roots, and typical symptoms were reproduced after inoculation with the strains. Consequently, the bacteria were identified as *Dickeya dianthicola*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Pectobacterium carotovorum* subsp. *odoriferum* based on further bacteriological characterization and phylogenetic analyses. These bacteria should be added along with the previously reported *Dickeya* (=*Erwinia*) *chrysanthemi* in Saitama prefecture, Japan, as causal pathogens of bacterial wilt in chicory.

# 学位論文審査の要旨

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## (チコリー軟腐症状とその病原細菌に関する研究)

本論文は図19，表10を含む５章、総頁数119からなり、別に参考論文１編が添えられている。

チコリーは、肥培した根から出させた芽を暗黒下で軟白栽培することで野菜として利用されるほか、様々な食品及び工業用原料として利用される多糖類の１種イヌリンの抽出・生産用に栽培される。主なイヌリンの原料国はオランダ、フランス、ベルギーなどであり、現在日本国内で使用されるイヌリンの多くはこれらの国からの輸入に依存している。近年、地域産業の振興を目的にイヌリンの機能性に着目した製品開発が進められ、原料の生産・供給までも視野に北海道へのチコリーの導入が試みられている。

本研究は、試験圃場で栽培したチコリーの根に発生した軟腐症状の病原細菌の同定を行ったものである。既に細菌性病害として報告のある「チコリー萎凋細菌病」の病原との細菌学的、生理学的及び系統学的比較から、北海道で発生したチコリー根軟腐症状は埼玉県で分離された病原細菌とは異なる３種の細菌により引き起こされることが明らかにされた。その詳細は，次のようにまとめられる。

**１．病徴**

軟腐症状チコリーは、滝川市（2010 年）、帯広市（2011 年）の試験圃場で発生が見られた。激しく発病した株は根の褐変または地上部の萎凋が見られ、さらには冠部近辺の葉柄が黒色に腐敗した。肥大根は部分的あるいは全体的に軟腐症状を呈し、根の病変部位では粘性物質が暗褐色あるいは黒色に変色した。

**２．病原性の確認**

分離した細菌株全てについて病原性を確認した。ほとんどの細菌株において、水浸状病斑が次第に根の内部あるいは外部に拡大し褐変を伴った軟腐症状となった。分離細菌２株には腐熟臭が伴っており、別の１株では明瞭な軟腐症状を示さず、接種部分にそうか状の病変が生ずることもあった。また、細菌を接種した土壌の温度を 30℃としたとき、20，25℃と比べ病徴の伸展は速やかで、症状も激しくなることが確認された。

**３．細菌学的及び生理学的特性**

病原細菌の細菌学的特性は、末端が円形の直鎖桿状、単一あるいは対の状態であること、細胞に運動性があること、４乃至６本の鞭毛が識別可能であるなどである。一方、生理学的特性

は、グラム陰性、嫌気状態での生長、37℃での生長及びカタラーゼ陽性、オキシダーゼ、色素生産は陰性など、いわゆる軟腐性細菌と一致する性状を示した。これらの表現型特性から、滝川市から分離された12菌株は*Dickeya*属細菌に属することが推定され、1菌株は*Pectobacterium carotovorum* subsp *carotovorum*に近く、帯広市から分離された2菌株は*Pectobacterium carotovorum* subsp. *odoriferum*であるとした。引き続き検討した近年まとめられた*Dickeya*属の分類基準とこの学名が使用される前の旧名*Erwinia chrysanthemi*の生物型による分類基準との比較照合から、先の12菌株は全て*Dickeya dianthicola*と同定された。

**4．系統学的解析**

*Dickeya*属を特異的に検出できるPCRプライマーセットにより、滝川市からの12菌株は全て*Dickeya*属であることが、また、別のPCRプライマーセットによりその他の菌株は、*Pectobacterium carotovorum* subsp *carotovorum*及び*Pectobacterium carotovorum* subsp. *odoriferum*であることが示唆された。さらに、malate dehydrogenase遺伝子あるいは16S rRNAの塩基配列に基づく系統学的解析から、*Dickeya dianthicola*、*Pectobacterium carotovorum* subsp *carotovorum*及び*Pectobacterium carotovorum* subsp. *odoriferum*との同定結果の妥当性が確認された。これらの結果より、「チコリー萎凋細菌病」には*Dickeya chrysanthemi*のほか、上記3種の細菌を病原として追加すべきであるとされた。

以上のように、チコリー軟腐症状には複数の細菌が関わっているという結果は、チコリーの導入・栽培にとって有益な情報になるとともに、「チコリー萎凋細菌病」の新たな病原として上記細菌が加えられるべきとの知見は植物病理学的に有意義な研究成果であり、応用上および学術上高く評価できる。よって 審査員一同は，兰巍巍が博士（農学）の学位を受けるに十分な資格を有するものと認めた。