Assessment of river environment using *Pythium* species

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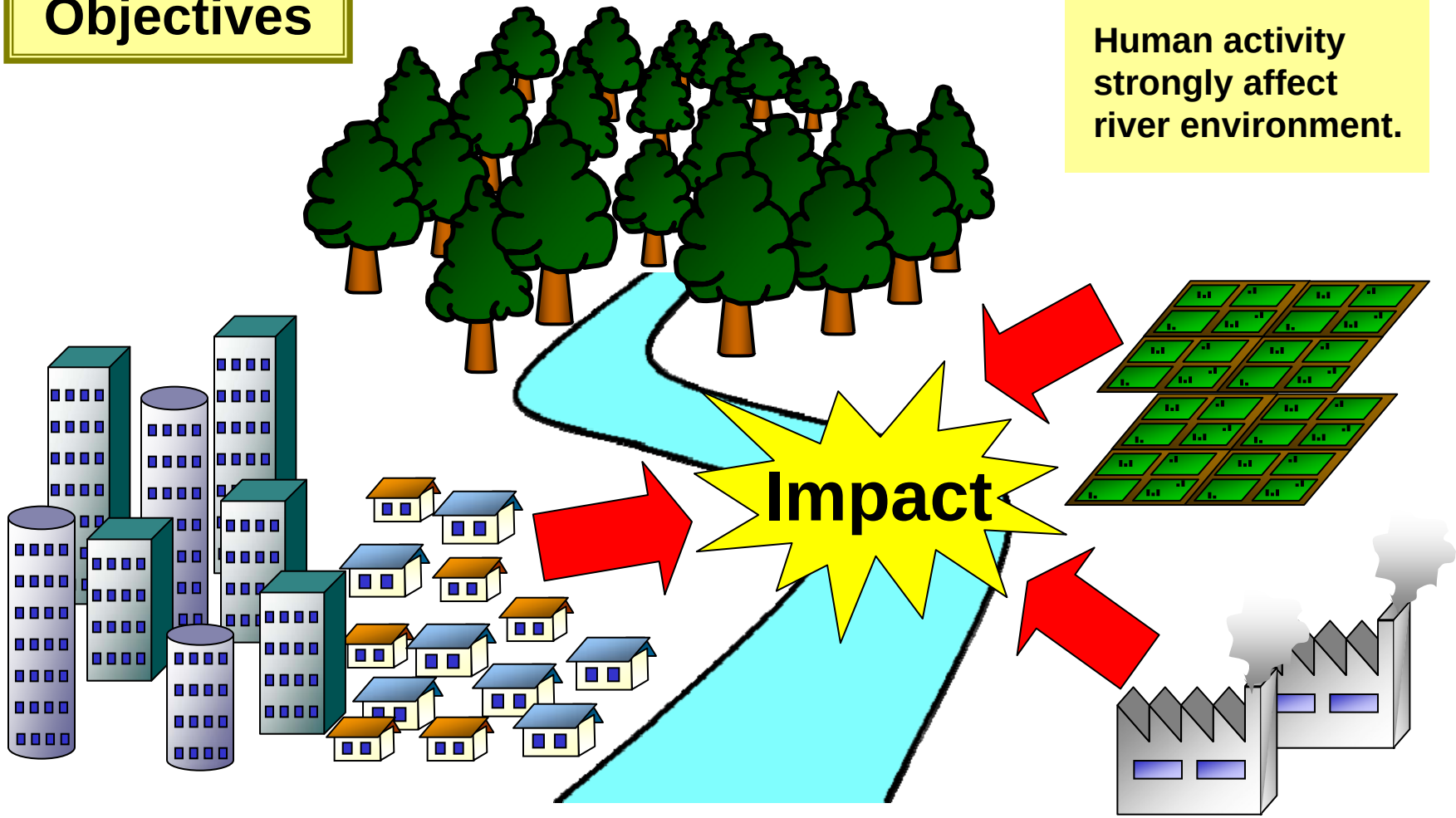
Research background

- **Recently, it is important to assess the effect of human activity on natural ecosystem.**
- **Previous researches have tried environmental assessment in view of changes on plant flora and animal fauna.**
- **There are few reports to be focused on soil microorganisms**
- **Soil microorganisms play important roles on preservation of natural ecosystem and have a potential to quickly response to environmental changes.**

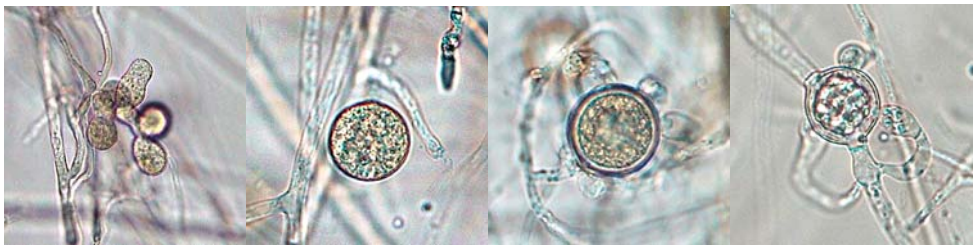
Objectives

Human activity strongly affect river environment.

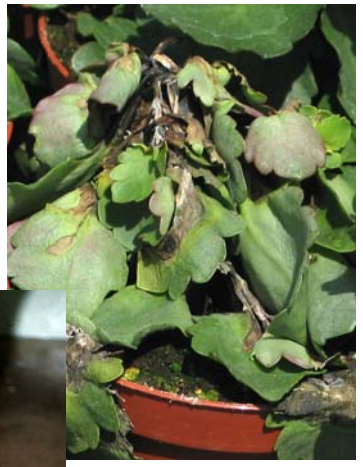


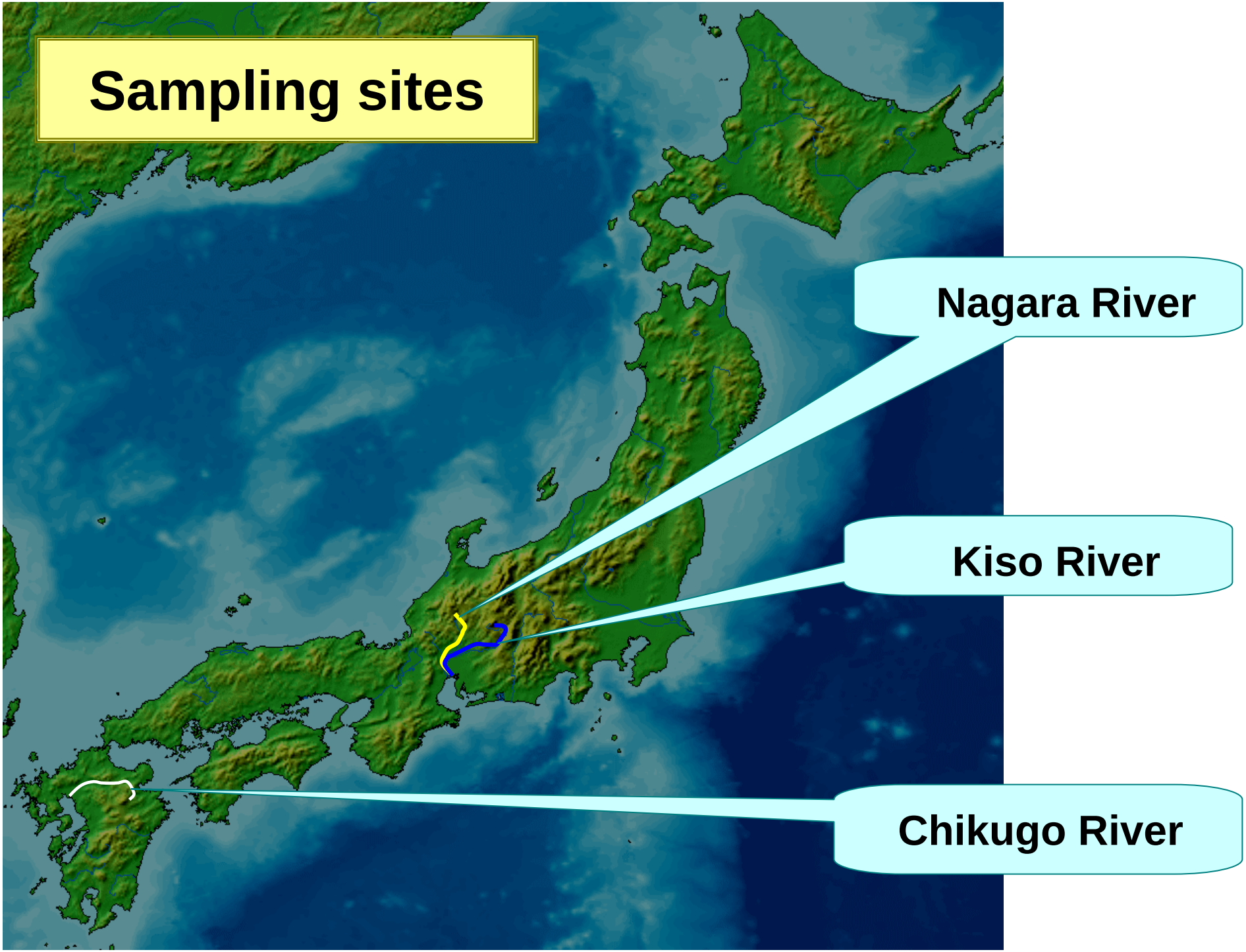
To determine potential assessment of human activity on river environment using soil microorganism ⇨ ***Pythium***

Why *Pythium* as target genus ?



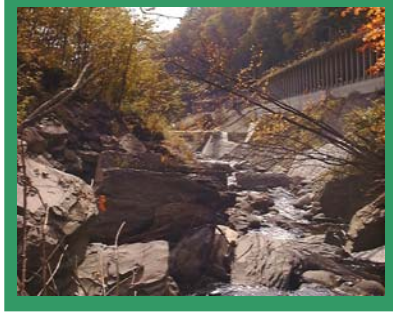
- **The genus consists of more than 120 species and inhabits in freshwater and seawater as well as soil.**
- **The genus have a wide range of functional species, such as saprophytes, animal and plant pathogens, mycoparasites.**
- **The selective medium for isolation from environmental samples is available.**
- **Identification of the species is practicable based on the morphological characteristics and molecular techniques.**




Sampling sites
Nagara River
Kiso River
Chikugo River

Nagara River

A: Owashi



B: Kamagahora

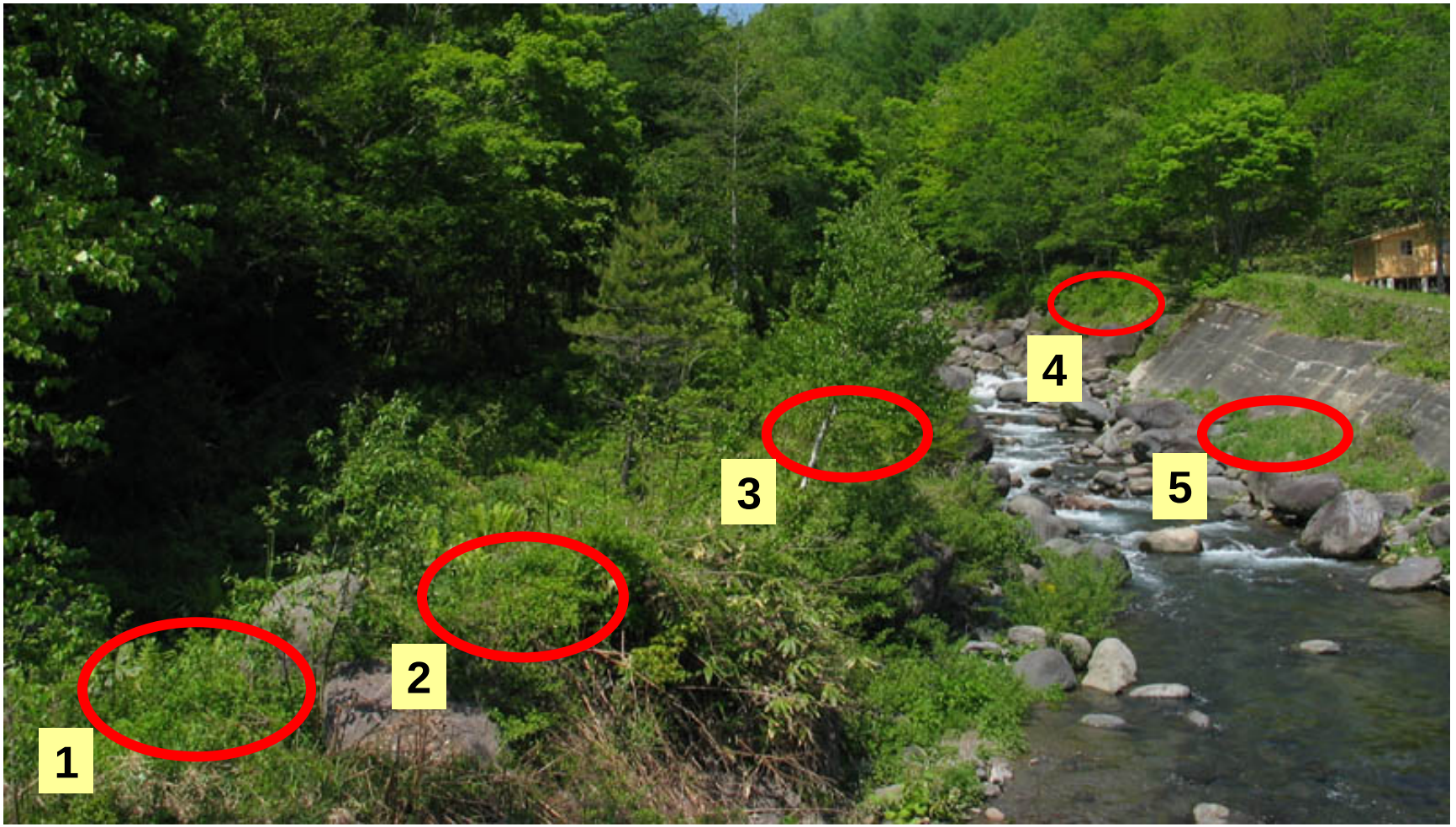
C: Yamato

D: Mino

E: Gifu

F: Kaizu

Soil Sampling



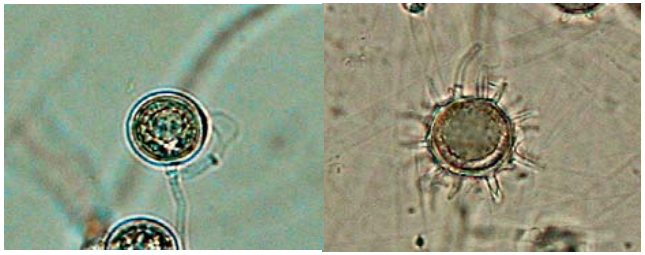
- Select five spots in 100m area along the river

Identification

Morphological characterization

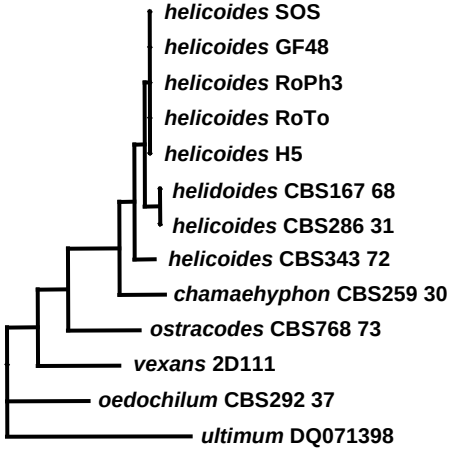
Asexual structure

Sexual structure

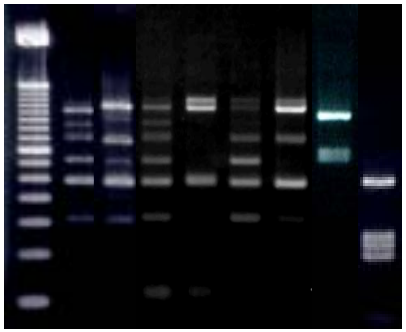


Molecular identification

Phylogenic analysis



PCR-RFLP



Pythium species from riverbed soils in three rivers

13 species including 3 new species and 4 groups

Chikugo River

P. nagaii

***Pythium* spp.**

P. spinosum

P. vexans

P. dissotocum

P. irregulare

P. rostratifingens

P. torulosum

Group F

Group HS

Nagara River

P. acanthicum

P. inflatum

P. perplexum

P. rostratum

P. vanterpoolii

P. parvum

Group P

Group T

***Pythium* spp.**

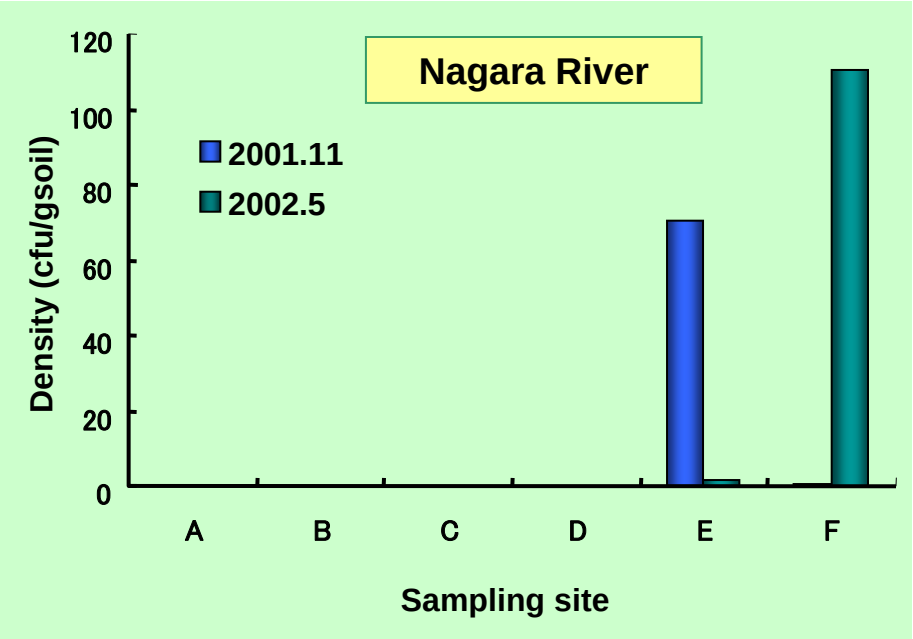
Kiso River

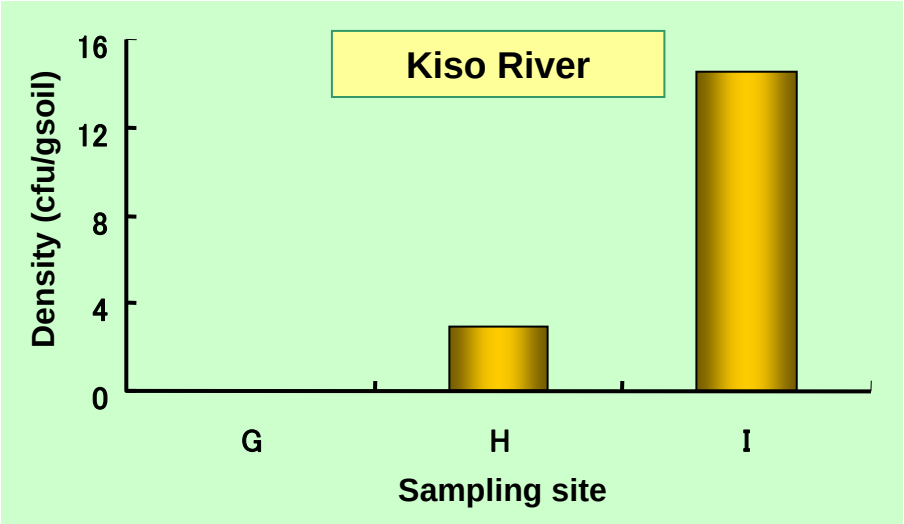
***Pythium* spp.**

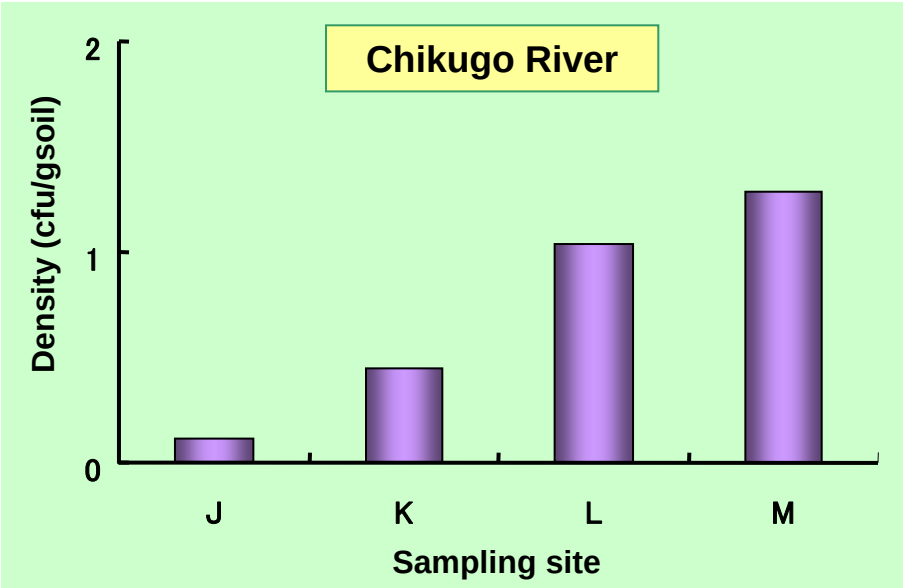
Population density of *Pythium* species in three rivers

Sampling site			Population density (cfu/g dry soil)[a]																	
			aca	dis	inf	irr	nag	par	per	rof	ros	spi	tor	van	vex	HS	P	F	T	spp.	Total
Nagara 2001.11	A	Up	0.0	3.3	0.0	0.0	0.0	0.0	0.0	8.6	0.0	0.0	2.7	0.0	44.7	156.2	0.0	0.0	0.0	2.6	218.0
	B		0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	3.1	0.0	0.0	42.9	0.0	15.7	0.6	20.7	84.3
	C		0.0	1.6	0.6	0.1	0.0	0.0	0.0	0.6	0.0	0.0	0.6	0.1	0.1	10.5	0.1	0.4	1.5	0.1	16.1
	D		0.0	0.8	0.0	0.2	0.0	0.0	0.0	3.6	2.7	0.0	0.1	0.0	0.8	3.7	0.0	0.0	0.0	0.1	11.9
	E		0.0	22.6	0.0	70.2	0.0	3.2	0.1	3.2	0.0	0.0	0.0	0.0	19.0	200.0	0.0	0.0	4.8	0.0	323.1
	F	Down	0.0	0.0	0.0	0.6	0.0	0.0	0.0	29.2	0.0	8.9	0.0	0.0	0.5	3.4	0.0	0.0	0.0	0.0	42.5
Nagara 2002.05	A	Up	0.0	18.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.7	2404.4	0.0	0.0	0.0	0.0	2513.5
	B		0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0	5.9	0.0	0.0	650.4	0.0	10.3	0.0	3.0	681.5
	C		0.0	5.4	0.0	0.0	0.0	0.0	0.0	2.4	0.0	2.4	0.0	0.0	1.3	30.9	0.0	0.0	0.9	0.1	43.2
	D		0.3	0.7	0.0	0.0	0.0	0.0	0.0	4.2	0.6	0.3	0.2	0.0	0.1	1.7	0.0	0.3	0.0	0.1	8.4
	E		0.0	3.4	0.0	1.5	0.0	0.0	0.0	59.6	0.0	0.0	0.0	0.6	0.0	40.3	0.0	0.0	0.0	0.0	105.7
	F	Down	0.0	37.6	3.1	110.5	0.0	0.0	12.3	0.0	0.0	0.0	15.0	81.6	0.0	753.8	0.0	0.0	56.4	8.9	1079.2
Kiso 2003.6	G	Up	0.0	57.8	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	16.7	0.0	0.0	1416.7	0.0	33.3	0.0	0.3	1526.5
	H		0.0	29.5	0.0	2.9	0.0	0.0	0.0	2.8	0.0	0.0	2.6	0.0	0.0	15.0	0.0	1.9	0.0	0.3	55.0
	I	Down	0.0	0.0	0.0	14.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	874.1	0.0	32.7	0.0	0.0	921.3
Chikugo 2003.6	J	Up	0.0	3.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	3.2	0.0	4.6	0.0	0.9	13.4
	K		0.0	0.3	0.0	0.4	0.0	0.0	0.0	0.1	0.0	0.5	0.1	0.0	0.1	2.7	0.0	0.4	0.0	0.9	5.7
	L		0.0	2.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.0	0.0	3.7	0.0	2.8	0.0	1.7	12.1
	M	Down	0.0	0.8	0.0	1.3	0.3	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	14.3	0.0	2.0	0.0	0.0	19.8

[a] aca, *P. acanticum*; dis, *P. dissotocum*; het, *P. heterothallicum*; inf, *P. inflatum*; irr, *P. irregulare*; nag, *P. nagaii*; par, *P. parvum*; per, *P. perplexum*; rof, *P. rostratifingens*; ros, *P. rostratum*; spi, *P. spinosum*; tor, *P. torurosum*; van, *P. vanterpoolii*; vex, *P. vexans*; HS, *Pythium* group HS; G, *Pythium* group G; P, *Pythium* group P; F, *Pythium* group F; T, *Pythium* group T; spp, *Pythium* spp.

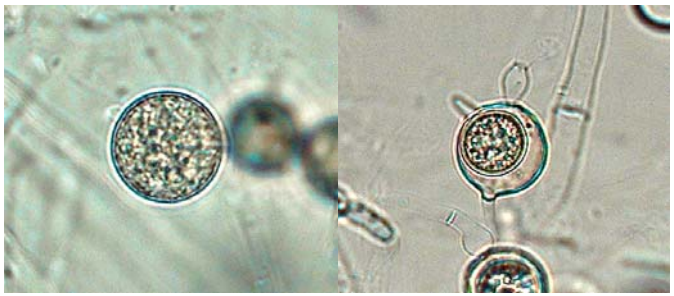






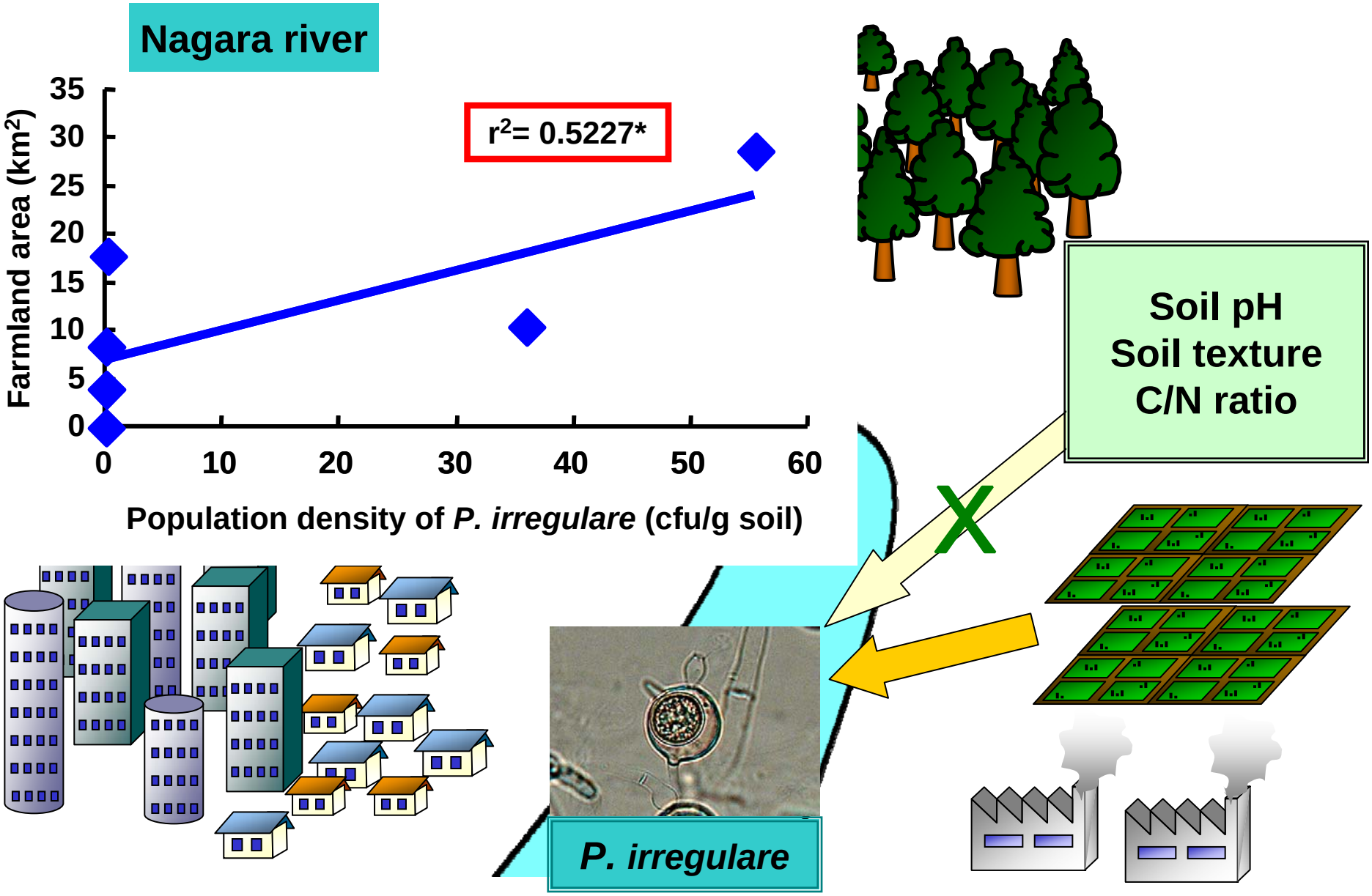
Population density of *Pythium irregulare* strain in riverbed soil

Is *Pythium irregulare* available as indicator of river environmental assessment ?



To examine the relationships between the distribution of* P. irregulare *and environmental factors

1. **Soil pH**
2. **Soil texture**
3. **Carbon/nitrogen ratio in soil**
4. **Farmland area**

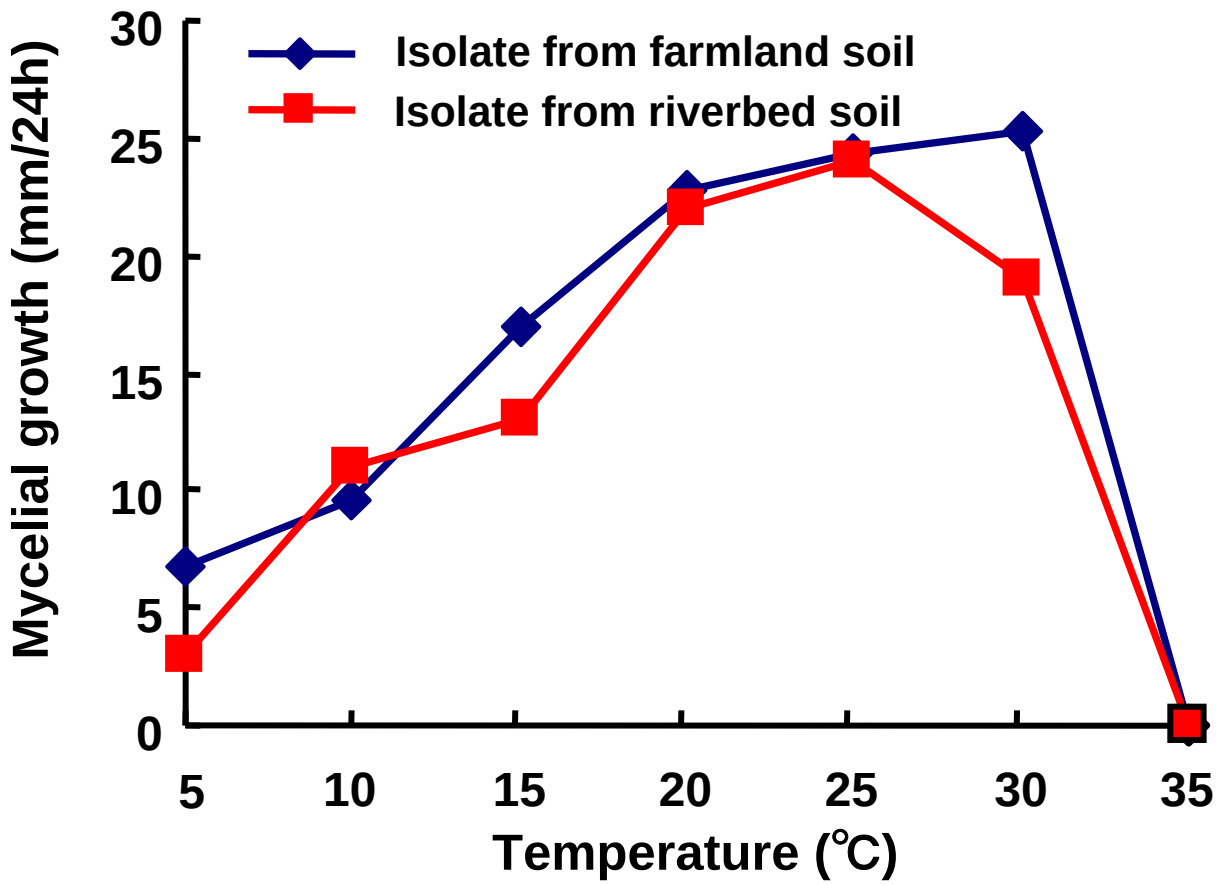


The distribution of *P. irregulare* will correlate with agricultural practice

To verify if* P. irregulare *will be available for assessment of the impact of agriculture

- **The growth rate of *P. irregulare* from riverbed soil**
- **Pathogenicity of *P. irregulare* from riverbed soil to crop plants**
- **The influence of *Pythium* flora in farmland soil on the flora in riverbed soil.**

Mycelial growth rate of *Pythium irregulare*



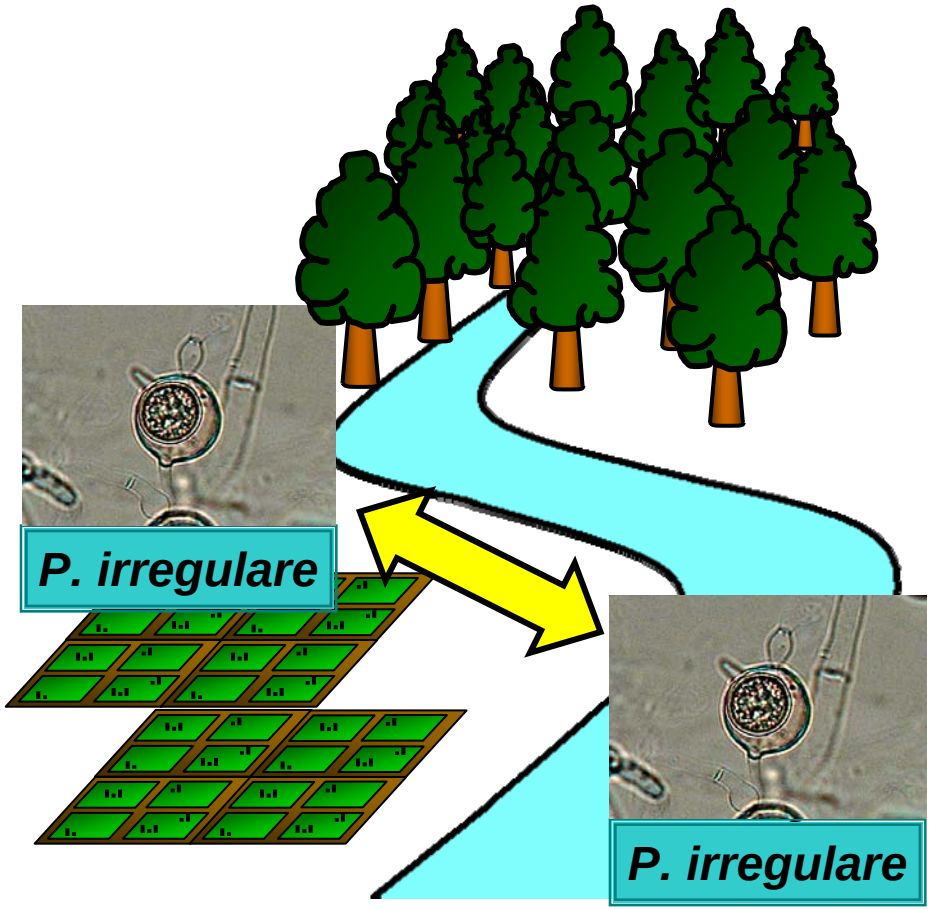
P. irregulare **in riverbed soil will have similar growth habit of that in farmland soil**

Pathogenicity of *Pythium irregulare* from riverbed soil

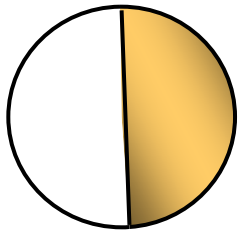
Isolate	Cucumber		Tomato		Chinese cabbage	
	EM[a]	DI[b]	EM	DI	EM	DI
Nagara isolate	0	0	17	100	90	0
Kiso isolate	73	59	5	100	75	0
Non-inoculated	67	0	93	0	90	0

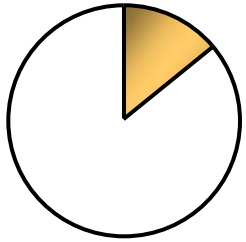
a: Percentage of emergency
b: Disease incidence

***P. irregulare* in riverbed soil is pathogenic to crops**



Population structure in farmland soil of the river basin





P. irregularе

The population structure of *Pythium irregularе* in farmland soil will affect that in riverbed soil.

Conclusion

- Population density of *P. irregulare* is correlated with
  - Soil pH ×
  - Soil texture ×
  - Soil Carbon/Nitrogen ratio ×
  - Farmland area ○
- *P. irregulare* from riverbed soil shows similar growth response to temperature to the response from farmland soil.
- *P. irregulare* from riverbed soil also has pathogenicity to crops.
- Population structure of *Pythium* species in farmland soil influences that in riverbed soil.

***P. irregulare* will be available as an indicator for assessment of the environmental impact of agriculture.**

Future perspective

More ecological studies are necessary to clarify impacts of human activities on environment including agriculture.

Molecular quantitative and qualitative detection methods are strongly required to make ecological studies easier and common.

Our Research Group

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