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ROOT NODULE BACTERIA OF *CYTISUS SCOPARIUS* LINK. AND *WISTARIA FLORIBUNDA* DC.

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Abstract

The rhizobial strains of *Cytisus scoparius* Link. and *Wistaria floribunda* DC. were studied on their cultural and physiological characters. In addition, the cross inoculation tests were conducted to determine the position of these plants.

The characters of the isolates from *Cytisus* were the same as those of the slow growers, whereas those from *Wistaria* differed in their growth rate from either growing type. Other characters of *Wistaria* isolates were as follows: No growth on potato and in bouillon; serum zone present and acid reaction in litmus milk; nitrate reduction positive; and raffinose or lactose was utilized as a carbon source.

From the results on the cross inoculation tests, it may be concluded that *Cytisus scoparius* belongs to the cowpea group. The isolates from *Wistaria* did not nodulate any plant of the major cross inoculation groups. Although the reciprocal relationship is unknown, *Wistaria* may not be included in any of the major groups. The relationship between *Wistaria* and the existing minor groups remains to be studied.

Introduction

Cytisus scoparius which was brought to Japan about three hundred years ago[9] and *Wistaria floribunda* which is widely distributed in Japan are both cultivated as an ornamental plant. Few studies seemed, however, to have been made on the microsymbionts of these plants. This is the reason why this study was undertaken.

I. Some Cultural and Physiological Characters

Materials and Methods

Isolation of rhizobia : Plants used for the isolation of rhizobia and the location where they were collected are as follows.

Cytisus scoparius Link.……
Rokkodai, Kobe
and Nishi-Ku, Yokohama.

Wistaria floribunda DC.……
Sasayama, Hyogo Prefecture.

Characters studied : Growth on yeast water mannitol agar slope, growth on patato, growth in bouillon, change in litmus milk, nitrate reduction, and availability of raffinose, lactose, glucose, xylose, and mannitol as a carbon source using the following basal solution.

K_2HPO_4…0.7 g, KH_2PO_4…0.3 g, $MgSO_4.7H_2O$…0.2 g, NaCl…0.2 g, $CaSO_4.2H_2O$…0.1 g, $FeCl_3.6H_2O$…Trace, Asparagine…0.32 g, Yeast extract…5 ml, Deionized water …995 ml.

Yeast extract was prepared by the following treatment. 15 g of dried yeast were added to 1,000 ml of deionized water, treated at 120°C. for 30 min., and then filtered.

The methods used were almost the same as those in the previous papers.[4,5]

Results

Accordind to the results shown in Tables 1 and 2, all isolates from *Cytisus* are quite similar to the so-called slow growing type such as *Rhizobium lupini*, *R. japonicum*, and

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some of cowpea bacteria. The isolates from *Wistaria* are neither similar to the slow grower nor to the fast grower in their growth rate, and rather intermediary between the two. They showed no growth on poato and in bouillon. These resuts are almost the same as those of most of rhizobia. The serum zone was produced and the reaction was turned acid in litmus milk. This reaction change is noticed, because the alkaline reaction seems rather popular in *Rhizobium* in general.

Among carbon sources, attention must be placed on lactose and raffinose, because rhizobia are divided into two groups depending upon whether lactose or raffinose is utilized or not.[6] The slow growers are unable to use them as a carbon source, and the reaction becomes usually alkaline in the media except xylose or arabinose medium.

Table 1. Some cultural and physiological characters

Rhizobial strain	Isolated from	Y.M. agar slope*	Potato	Bouillon	Litmus milk S.z.**	R.***	Nitrate reduction
C-1-Y	*Cytisus* (Yokohama)	growth : slow streak : raised, glistening, opaque, and white	slight growth	no growth	absent	−†	positive
C-2-Y	〃	〃	〃	〃	〃	〃	〃
C-3-K	*Cytisus* (Kobe)	〃	no growth	〃	〃	〃	〃
C-4-K	〃	〃	〃	〃	〃	〃	〃
W-1	*Wistaria*	growth : fairly rapid, but not so rapid as fast grower. streak : raised, glistening, translucent, and white.	no growth	no growth	present	+‡	positive
W-2	〃	〃	〃	〃	〃	〃	〃

* : Yeast water mannitol agar slope.
** : Serum zone.
*** : Reaction. † : Alkaline. ‡ : Acid.

Table 2. Availability of various sources of carbon

Rhizobial strain	Basal medium		Raffinose		Lactose		Glucose		Xylose		Mannitol	
	R*	G**	R	G	R	G	R	G	R	G	R	G
C-1-Y	−	⊕	−	⊕	−	⊕	−	++	±	++	−	++
C-2-Y	−	⊕	−	⊕	−	⊕	−	++	±	++	−	++
C-3-K	−	⊕	−	⊕	−	⊕	−	++	±	++	−	++
C-4-K	−	⊕	−	⊕	−	⊕	−	++	±	++	−	++
W-1	−	⊕	±	++	±	++	+	++	+	++	+	++
W-2	−	⊕	±	++	±	++	+	++	+	++	+	++

* : Reaction. − : Alkaline. + : Acid. ± : No change.
** : Growth. ⊕ : Slight growth. ++ : Good growth.

The growth of each strain of *Cytisus* isolates was poor in lactose or raffinose medium, and the change of reaction was the same as that of slow growers. On the other hand, the strains of *Wistaria* isolates grew well in raffinose as well as in lactose medium and showed acid reaction in the media except those containing raffinose or lactose.

From these results, it may be expected that the isolates from *Wistaria* are different from either growing type, though those from *Cytisus* are included in the slow growing type.

II. Cross Inoculation Tests

Materials and Methods

Following plants and rhizobia were used.

Plants : *Cytisus scoparius* Link., *Vicia villosa* Roth., *Trifolium repens* L., *Phaseolus vulgaris* L., *Medicago sativa* L., *Lupinus luteus* L., *Glycine Max* Merr., *Vigna sinensis* Endl., and *Astragalus sinicus* L.

Rhizobia : Isolates from *Cytisus*, isolates from *Wistaria*, *Rhizobium leguminosarum*, *R. trifolii*, *R. phaseoli*, *R. meliloti*, *R. lupini*, *R. japonicum*, types A and B of cowpea bacteria, and isolate from *Astragalus*.

Method of cultivation : Small seed plants (*Cytisus*, *Trifolium*, *Medicago*, and *Astragalus*) were grown in a bottle (500 ml vol.) containing sand, whereas for large seed plants (*Vicia*, *Phaseolus*, *Lupinus*, *Glycine*, and *Vigna*) "bottle-jar"[10] was used. Nutrients were supplied with CRONE's solution.[3]

Season and period of cultivation differed depending upon the kind of plant.

Vigna, *Phaseolus*, and *Glycine* : 29/VIII-14/IX, 24/IX-8/XI.

Cytisus : 7/IX-16/X, 20/X-29/XII.

Medicago and *Astragalus* : 25/XI-15/II.

Vicia and *Lupinus* : 25/XI-20/III.

Inoculant was prepared from the growth on yeast water mannitol agar slope.

Results

Isolates from *Cytisus* nodulated *Vigna* and both types of cowpea bacteria nodulated *Cytisus vice versa* (Tables 3 and 4). In the relationship between *Lupinus* and *Cytisus*, there existed some irregularities. All isolates from *Cytisus* did not nodulate *Lupinus*, whereas *R. lupini* did *Cytisus*. Nodule production was not observed in any other combination.

Both isolates from *Wistaria* could not nodulate any plant used.

III. Discussion

Cytisus was placed in the cowpea group by FRED *et al*,[3] though with a question mark. In addition, some descriptions on the rhizobia of *Cytisus* are observed in the "IBP World Catalogue of *Rhizobium* Collections"[1] recently published. Among them, the strain of *Cytisus prolifer* in the collection of Rhodesia is highly effective on *Phaseolus lathyroides*, poorly effective on *Crotalaria juncea*, and does not produce nodule on *Mucuna deeringianum*, *Phaseolus radiatus* and *Vigna sinensis*. These descriptions appear to differ from the authors' results in the nodulation on cowpea (*Vigna*), but the host plants, which are nodulated by the Rhodesian strain, are included in the cowpea group. Although the characters of the Rhodesian strain are unknown, such irregularities may be found in some combinations between the kind of host and the strain of rhizobia in the cowpea group.[7,8]

In the authors' results, the isolates were quite similar to the type B of cowpea bacteria[6] in the cultural and physiological characters, and the reciprocal relationship between *Vigna* and *Cytisus* was found in the cross inoculation tests. From these results, it may be stated that *Cytisus* belongs to the cowpea group. The presence of some irregularities between lupine- and cowpea-group has long been known.[7] The non-reciprocal relationship between *Lupinus* and *Cytisus* in Tables 3 and 4 may be shown as one of examples of such irregularities stated above.

Concerning the irregularities within the cowpea group, a device of dividing it into subgroups will be needed because of its exceptionally large group among the existing cross inoculation groups. Rhizobial strain of *Cytisus scoparius* is observed, too, in the collection of Czechoslovakia,[1] but description

Table 3. Nodule production of the isolates from *Cytisus* and *Wistaria* on the plant of each inoculation group

Plant \ Rhizobia	C-1-Y (Yokohama)	C-2-Y (Yokohama)	C-3-K (Kobe)	C-4-K (Kobe)	W-1	W-2
Vicia villosa	—*	—	—	—	—	—
Trifolium repens	—	—	—	—	—	—
Phaseolus vulgaris	—	—	—	—	—	—
Medicago sativa	—	—	—	—	—	—
Lupinus luteus	—	—	—	—	—	—
Clycine Max	—	—	—	—	—	—
Vigna sinensis	+**	+	+	+	—	—
Astragalus sinicus	—	—	—	—	—	—
Cytisus scoparius	+	+	+	+	—	—

* : Nodule was not produced.
** : Nodule was produced.

Table 4. Nodule production of rhizobia of main cross inoculation groups on *Cytisus scoparius*

Rhizobia	*Cytisus scoparius*	Rhizobia	*Cytisus scoparius*
Rhizobium leguminosarum	—*	*Rhizobium japonicum*	—
R. trifolii	—	*Rhizobium* of cowpea (A)	+
R. phaseoli	—	*Rhizobium* of cowpea (B)	+
R. meliloti	—	*Rhizobium* of *Astragalus*	—
R. lupini	+**		

* : Nodule was not produced.
** : Nodule was produced.

is made neither on the characters nor on the nodulation pattern on other plants.

Although the isolates from *Cytisus* were only type B of cowpea bacteria, rhizobia of cowpea group are so diverse[6] that type or types other than type B will certainly be isolated when isolation is tried using *Cytisus* from different districts.

On the rhizobia of *Wistaria*, it appears that no description has been made, though there is a report in which *Wistaria Chinensis* is an individual group,[2] and from this it may be suggested that the rhizobia of *Wistaria* are specific. But their characters are unknown.

According to the authors' results, the isolates from *Wistaria floribunda* are rather specific in the growth rate and in the change of litmus milk as compared with the so-called fast or slow growers. And from the cross inoculation tests, it is known that both isolates from *Wistaria* do not nodulate any plant of major cross inoculation groups. Although the reciprocal relationship is not known, it may be stated that *Wistaria floribunda* is a separate group different from the major cross inoculation groups (pea-, clover-, bean-, alfalfa-, lupine-, soybean- and cowpea-group). Whether *Wistaria* is really

an individual group or not must be determined after the study on the relationship between *Wistaria* and existing minor groups.

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エニシダ（*Cytisus scoparius* Link.）及び フヂ（*Wistaria floribunda* DC.）の根粒菌について

石沢修一・細見澄子

要旨

日本にかなり広くみられるエニシダ，フヂの根粒菌については，研究がひじょうに少ないという事情にかんがみ，その根粒菌の性状を調べるとともに，交互接種試験によって両植物の所属を検討した。

エニシダの菌はダイズなど生育の遅い型に類似し，交互接種試験の結果，カウピー群に含めうることが知られた。一方，フヂの菌はその生育の早さは，生育の遅い型とエンドーなど生育の早い型の中間にあり，ポテト，ブイヨンに発育せず，硝酸還元陽性，リトマスミルクでは透明帯を生じ，酸性を示す。また，ラフィノース，ラクトースをもよく利用する。この菌は主なる接種群の植物に根粒を形成せず，逆の関係は不明であるが，これらの群のいずれにも入らぬものとみられる。しかし，独立の群とするかどうかは，他の小さな群との関係をみてから決定しなければならない。