Biotechnology for Recovery of Rare Metals in Wastewater

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Rare-metal Bioremediation and Recycling using Biotechnology

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AGENDA

Selenium as a resource and a pollutant

Double benefit concept for selenium recovery from wastewater

Selenium-reducing bacterium (1)
Bacillus selenatarsenatis SF-1

Selenium-reducing bacterium (2)
Pseudomonas stutzeri NT-I

Possible applications of metal biology to other rare metals

Rare Metals

Group	1	2		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Period																			
1	1 H																		2 He
2	3 Li	4 Be												5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg												13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca		21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr		39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	*	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	**	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Uub	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo
*Lanthanoids			*	57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb		
**Actinoids			**	89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No		

☐ Rare Metal ☐ Rare Earth

Consumption of metals is increasing in hi-tech Industries.

Environmental Issues Related to Rare Metals

Rare metals are emitted into environment via wastes and wastewater

⇩

Environmental Contamination

Elevated concentration of rare metals can cause toxic or adverse effects on the environment.

Decay/Depletion as Resource

Shortage as resource has started due to loss as wastewater and wastes, soaring market price of elements.

⇩

Recovery of rare metals from wastes/wastewater is necessary for sustainable society.

Selenium as Example

As Environmental Contaminant

Se-Wastewater

Selenate (SeO_4^{2-}) : Soluble Se
Selenite (SeO_3^{2-}) : Toxic

Environmental standard on water quality ≦ 0.01mg-Se/L

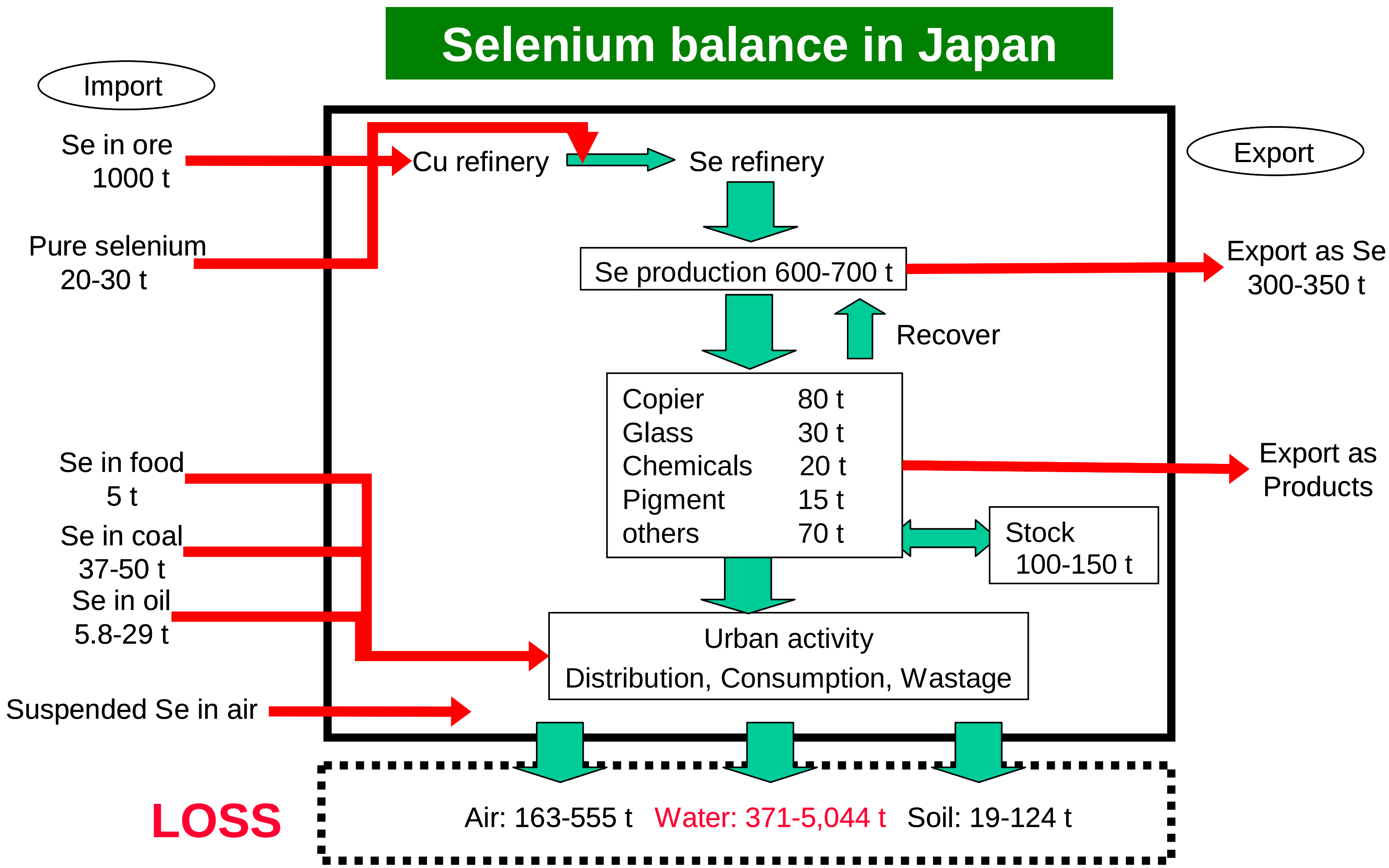
Regulation of industrial wastewater ≦ 0.1mg-Se/L

Provisional regulation for Se industry ≦ 0.3 mg-Se/L

Industry	Se conc.
Oil refinery	50-300 μg/l
Copper purification plant	>20 mg/l
Chemical plant	250 mg/l

Selenium as Example

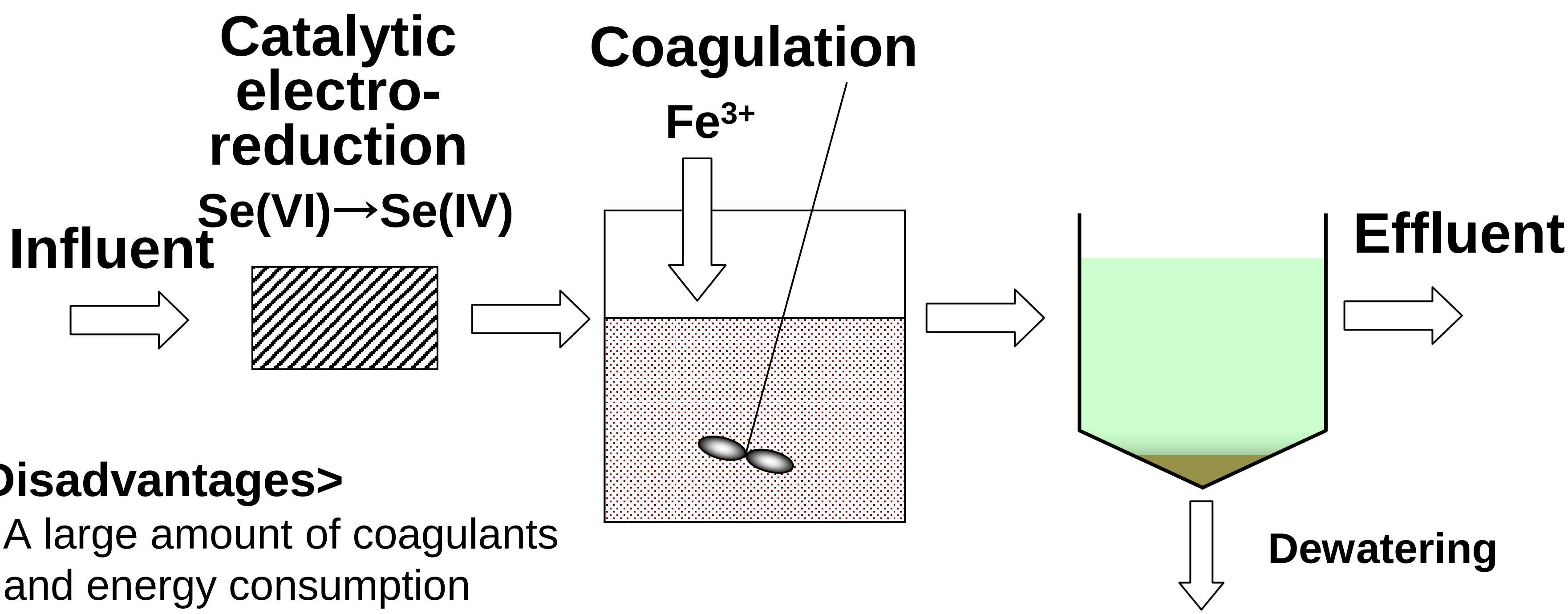
As Resource



Market Price: 2.01 $/lb in 1996 ➡ 47.4 $/lb in 2005

Existing Se Wastewater Treatment

Physico-Chemical Process



Chemical sludge (Se <1%)

*** Cannot be recycled due to low Se content**

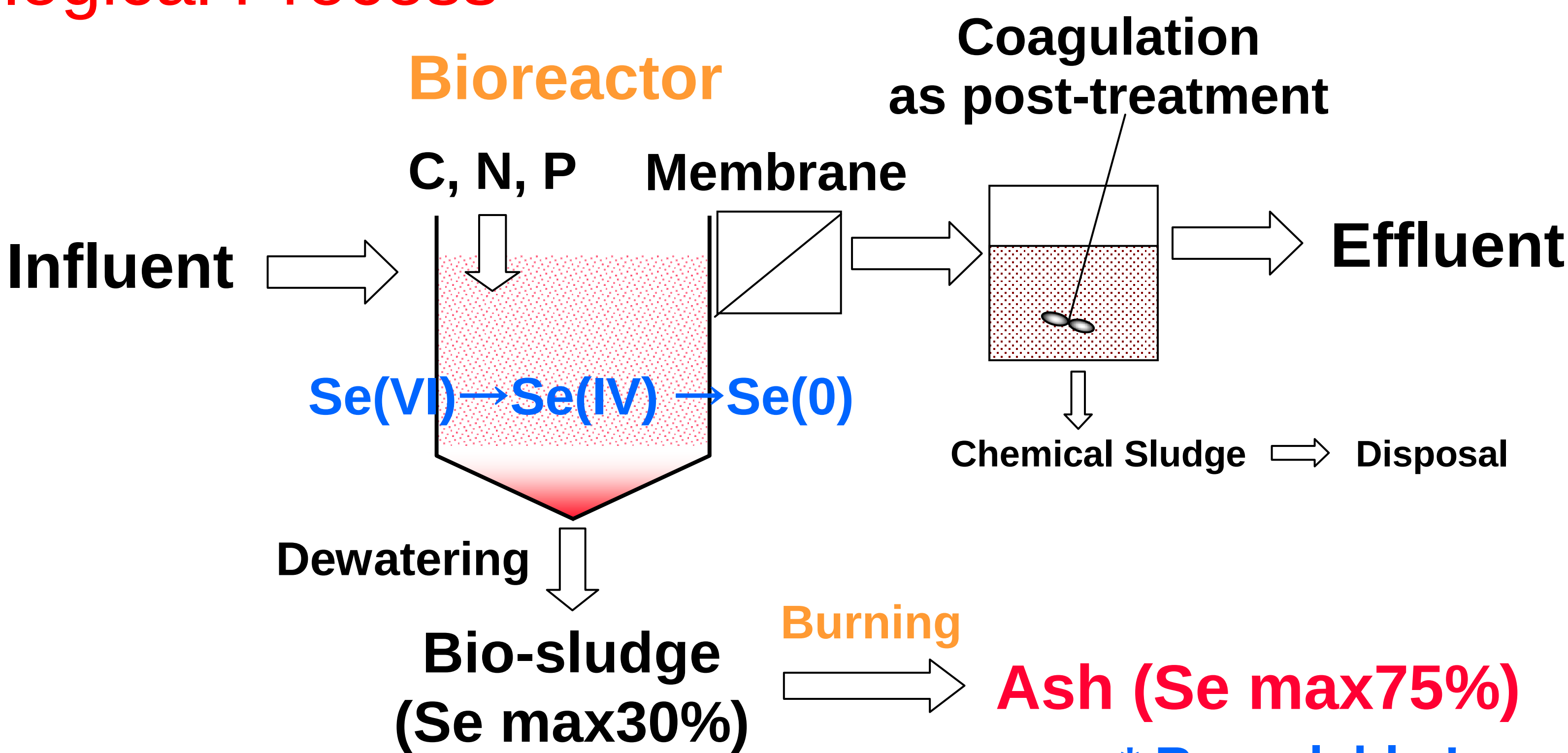
Waste disposal cost ¥30,000/ton-sludge!

<Disadvantages>

- A large amount of coagulants and energy consumption
- Generation of a large amount of sludge
- Low resource value of sludge due to low Se content
- High-cost (including waste disposal cost)

Novel Concept for Se Recovery from Wastewater

Biological Process



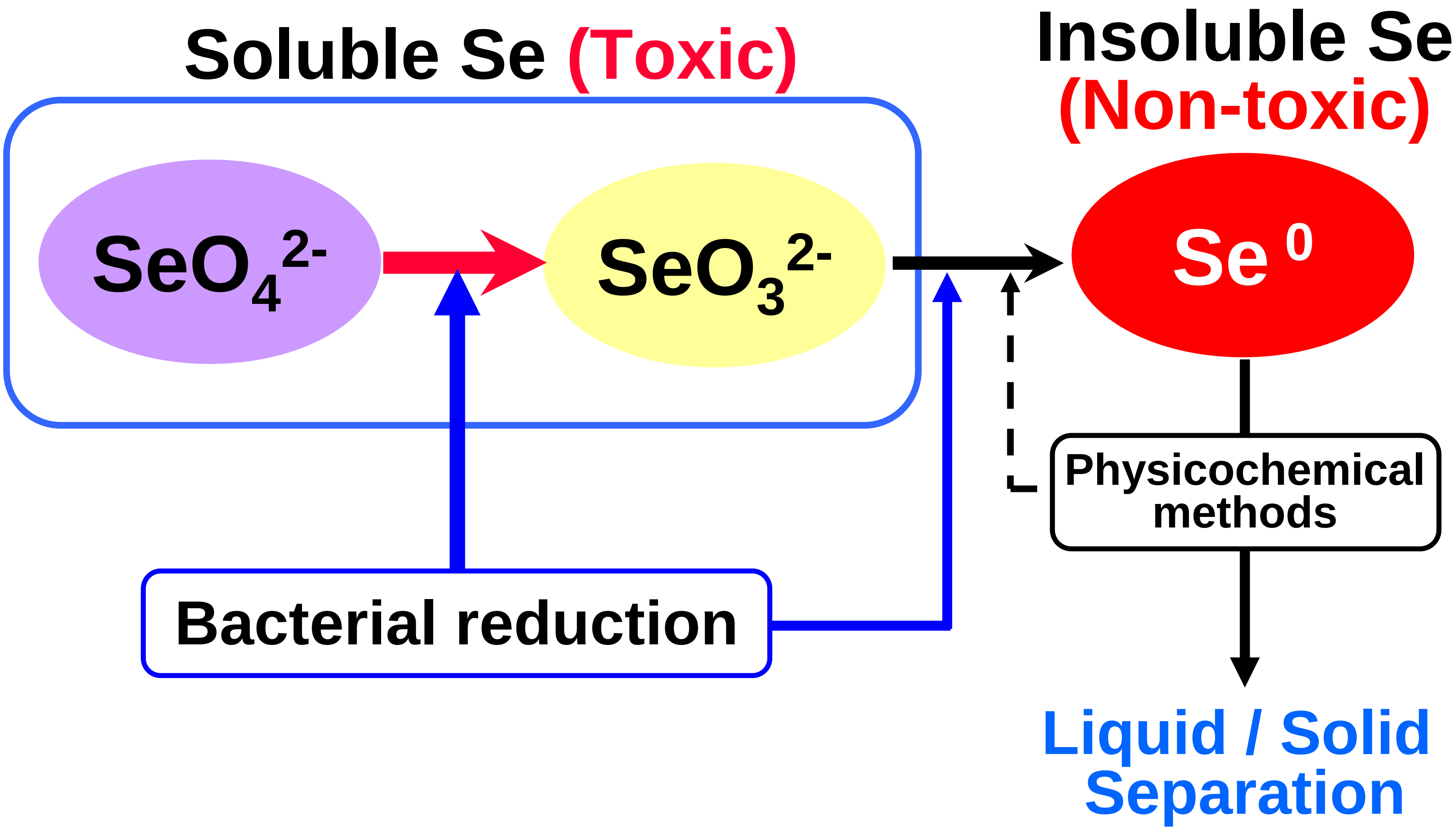
*** Recyclable !**
Due to high Se content
Benefit ¥ 650,000/ton-ash

<Advantages>

- Low energy and reagent consumption
- Low sludge generation
- High resource value of bio-sludge due to high Se content
- Disposal cost turns a profit by Se recycle

Key Microbial Reaction

Selenate/Selenite Reduction into Elemental Se

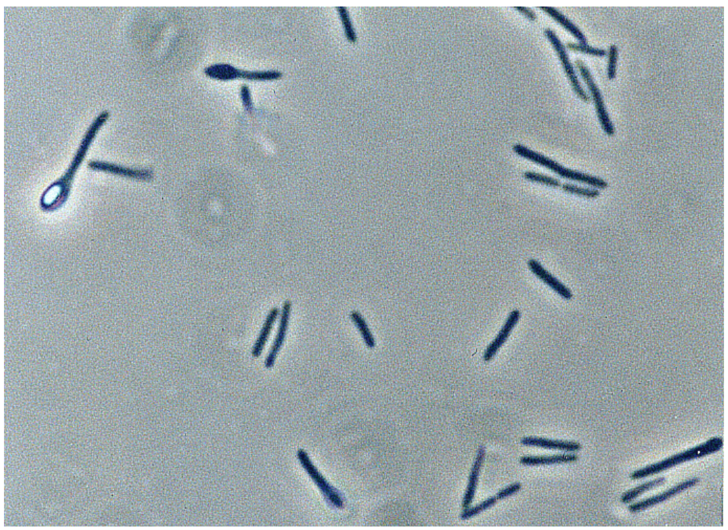


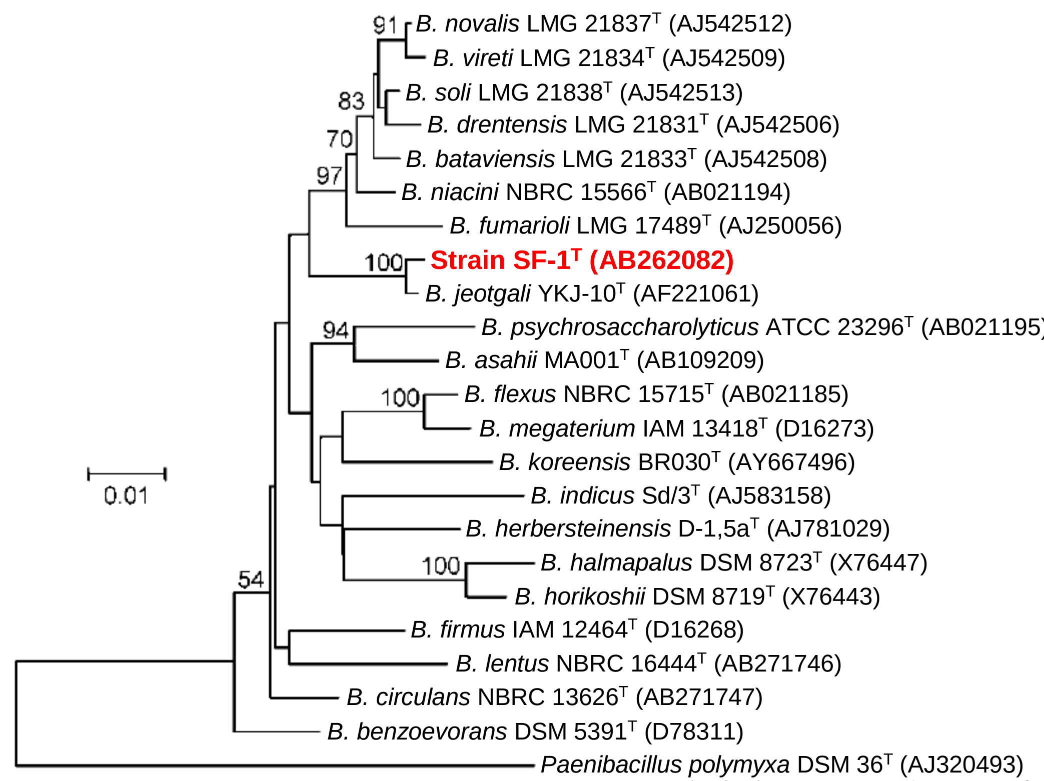
Selenate-Reducing Bacterium (1)
Bacillus selenatarsenatis SF-1

Origin: Se-contaminated sediment

Taxonomy: gram positive, facultative anaerobe

Selenate reduction: respiration with selenate as e^- acceptor



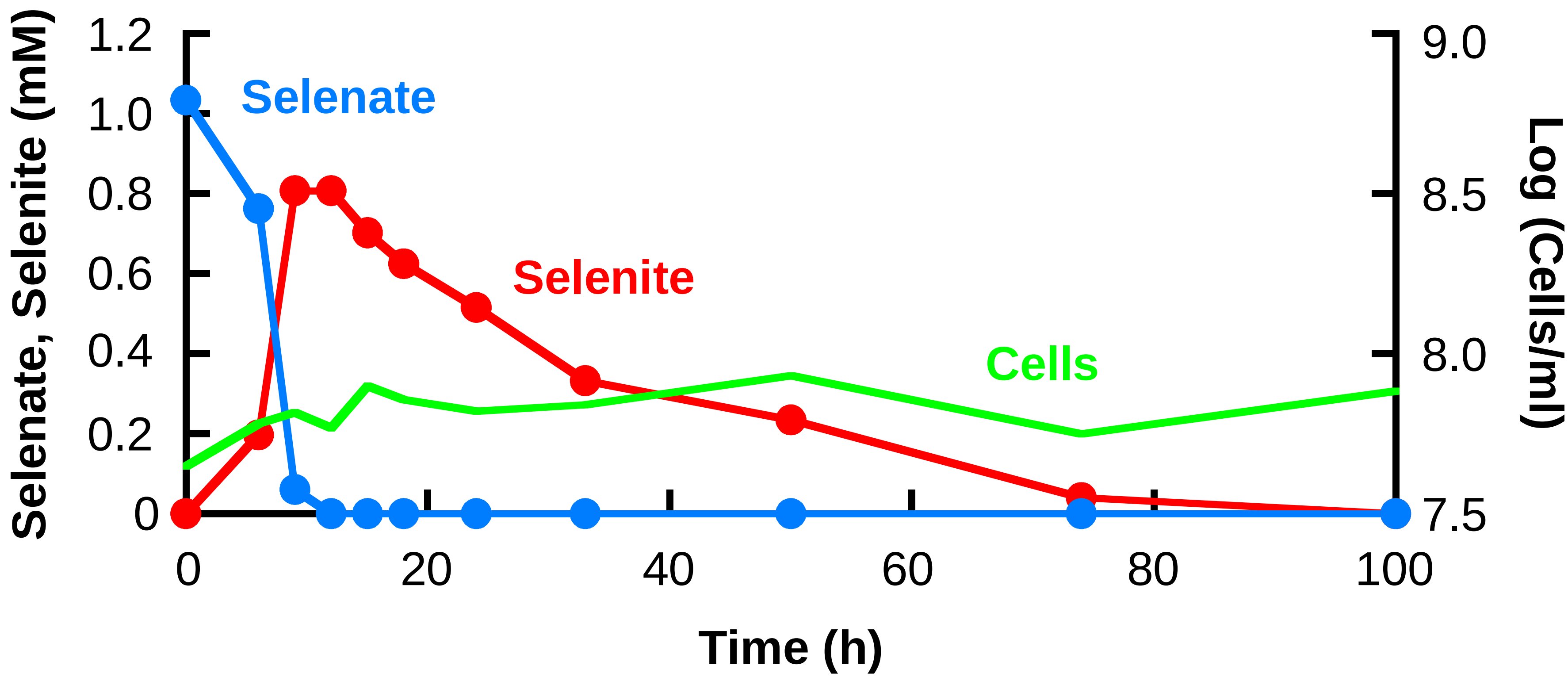


Novel species of *Bacillus*

Bacillus selenatarsenatis

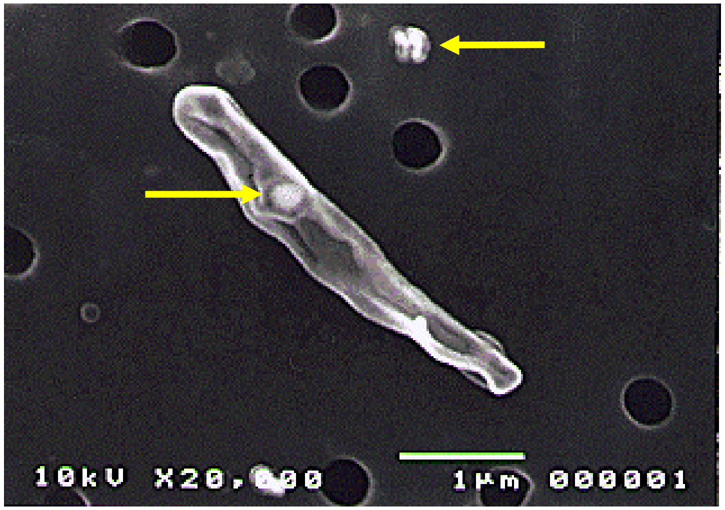
Fujita et al. (1997) J. Ferment. Bioeng., 83, 517-522;.
Yamamura et al. (2007) Int. J. Syst. Evol. Microbiol., 57, 1060-1064.

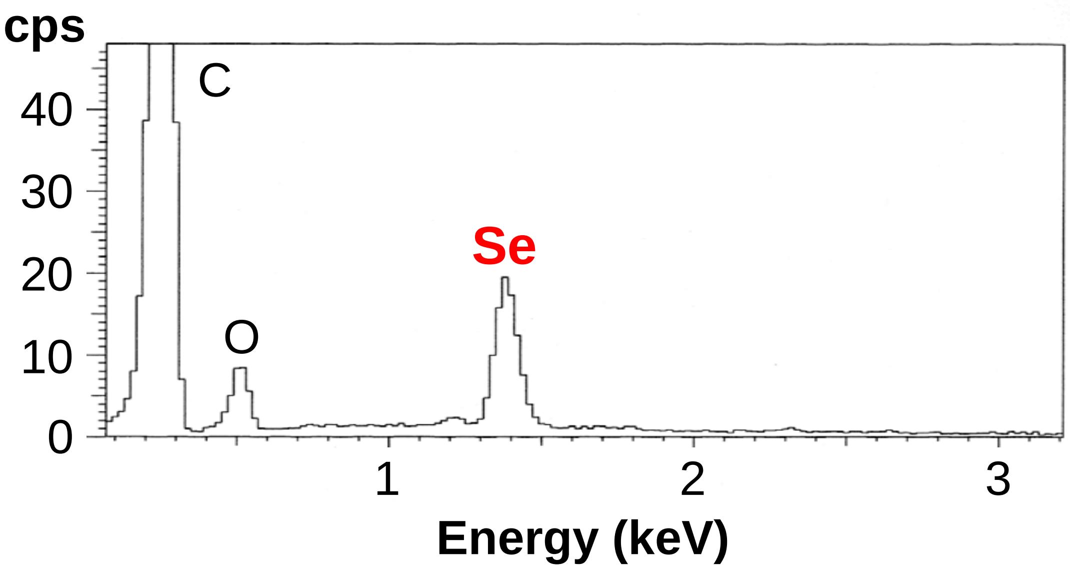
Selenate Reduction by *B. selenatarsenatis* SF-1



Under anoxic condition (energy saving)
Rate-determining step in selenite reduction

Deposition of Elemental Se by *B. selenatarsenatis* SF-1





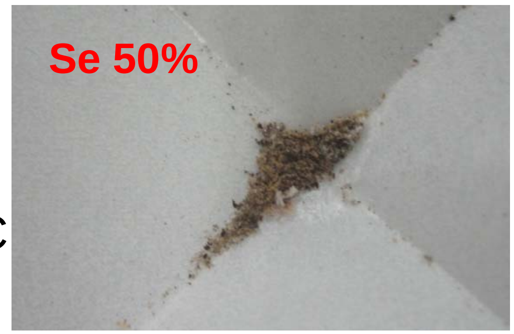
Se deposition in cells (need for extraction from biomass)

Lab-scale Se Recovery Process with *B. selenatarsenatis* SF-1

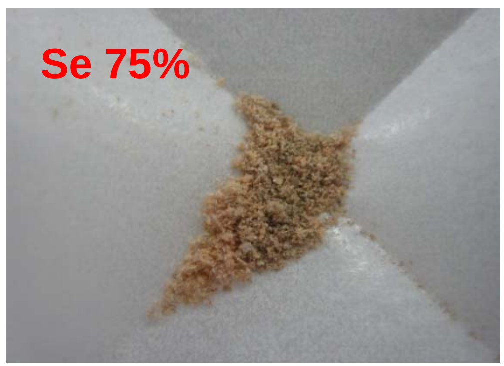
Se Recovery from Biomass

Se-enriched Biomass

Burn at 500ºC (Acidic)

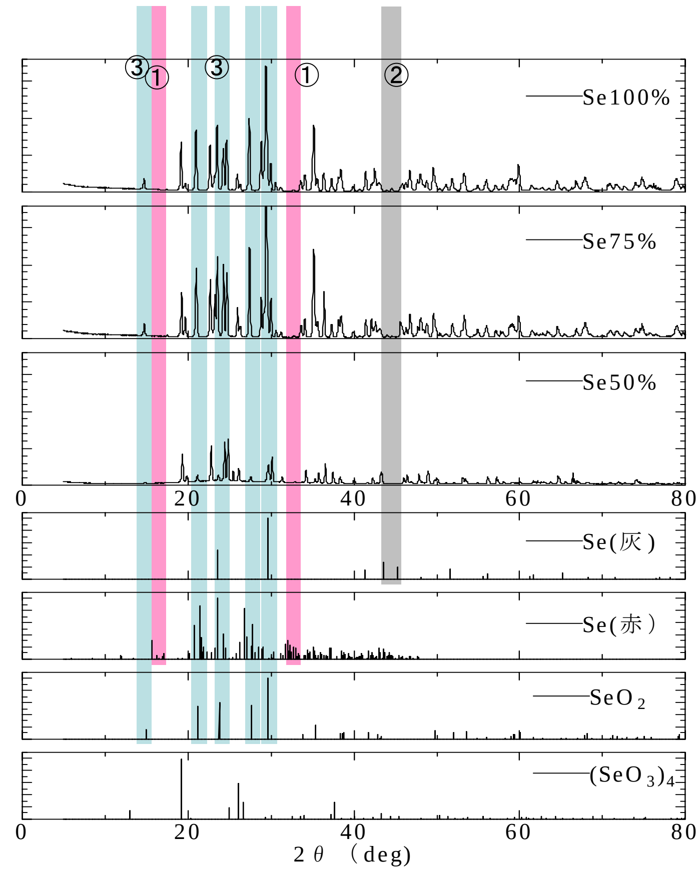


→ Se



→ SeO_2

X-Ray Diffraction (XRD) analysis



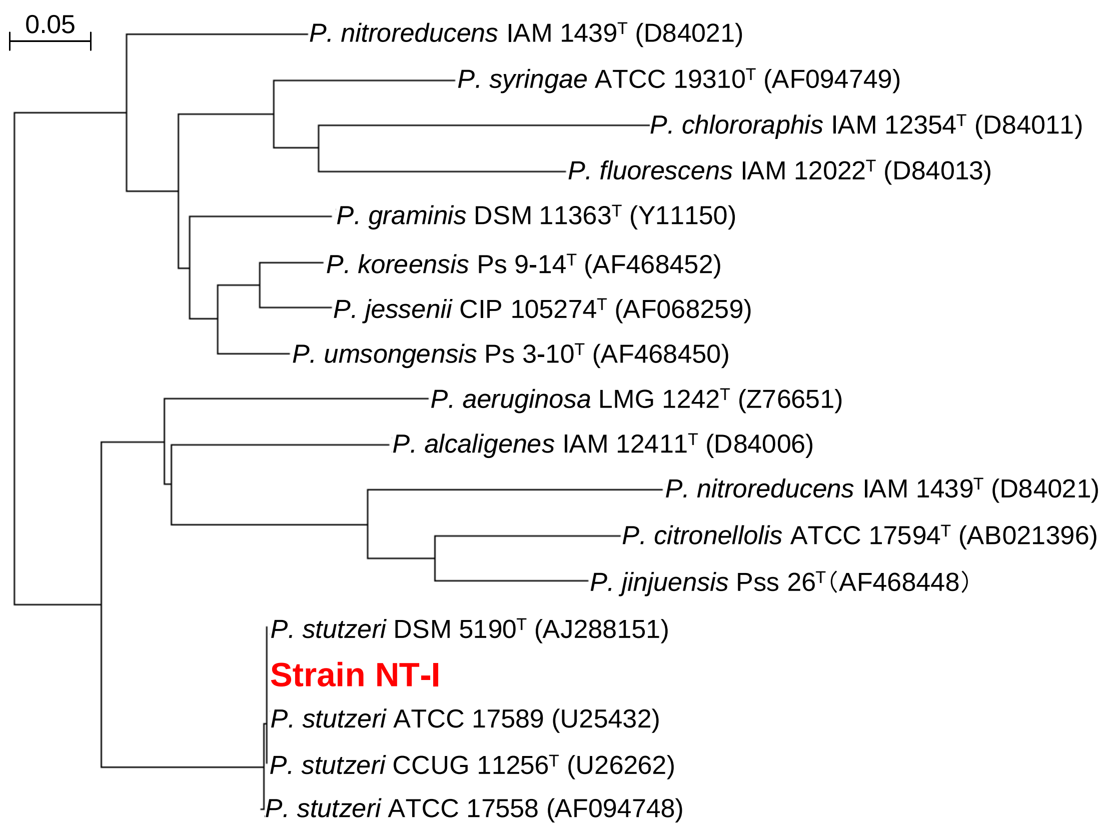
Se metal or SeO_2 can be recovered from the biomass

Selenate-Reducing Bacterium (2)
Pseudomonas stutzeri NT- I

Origin: Biofilm from Se wastewater drainage

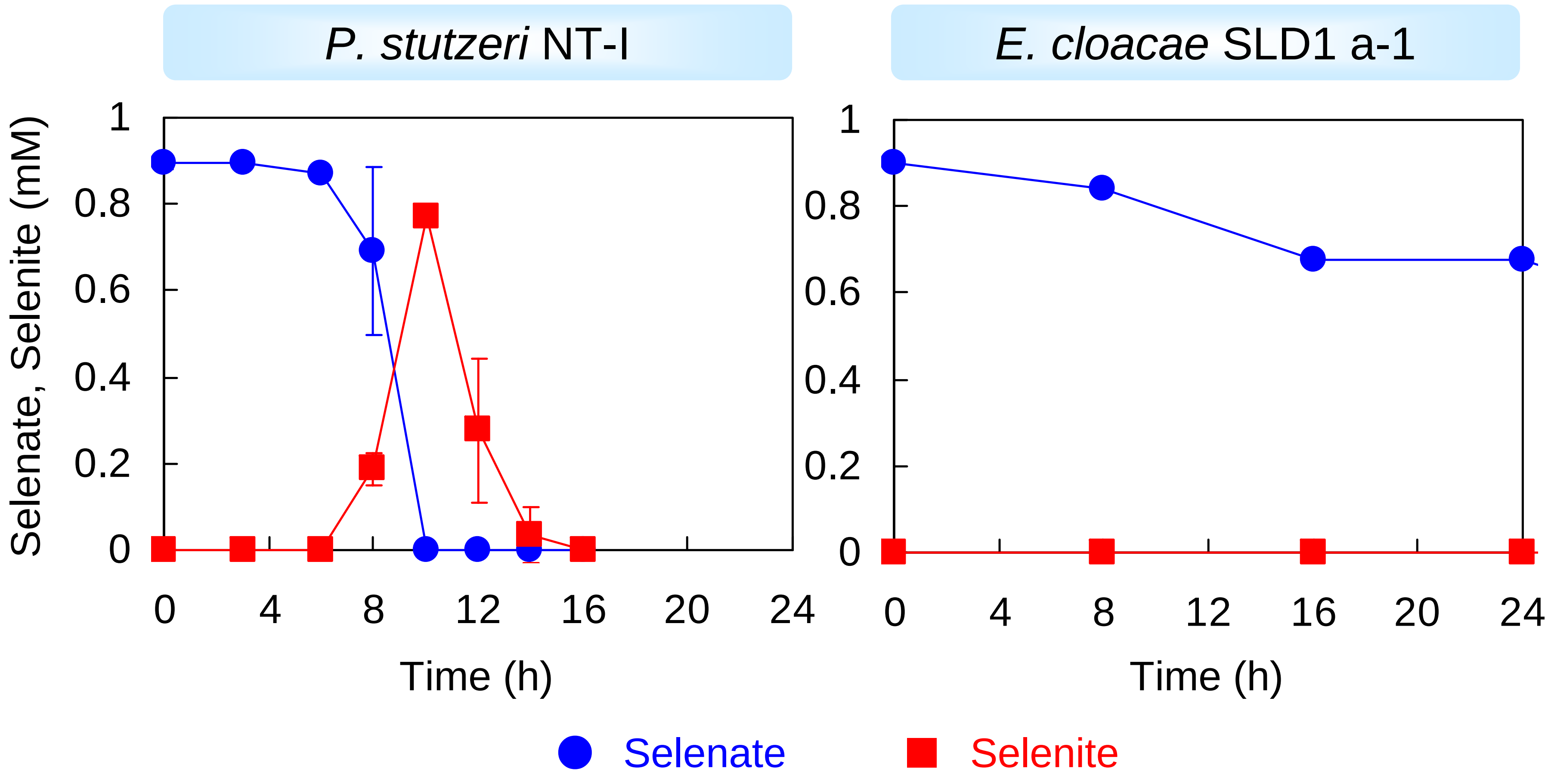
Taxonomy: gram negative, aerobe

Selenate reduction: in the presence of oxygen (detoxification?)



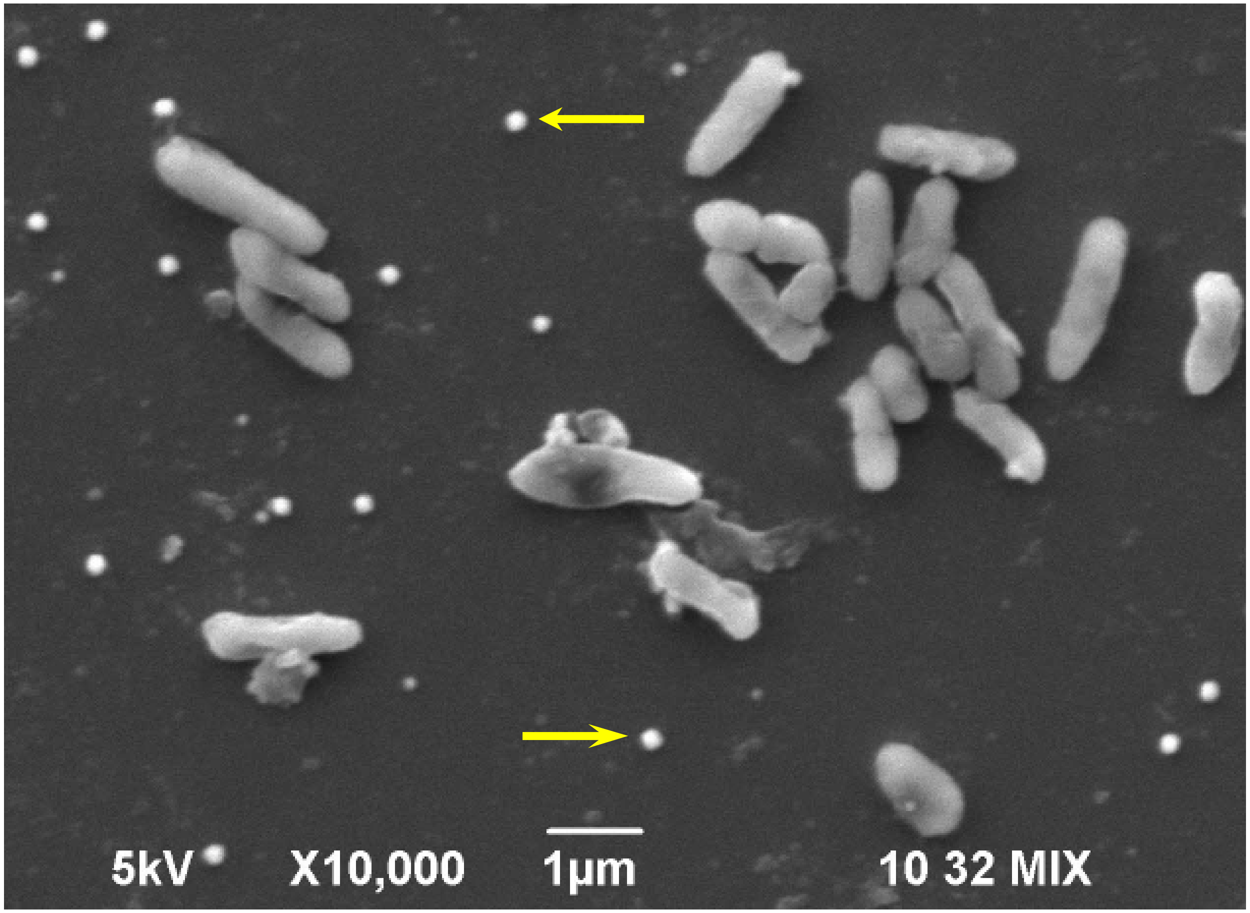
Soda et al. (2008) 5th SETAC World Congress.

Selenate Reduction Property by *P. stutzeri* NT- I



Under aerobic condition (energy consuming)
Very effective reduction (the fastest reduction rate reported)

Deposition of Elemental Se by *P. stutzeri* NT- I



Se deposition outside cells (simple separation may be possible)

Pilot-scale Se Recovery Process with *P. stutzeri* NT- I

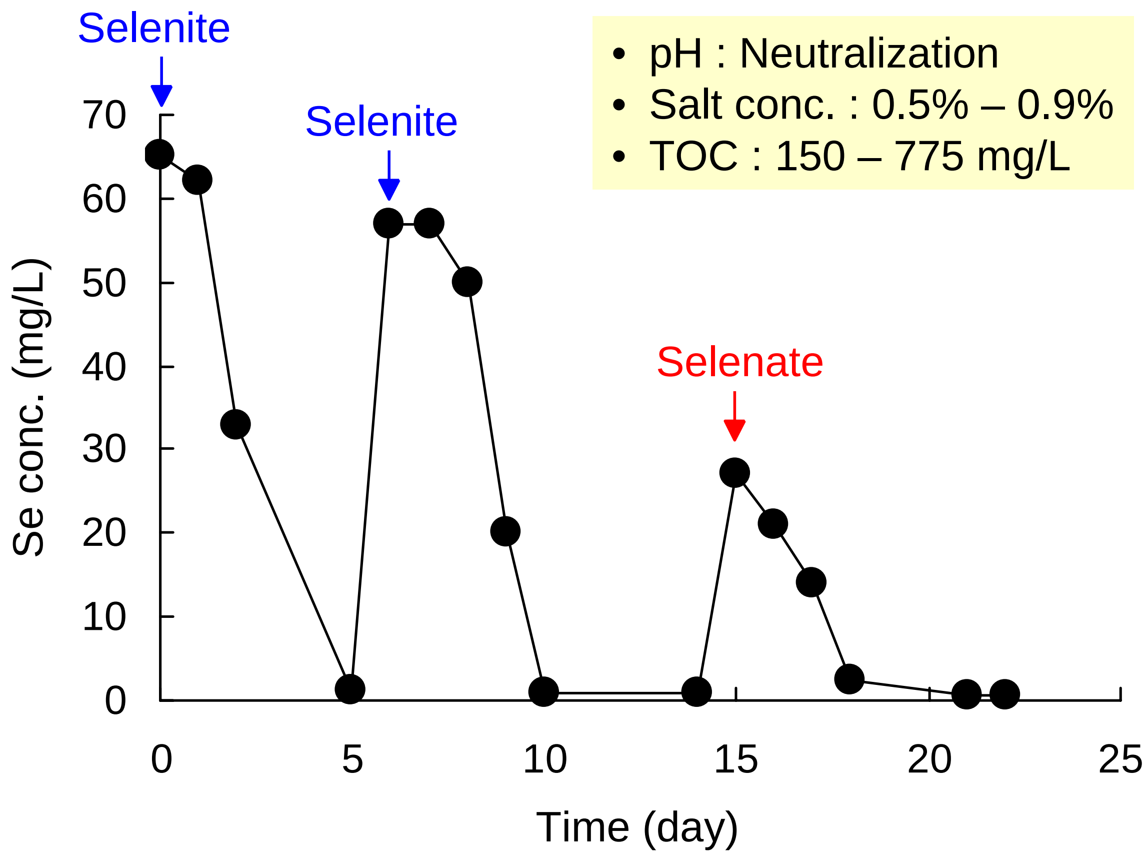
Pilot-scale Reactor at Shinko Chemical Co Ltd.

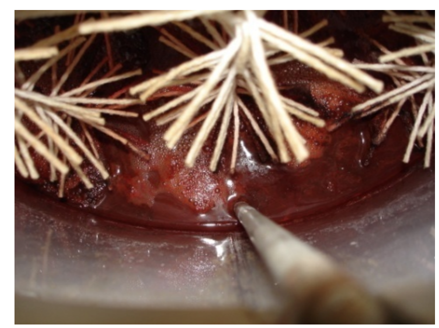
Reactor Overview (0.2 million yen for 2 reactors, 200L × 2) UASB

Pilot-scale Se Recovery Process with *P. stutzeri* NT-I

Sequential batch trial using real industrial wastewater with NT-I attached onto biomass carrier

- pH : Neutralization
- Salt conc. : 0.5% – 0.9%
- TOC : 150 – 775 mg/L

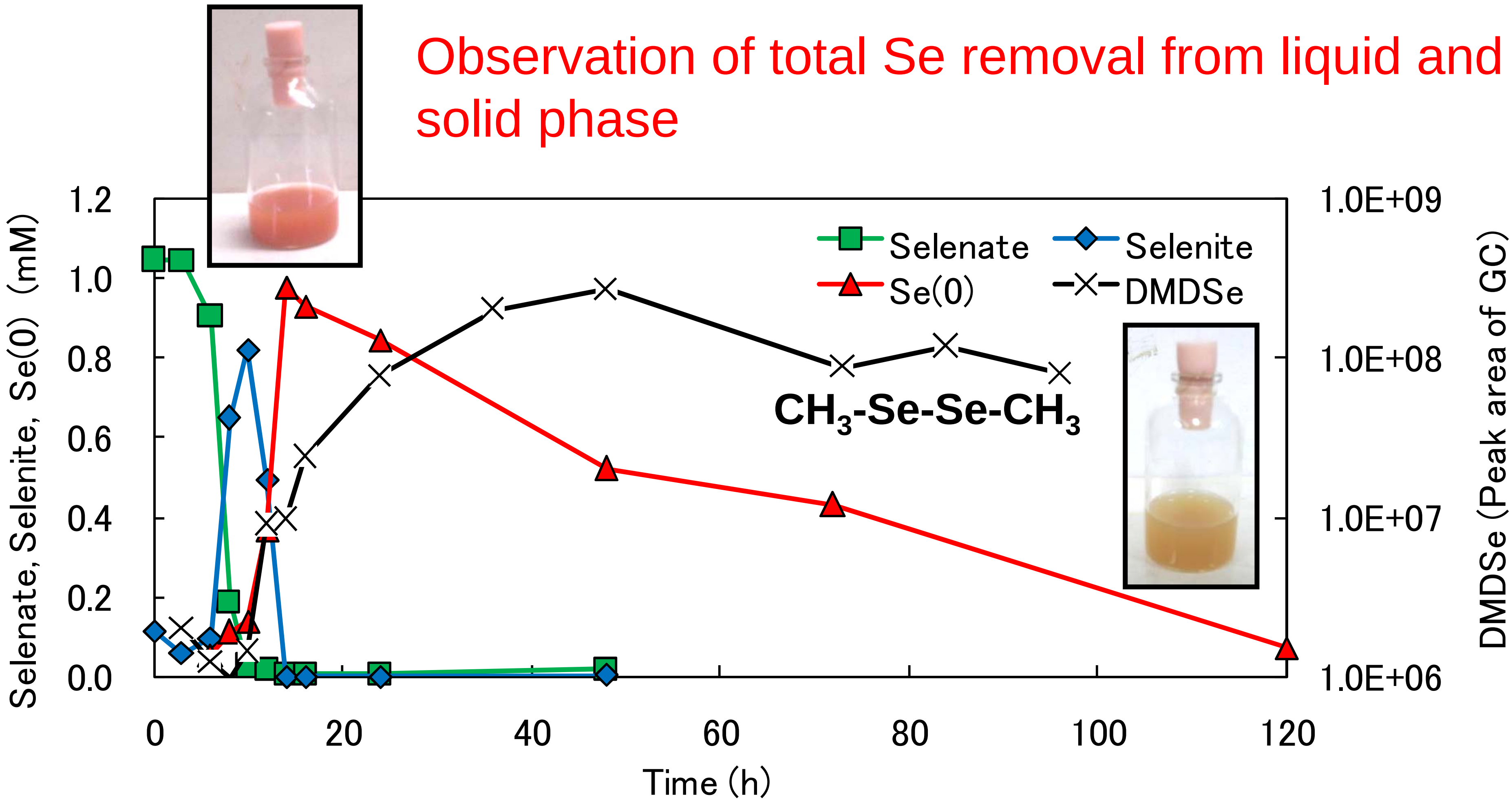




Efficient removal of soluble Se is achieved with weak aeration.

Possibility of Se Recovery by Volatilization (DMDSe)

Observation of total Se removal from liquid and solid phase



More cost-effective and simple Se recovery ?

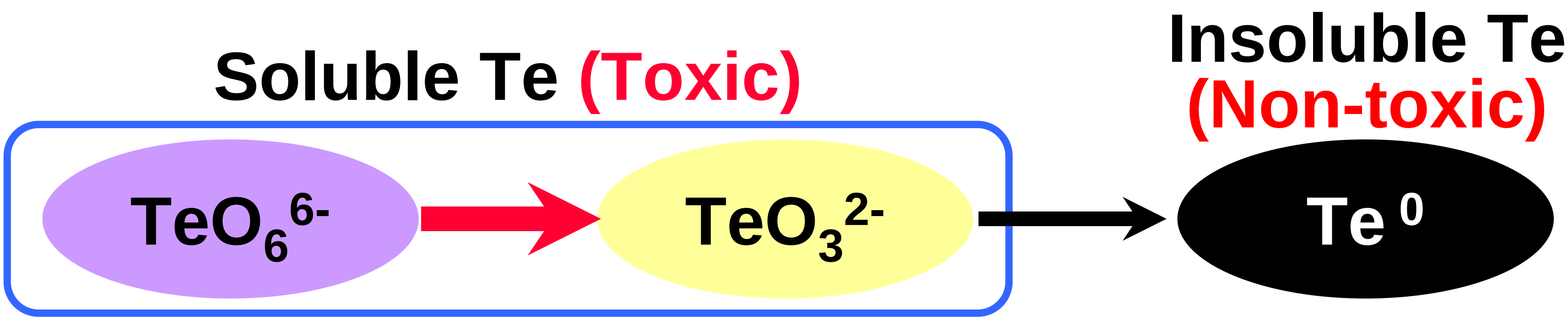
Possible Application to Other Rare Metals

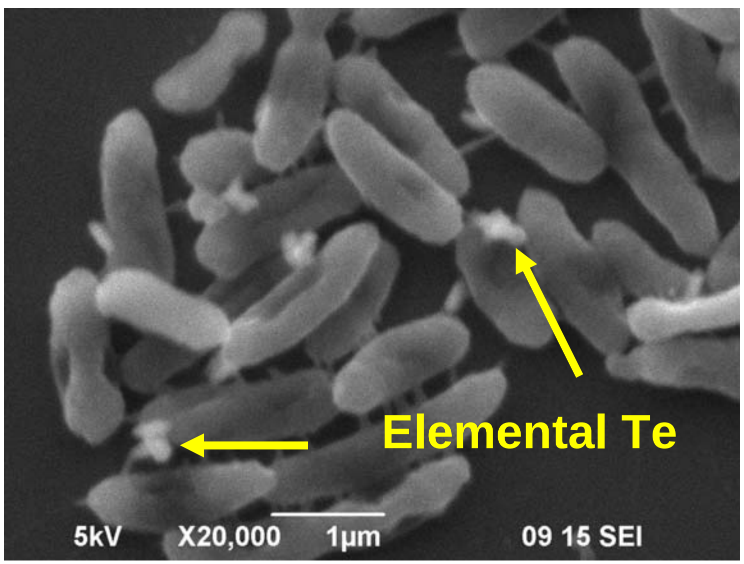
Transformation to Insoluble or Less-soluble Forms by Reduction of Metal Oxyanions

Soluble		Insoluble/less-soluble
Se(VI) → Se(IV)	→	Se(0)
Cr(VI)	→	Cr(III)
Te(IV)	→	Te(0)
Pd(II)	→	Pd(0)
Pt(IV)	→	Pt(0)
U(VI)	→	U(IV)
Tc(VII)	→	Tc(IV)

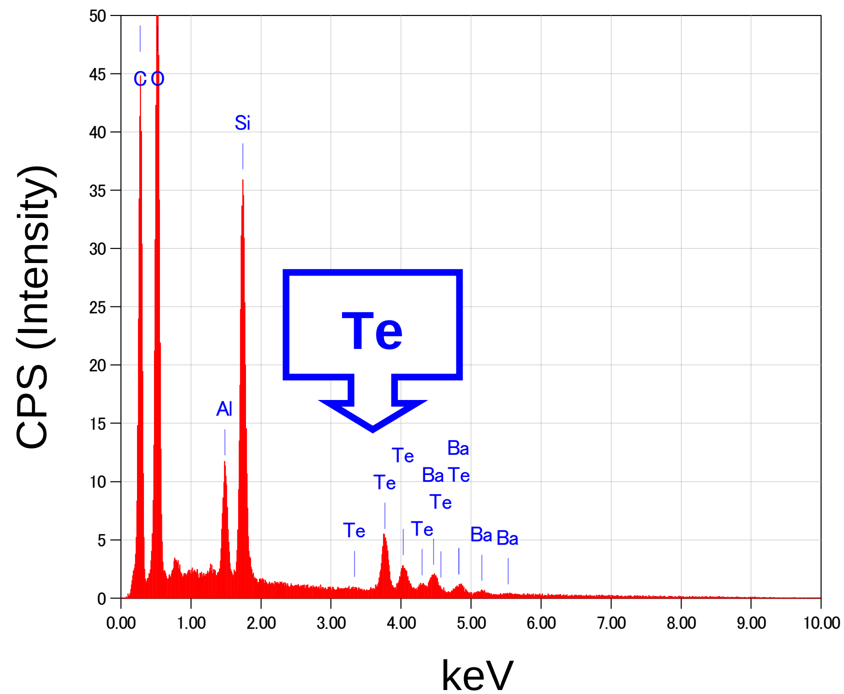
Possible Application to Other Rare Metals

Screening of Tellurate/Tellurite-Reducing Bacteria





Stenotrophomonas maltophilia TI-1



Possible Applications of Metal-Biotechnology

Wastewater treatment
for metal-containing
wastewater

Bioremediation
for polluted wetlands

Bioremediation
for polluted soils

Double Benefit Metal-Biotechnology

Metal recovery, recycling, processing

Low energy consumption, Speed up, New pathways, New compound (nano-particles), etc.

“Metal-Biotechnology” for Conservation of Environment and Resource

Bioprocess including reduction of metal-oxyanions has a great potential for concentration, extraction, and recover of rare metals from wastewater, wastes, and natural environment in cost-effective and eco-harmonizing manner.

Metal bioprocess not only in wastewater treatment and resource recovery (end-of-pipe) but also in mining, production, and processing (upper-stream application) can establish sustainable metal industries for future.

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FOUNDATION

Regional New Consortium Project, the Ministry of Economy, Trade and Industry, Japan, the Kansai Bureau Economy, Trade and Industry (2007)
Feasibility Study, Innovation Plaza Osaka, Japan Science and Technology Agency (2006)

Biotechnology for Recovery of Rare Metals in Wastewater

■INVESTMENT FOR A TRIAL PRODUCT FOR A SPECIFIC MARKET

1.Selenium: treatment & recovery:

Investment fee : About $ 0.4 Million

(Royalty, any cost for patent is not included)

Period : 3 years

2. Other metals: treatment & recovery

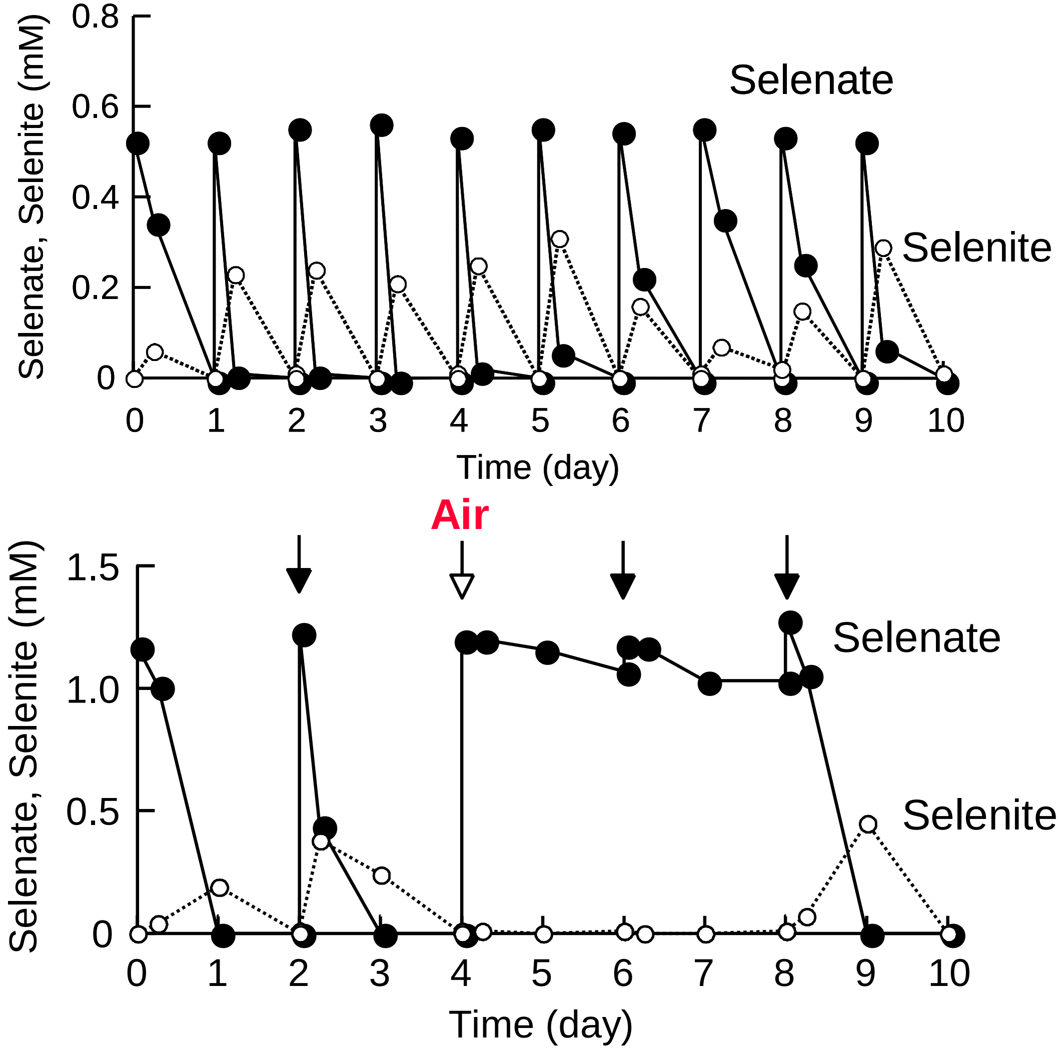
Investment fee : About $ 0.8 Million

(Royalty, any cost for patent is not included)

Period : 5 years

Lab-scale Se Recovery Process with *B. selenatarsenatis*SF-1

Sequential
Batch Tests

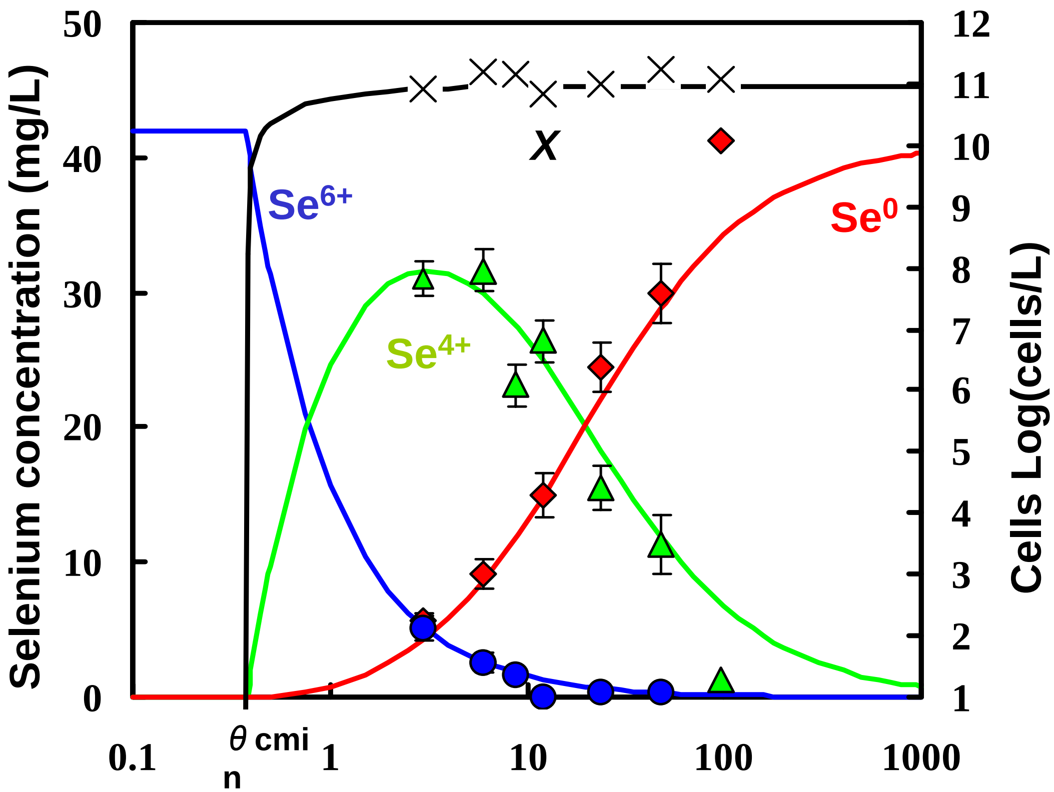


Strict control of anoxic condition is required.

Kashiwa et al. (2000) J. Biosci. Bioeng., 89, 528-533;. Kashiwa et al. (2001) Biofactors, 14, 261-265;

Lab-scale Se Recovery Process with *B. selenatarsenatis*SF-1

Continuous Flow Tests



Kinetics of the reactor

$$[\mathrm{Se}^{6+}] = \frac{1}{k_1 Y_1 \theta_c}$$

$$[\mathrm{Se}^{4+}] = \frac{k_1 Y_1 [\mathrm{Se}^{6+}]_{\mathrm{in}} \theta_c - 1}{Y_1 \theta_c (k_1 - k_2 + k_1 k_2 Y_1 [\mathrm{Se}^{6+}]_{\mathrm{in}} \theta_c)}$$

$$[\mathrm{Se}^{0}] = \frac{k_2 (k_1 Y_1 [\mathrm{Se}^{6+}]_{\mathrm{in}} \theta_c - 1)^2}{k_1 Y_1 \theta_c (k_1 - k_2 + k_1 k_2 Y_1 [\mathrm{Se}^{6+}]_{\mathrm{in}} \theta_c)}$$

$$[X] = Y_1 [\mathrm{Se}^{6+}]_{\mathrm{in}} - \frac{1}{k_1 \theta_c}$$

Fujita et al. (2002) Biotechnol. Bioeng., 80, 755-761.

Rare-metal Bioremediation and Recycling using Biotechnology

Mitsuo YAMASHITA
Shibaura Institute of Technology
Faculty of Engineering

Raremetals

○a few resource, ○ hard to mine and refine in technical and economical, ○ a rapid increasing in use depending on the development of high technology such as IT, Space, Car, Energy Industry, ○ harmful contaminant

Possibility of Depleting Resource

Expensive in market, nation’s reservation：

Ga, Ge, Se, Te et al. 31 kinds；Ni, Cr, W, Mo, Co, Mn, V

Environmental pollution in the century

Environmental quality standards for contamination and monitoring substances：

Cd, Pb, Cr, As, Hg, Se, B, Cu, Zn, Ni, Mo, Sb, Mn

Problem of Rerametals

Depleting resource a few resource, expensive in market

In：6.2–folds、Mo：4,1–folds、Sb：2.4–folds increased

for 5 years (2000～2005)

Environmental pollution new environmental quality standards for contamination

ex)

molybdenum（Mo）	＜ 0.07 mg/l
antinomony（Sb）	＜ 0.02 mg/l
uranium（U）	＜ 0.002 mg/l

From 2006 in Japan

↓

Remove and Recover from Environment

Remediation･Recover works

Physical and chemical treatment（coagulation-sedimentation, ion exchanger）

merit：high efficiency from high conc. contaminant, short time

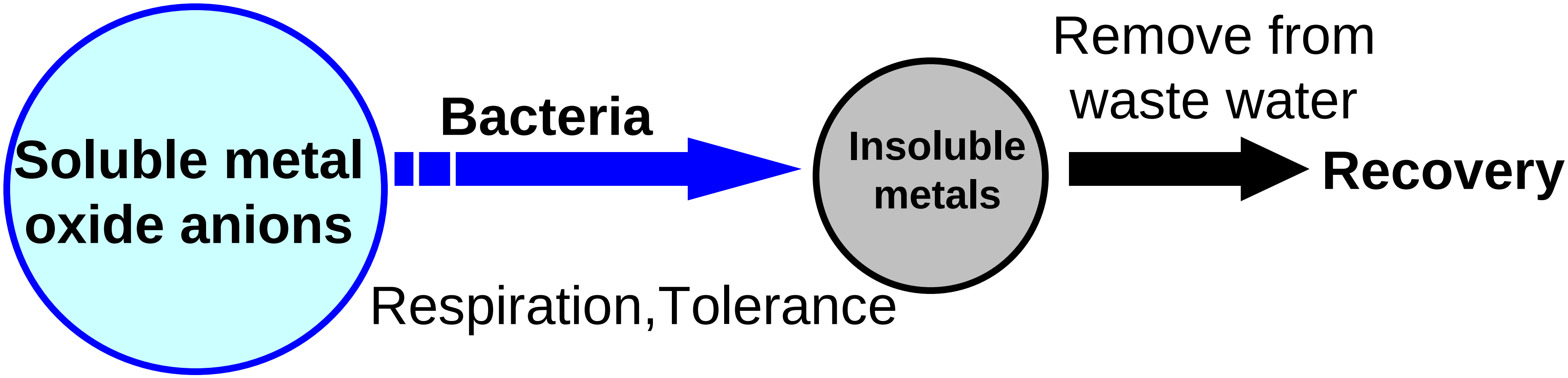
demerit：expensive

Biological treatment（bioremediation）

merit：high efficiency from low conc. contaminant, low cost

demerit：long time

Focus on Bacteria Metabolizing Metals

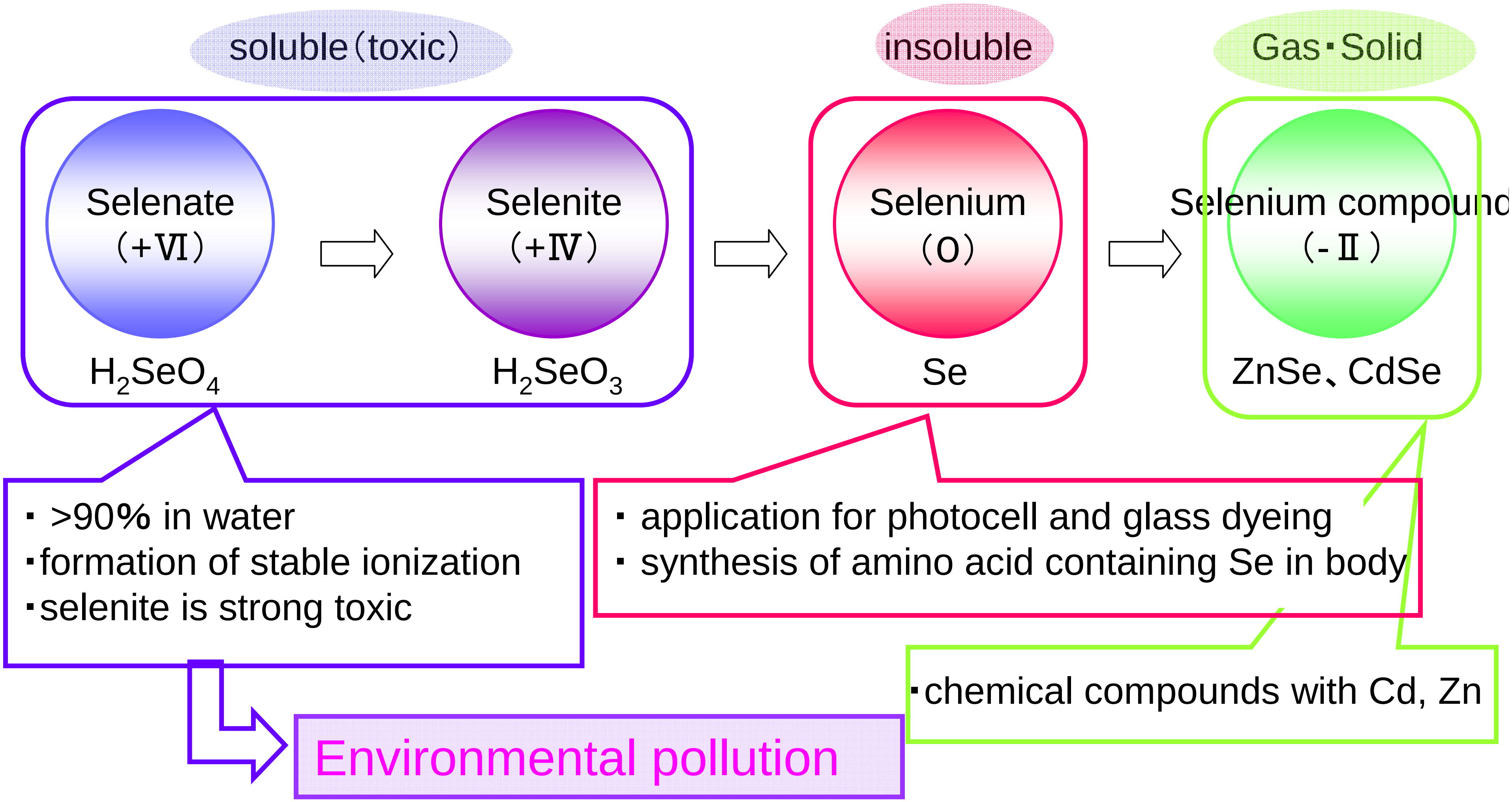


Purpose

- Screening and isolation bacteria available metabolizing rerametals（Se,Te et al.) and recover them
- Analysis the genes and enzymes related to metabolite rerametals
- Construction of platform to enlarge rerametals recover process

Selenium(Se)

- Application for electronic part ➡ **possibility of depleting resource**
- Essential element for organism



Selenium（Se）

Bacillus sp. SF-1

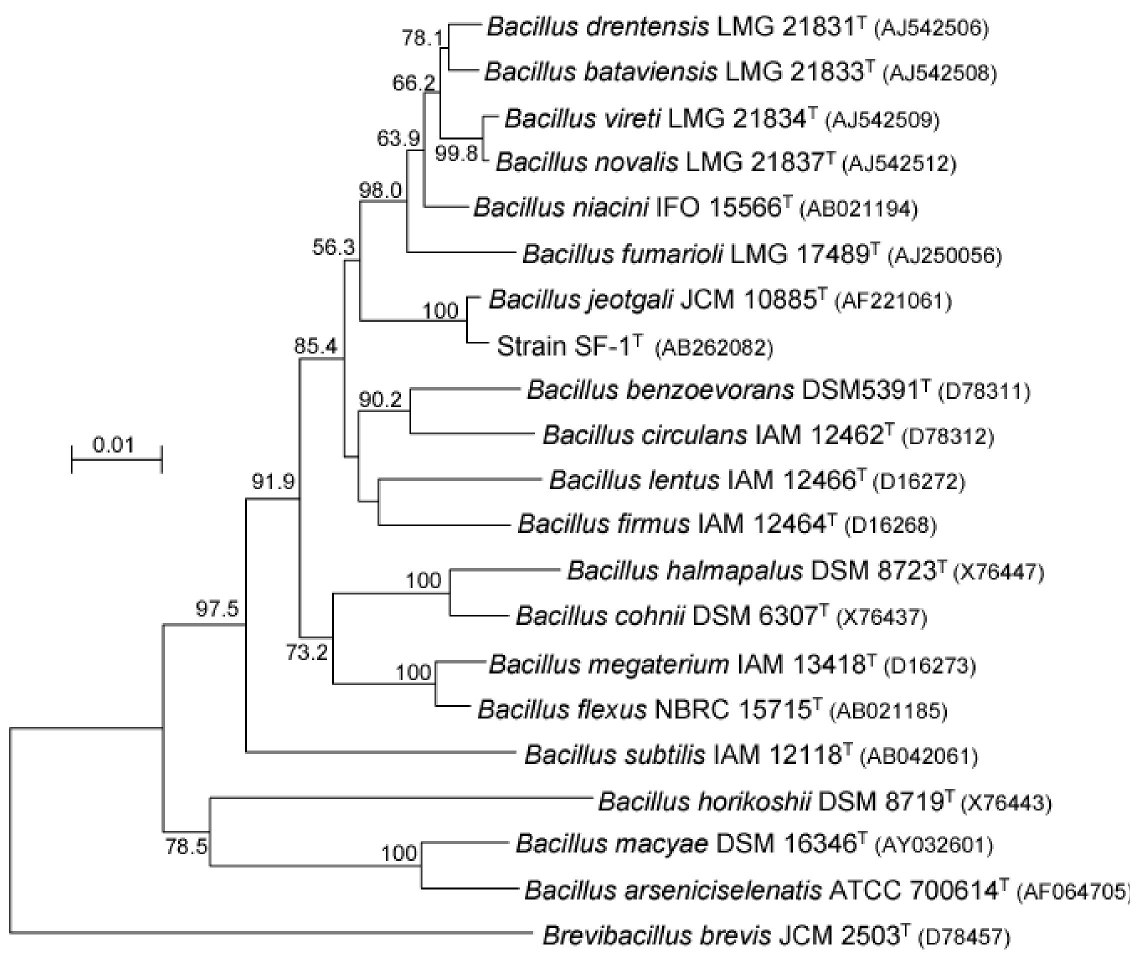
- **Isolation from selenium-contaminated sediment（M. Fujita et al., 1997）**
- **selenate ⇒ selenite （anaerobic）**
  selenite ⇒ selenium
- **High growth rate under aerobic condition**
- **Enzyme complex of selenate reduction（S. Yamamura et al., 2004）**
  - **membrane-bound enzyme**
  - **Mo containing enzyme**
  - **induction by selenate under anaerobic**
- **No Host-vector system**

Selenate reduction by SF-1
Cause red color

Purpose ⇒ To construct Se remove process by SF-1, molecular mechanism of selenate reduction is examined.

Phylogenetic trees of 16S rRNA between *Bacillus* sp. SF-1 and related *Bacillus* species

16S rDNA of SF-1 is 99.6% similar to that of *Bacillus jeotgali* JCM 10885^T



Phenotype between strain SF-1 and JCM 10885^T

Different phenotype in selenate and arsenate reduction

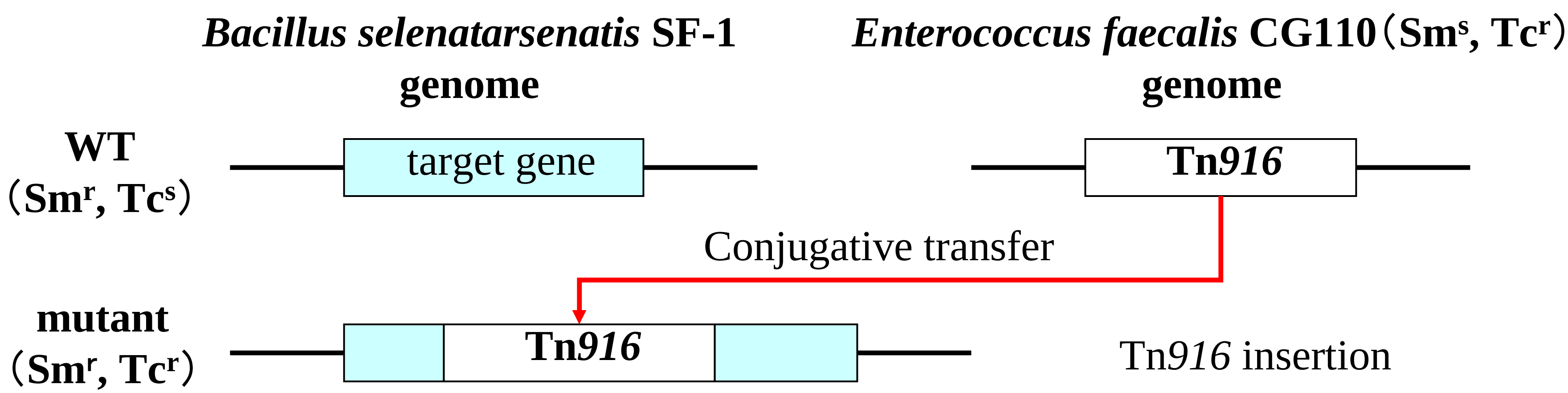
	Strain SF-1	*B. jeotgali* JCM10885^T
Selenate reduction	+	−
Arsenate reduction	+	−
Gram stain	Positive	Variable
7% NaCl tolerance	−	+

DNA-DNA reassociation value, 14% between SF-1 and JCM 10885^T

Novel species

Bacillus selenatarsenatis SF-1

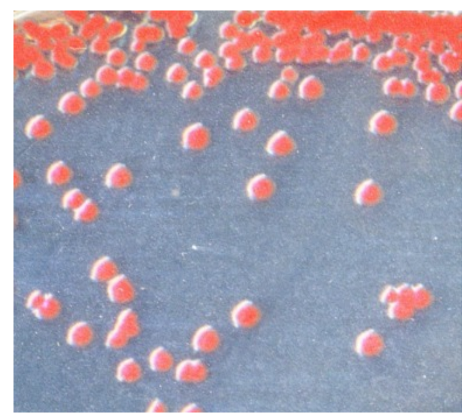
Screening of selenium synthesis-defective mutants



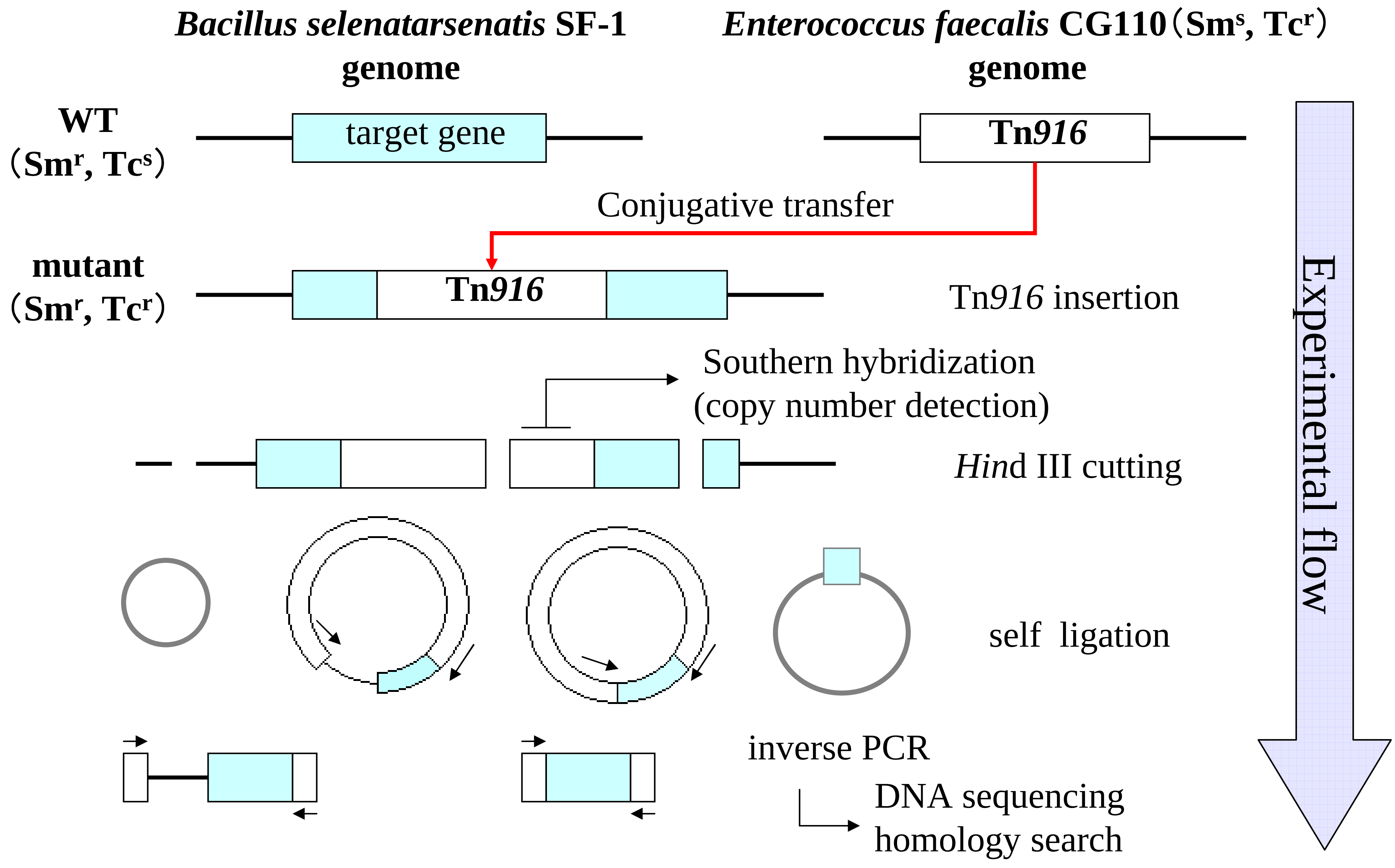
Selenium synthesis-defective mutants
7 colonies（white）

Selenium synthesis-leaky mutants
10 colonies（pink）

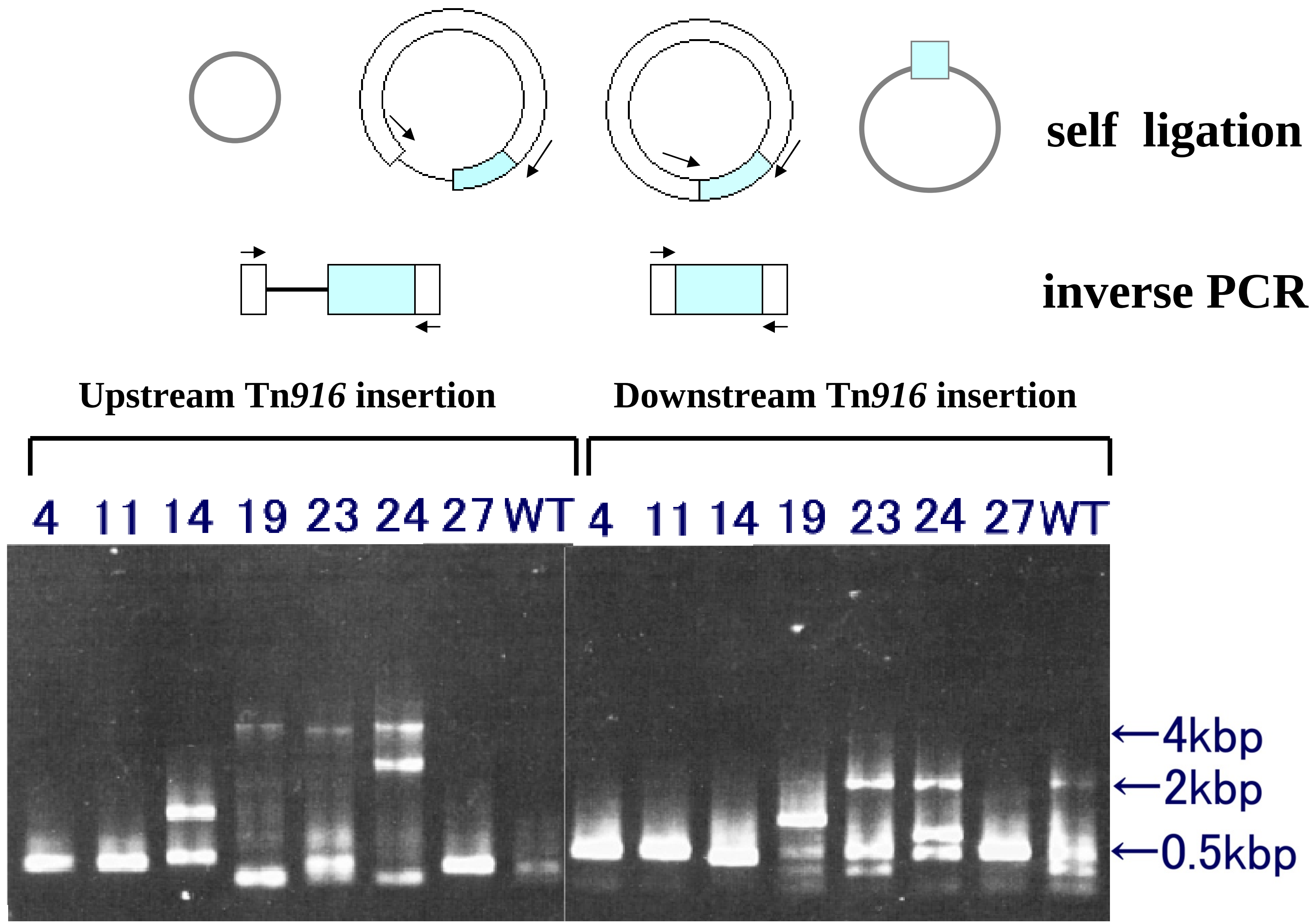
Wild-type
（red）



Cloning of target genes using a conjugating transposon Tn*916*



inverse PCR


self ligation
inverse PCR
Upstream Tn*916* insertion
Downstream Tn*916* insertion
4 11 14 19 23 24 27 WT
4 11 14 19 23 24 27 WT
←4kbp
←2kbp
←0.5kbp

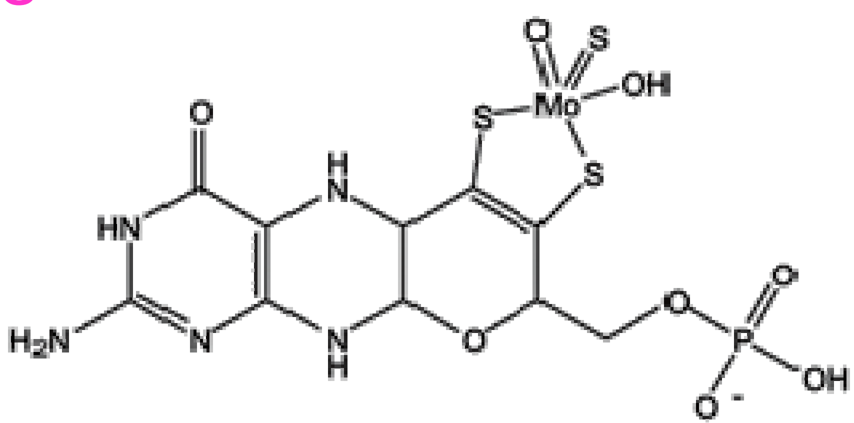
Homology search

- Molybdopterin oxidoreductase
- Diguanylate cyclase / phosphodiesterase（GGDEF domain）

From mutants carrying one copy of Tn*916*

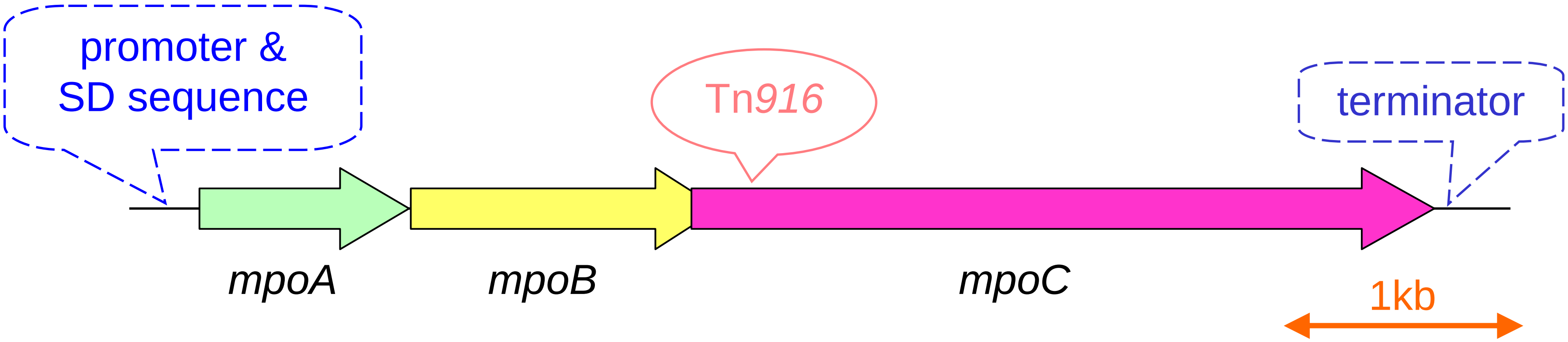
Molybdopterin oxidoreductase

- Generic name for oxidoreductase containing Mo, pterin, Fe-S cluster
- Oxidoreductase related in anaerobic respiration (ex　nitrate reductase）



molybdopterin

Analysis of the Genes coding Molybdopterin Oxidoreductase

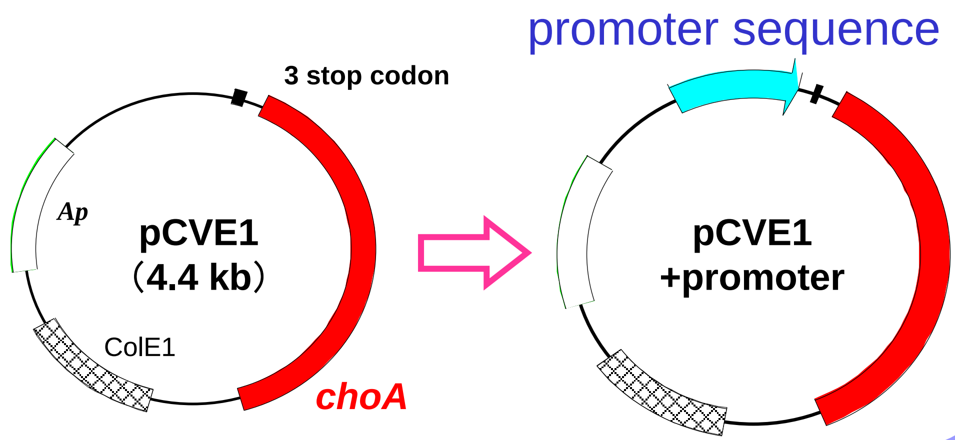


molybdopterin oxidoreductase, iron-sulfur binding subunit	→ ***mpoA***	(0.9 kb)
molybdopterin oxidoreductase membrane subunit	→ ***mpoB***	(1.3 kb)
molybdopterin dinucleotide-binding region	→ ***mpoC***	(3.2 kb)

Identification of Promoter region

pCVE1

Promoter proving vector with cholesterol oxidase gene（*choA*）as a reporter gene

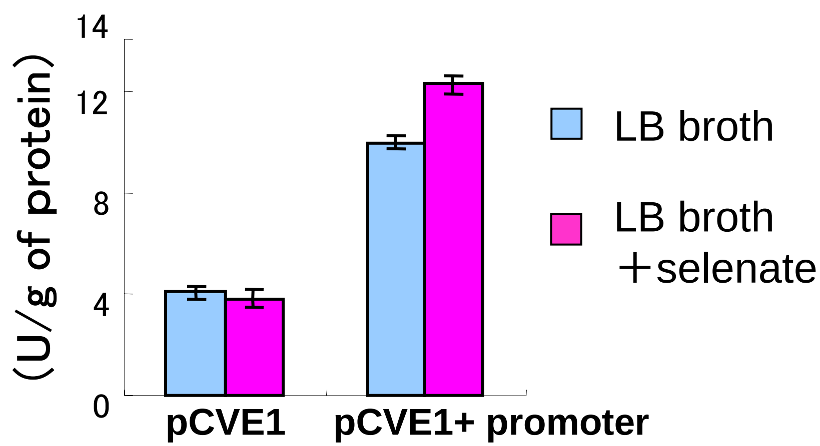


ChoA assay

①Transform into *E. coli*

② Culture・Cell extracts

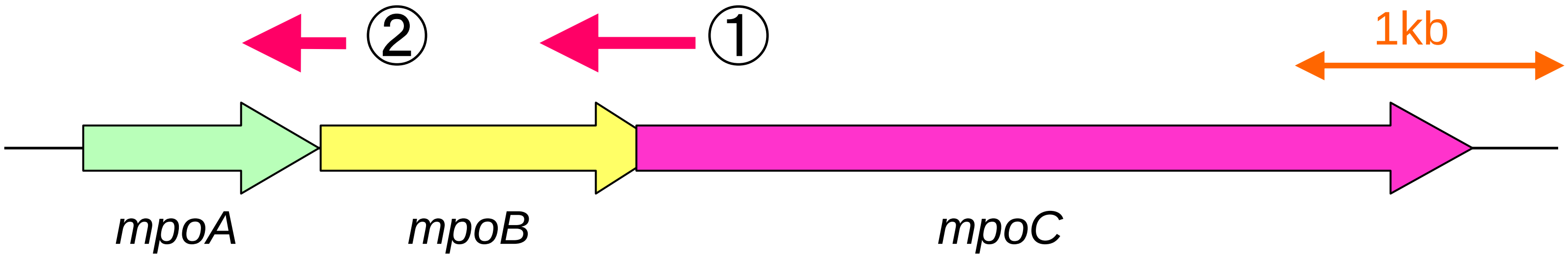
③ ChoA assay with supernatant



Upstream region *mpoA* is promoter

Identification of *mpoABC* operon

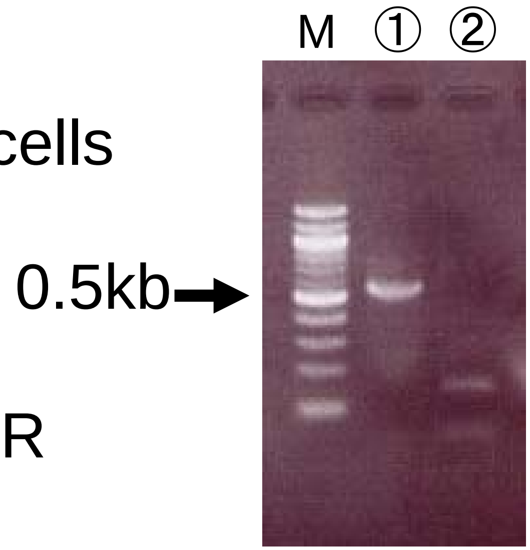
Analysis of Transcripts by RT-PCR



① RNA extracts from SF-1 cells

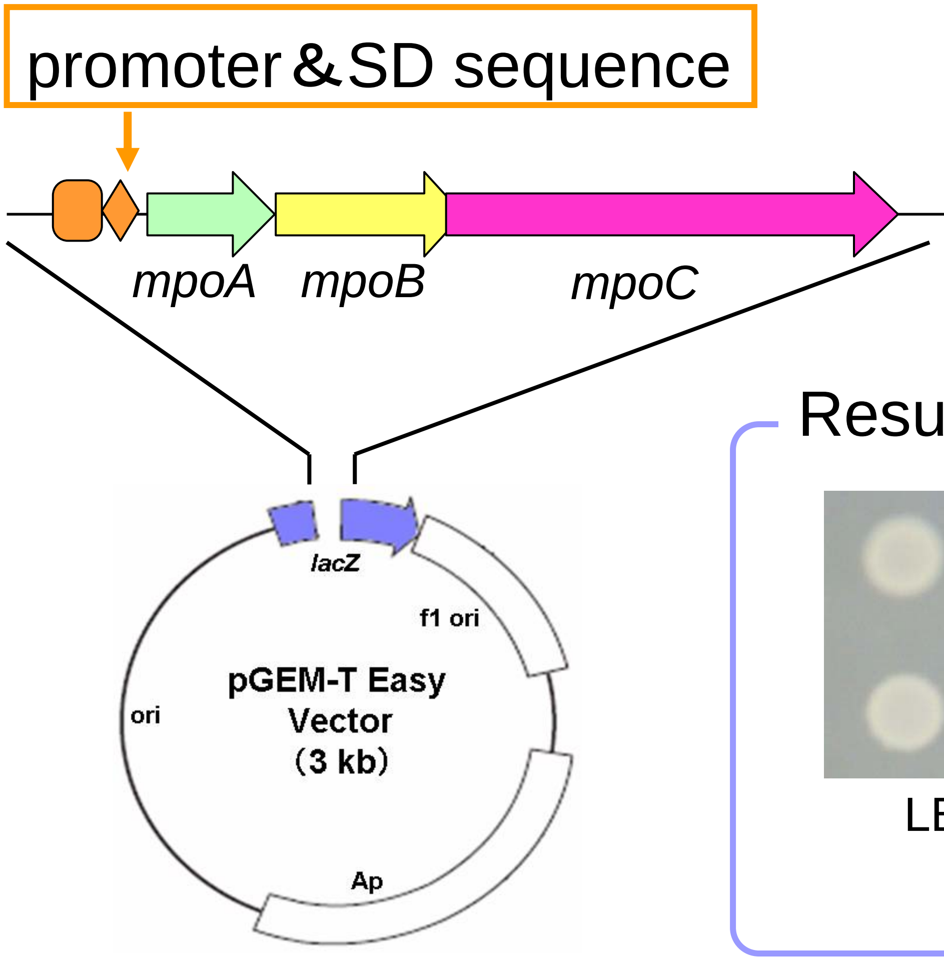
② cDNA synthesis

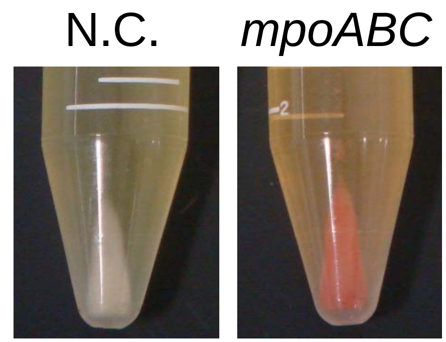
③ DNA Amplification by PCR

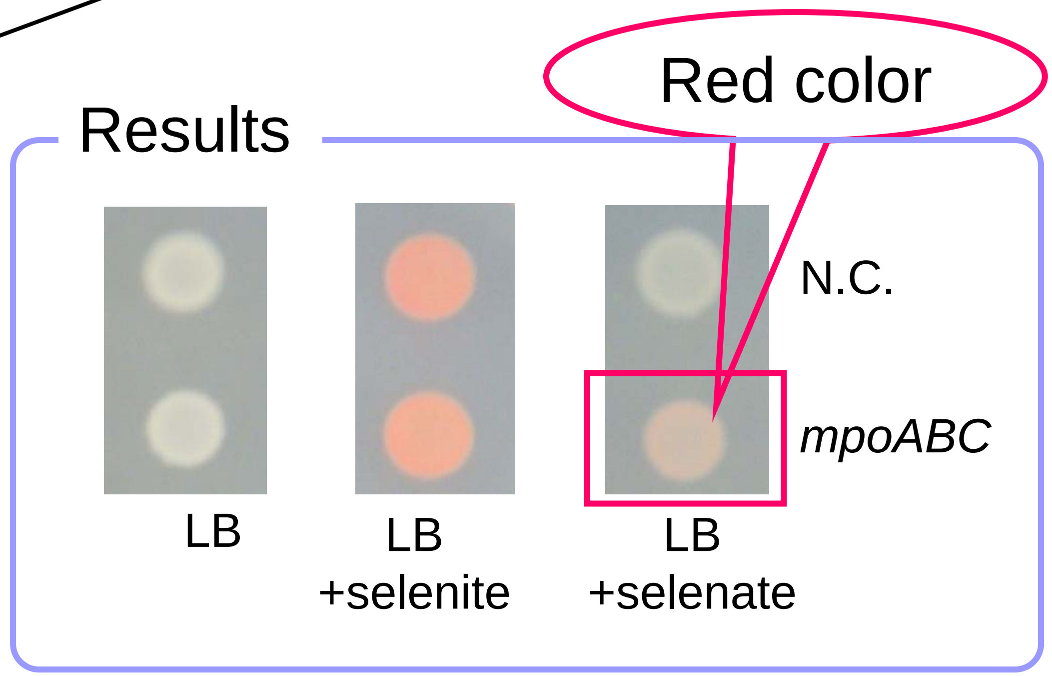


mpoABC is operon

Selenate reduction *in E. coli* carrying *mpoABC*







mpoABC are selenate reducing genes

Back ground Reduction process of selenate by microorganisms

Se(+Ⅵ) selenate → Se(+Ⅳ) selenite → Se(0) elemental

Toxic, soluble — Atoxic, insoluble

- Anaerobic condition ･･･ terminal electron acceptor
- Aerobic condition ･･･ detoxification

Merit of aerobe

- easy works （culture･treatment）
- high reaction rate and remove for short time

Construction of Aerobic waste water treatment?

Few reports of aerobic selenate reducing bacteria

Purpose Construction of new selenate remediation system

Isolation of novel aerobic selenate high-reducing bacteria and characterization of reduction

Examination Items

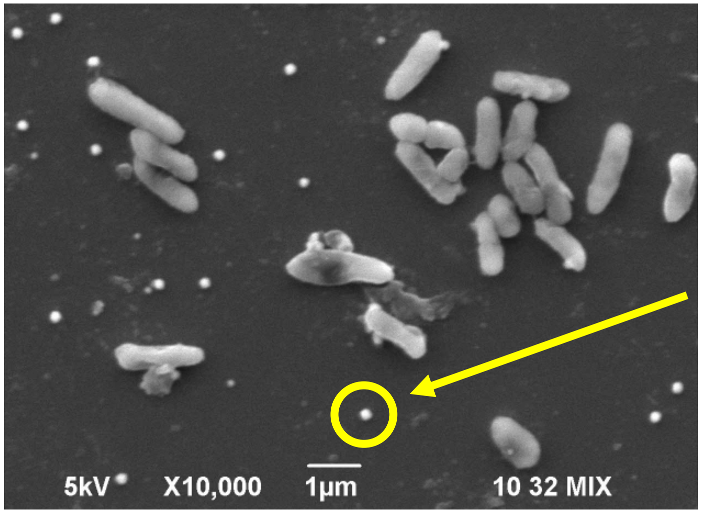
- isolation and identification of selenate reducing bacteria
- physiology test
- characterization of reduction（growing cell）

Experiment Isolation

- Isolation of selenate reducing bacteria, NT-I from an effluent drain of a selenium recycle factory

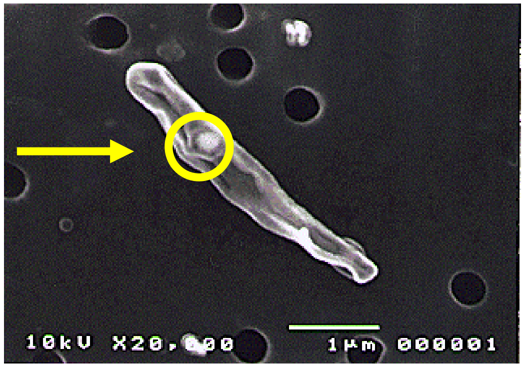
Selenate reducing bacteria

- SEM and elemental analysis of selenate reducing NT-I and SF-1



strain **NT-I** (Se is outside of cells)

elemental Se



strain **SF-1**(Se is inside of cells)

Separation of Se element and NT-I cells by filtration

➡ Effective recovery of Se element

Identification

■Physiology test

cell shape	rod
Gram stain	−
motile	+
O-F test	O
catalase reaction	+
oxidase reaction	+

■16S rDNA sequence analysis

100% similarity to that of *Psedomonas stutzeri* DSM 5190^T

Physiology and Molecular biology test

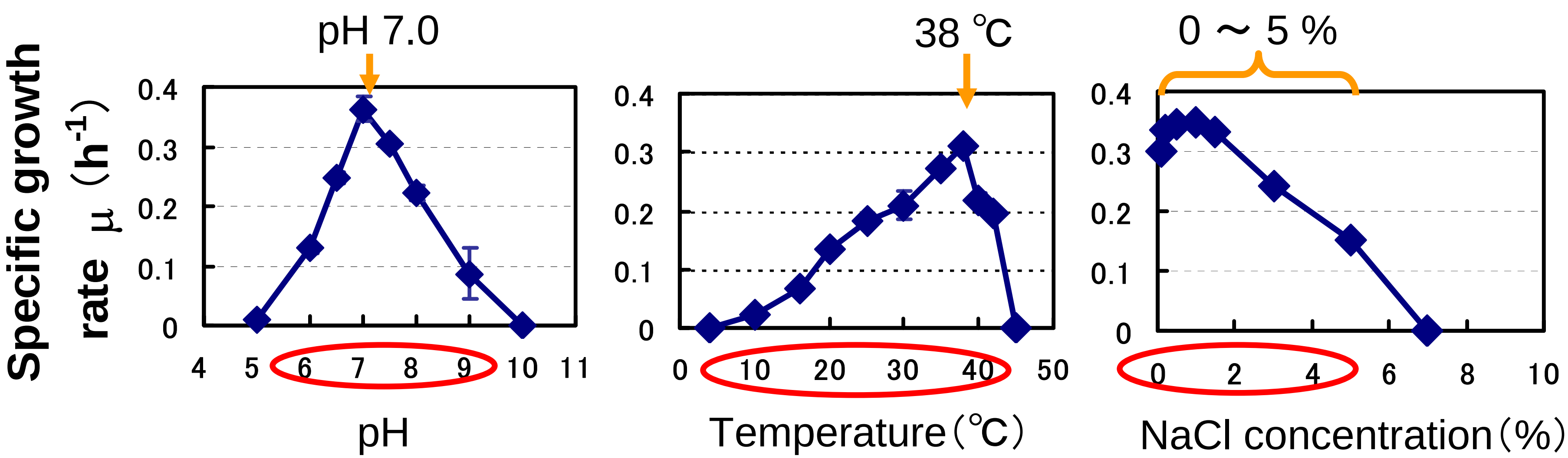
→ ***Pseudomonas stutzeri* NT-I**

Cell physiology Physiological characterization of NT-I

■Carbon Assimilation test (Biolog GN Microplates) → a variety of C source (80 kinds) (sugar,amino acids,organic acids)

■Growth rate

Medium ・・・ Glucose +mineral



Growth:pH6.0-9.0, Temp.10-42℃, NaCl tolerance <5 %
Optimum: pH 7.0, Temp. 38℃

***Pseudomonas stutzeri* NT-I**

Experiments　Characterization of reduction（growing cells）

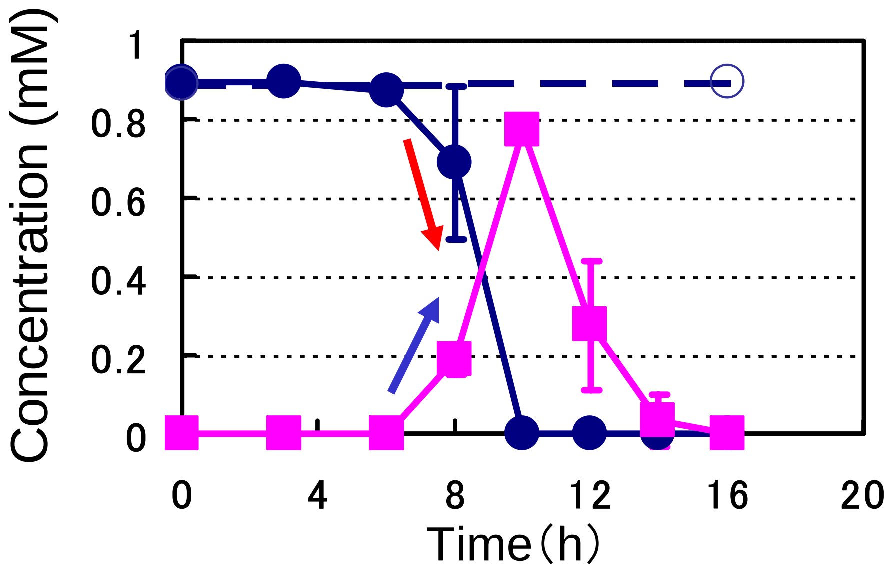
- Medium　:　TSB + selenate or selenite
- Conditions　:　pH 7.2, Temp. 28℃, Aerobic

- Comparison　: known aerobic selenate reducing bacteria
  - *Pseudomonas stutzeri* ATCC 51152 (Lortie *et al.*, 1992)
  - *Enterobacter cloacae* subsp. *cloacae* SLD1 a-1（ATCC 700258 ）　(Losi *et al.*, 1997)

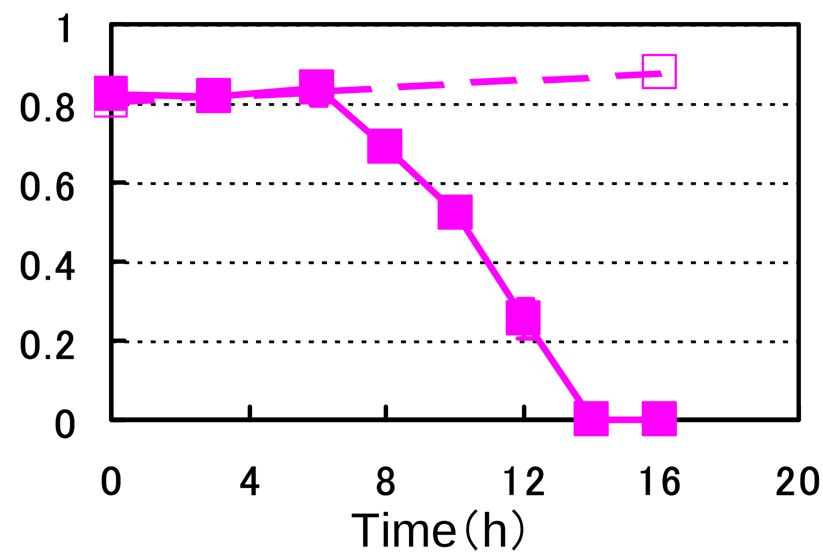
Results

Characterization of reduction in NT-I

selenate

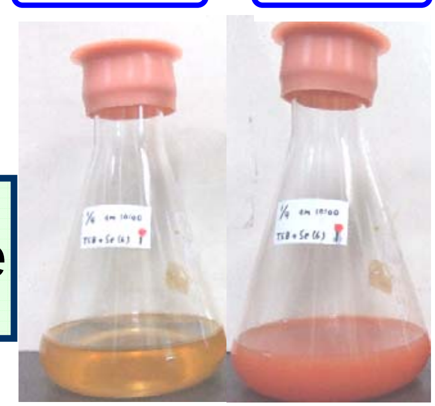


selenite



● : selenate
○ : selenate (control)
■ : selenite
□ : selenite (control)

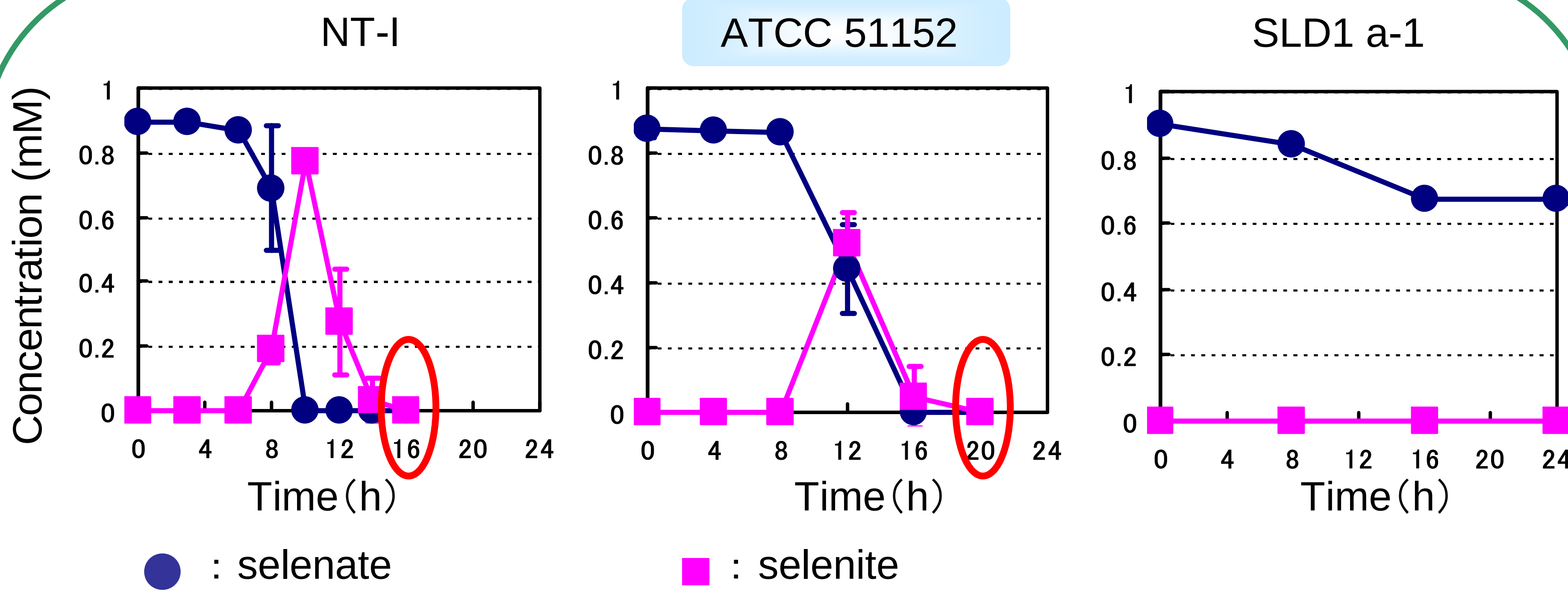
before after



Reduce selenate to Se and Remove from culture

Results

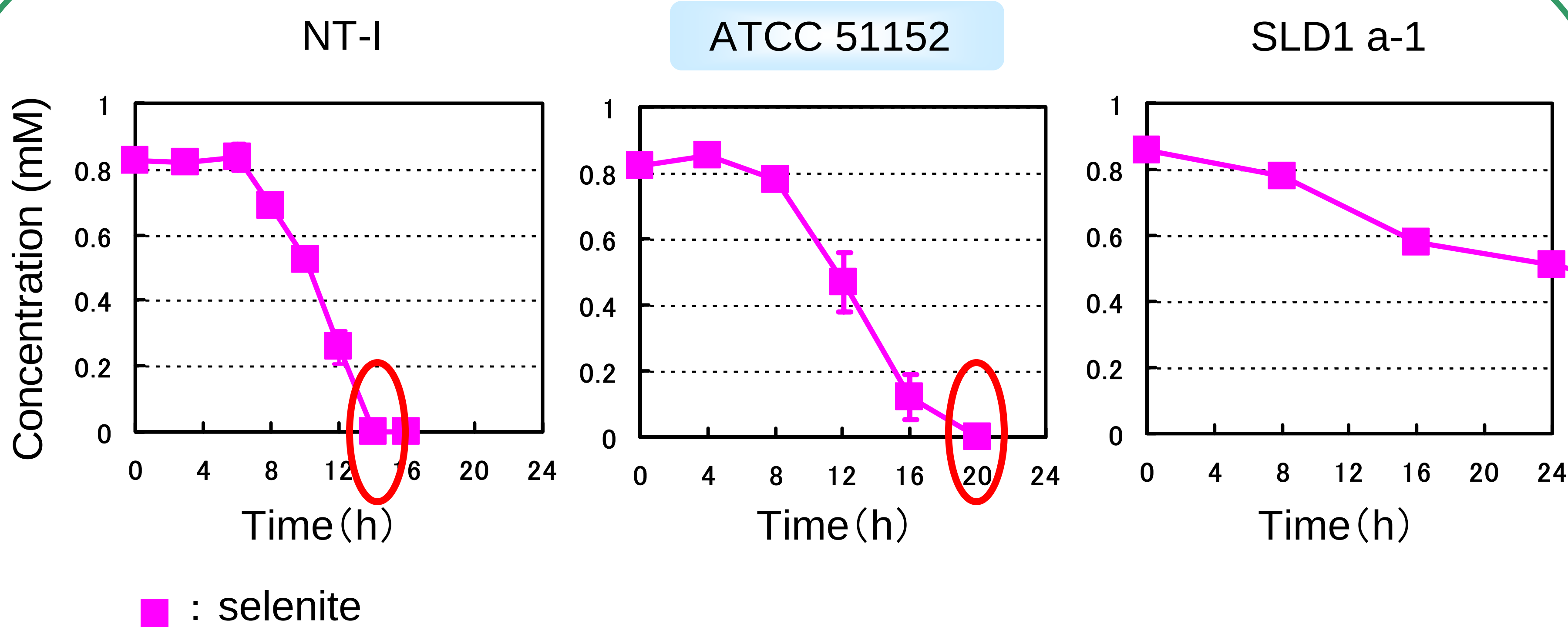
Selenate reduction between three strains



- NT-I ,ATCC 51152 ･･･100 % reduction(for 10-16h)
- SLD1 a-1･･･25 % reduction(for 24h) ,45 % reduction(for 48h)

Results

Selenite reduction between three strains



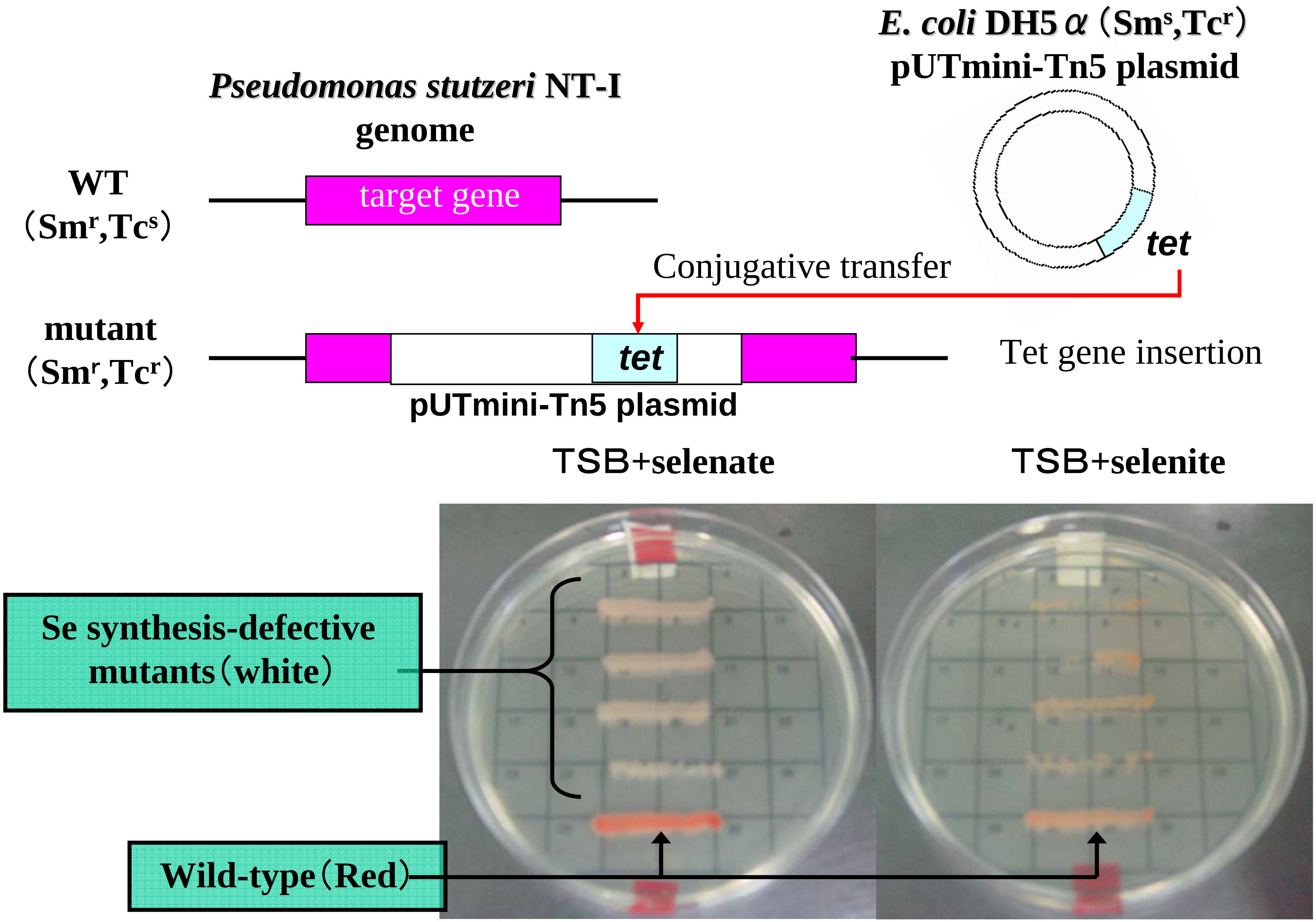
- NT-I , ATCC 51152 ・・・ 100 % reduction (for 14-20 h)
- SLD1 a-1・・・40 % reduction(for 24h) ,100 % reduction(for 48h)

Summary Comparison of reduction between three strains

	ATCC 51152	SLD1 a-1	NT-I
1 mM selenate reduction rate（mol/h）	1.1×10^{-4}	1.2×10^{-5}	2.2×10^{-4}
1 mM selenite reduction rate（mol/h）	8.2×10^{-5}	1.8×10^{-5}	1.1×10^{-4}
maximum selenate reducing concentration	50 mM (Lortie *et al.*, 1992)	1 mM (Losi *et al.*, 1997)	≧400 mM (visual observation)

NT-I has the most selenate reducing activity

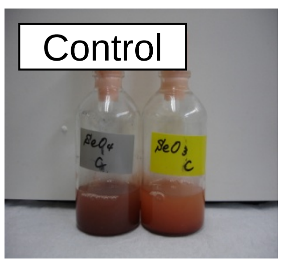
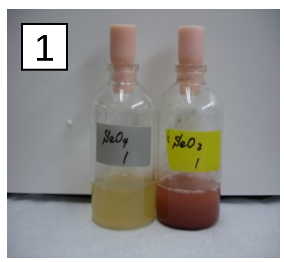
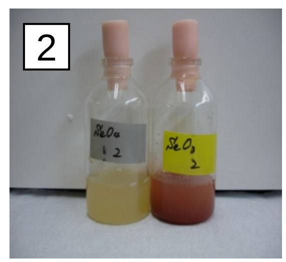
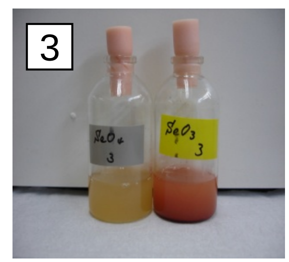
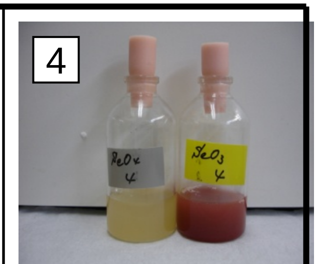
Screening of selenate reduction-defective mutants



Selenate and Selenite reduction of transposon mutagenesis of NT- Ⅰ

TSB+H_2SeO_4

TSB+H_2SeO_3

		 Control		 1		 2		 3		 4	
		H_2SeO_4	H_2SeO_3	H_2SeO_4	H_2SeO_3	H_2SeO_4	H_2SeO_3	H_2SeO_4	H_2SeO_3	H_2SeO_4	H_2SeO_3
Conversion to Se (%)	12h	99	33	3.2	34	2.3	40	0.0	37	2.9	39
	24h	99	74	1.1	91	2.4	82	1.4	75	5.9	95

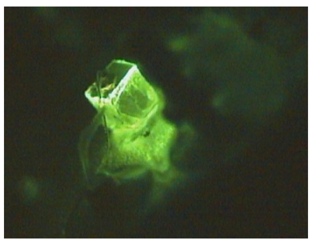
Definite conversion as 0 % from selenate or selenite at 5 mM in initial time to Se.

Tellurium (Te)

・16 family element as Se and application of semiconductor material and glass-dyeing

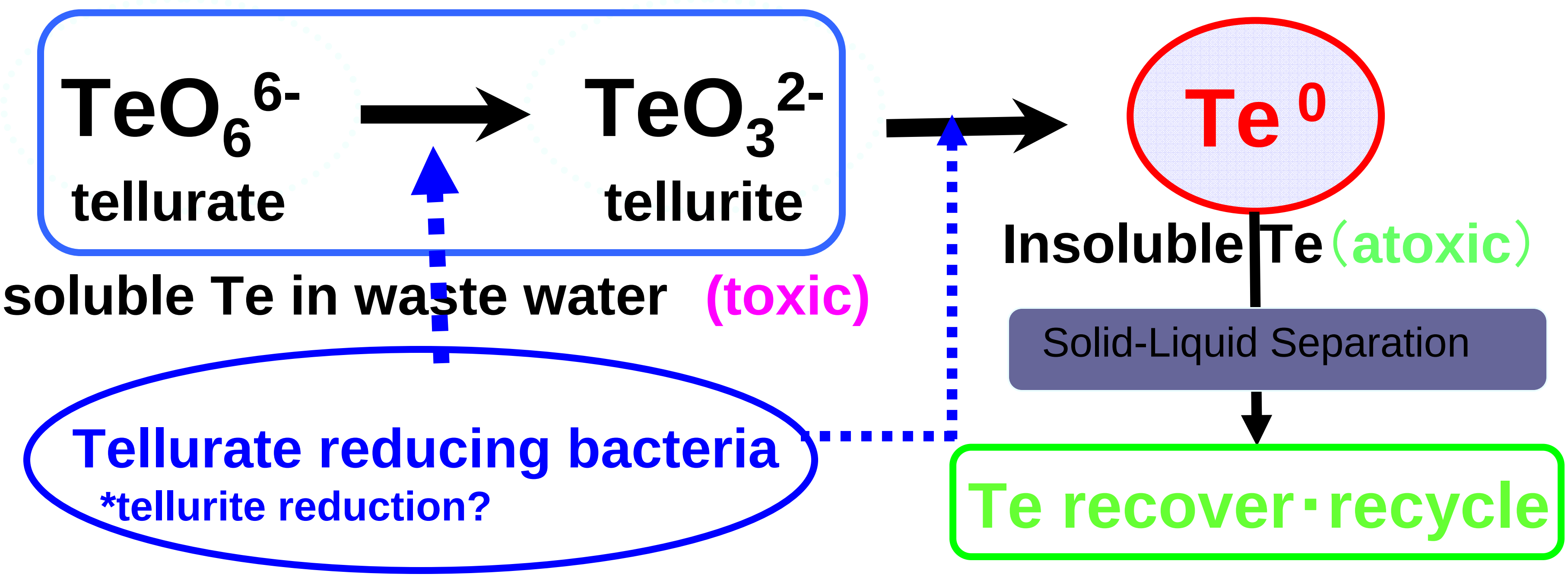
・Soluble oxide and Toxic

・Environmental quality standards for monitoring substances (in Japan)



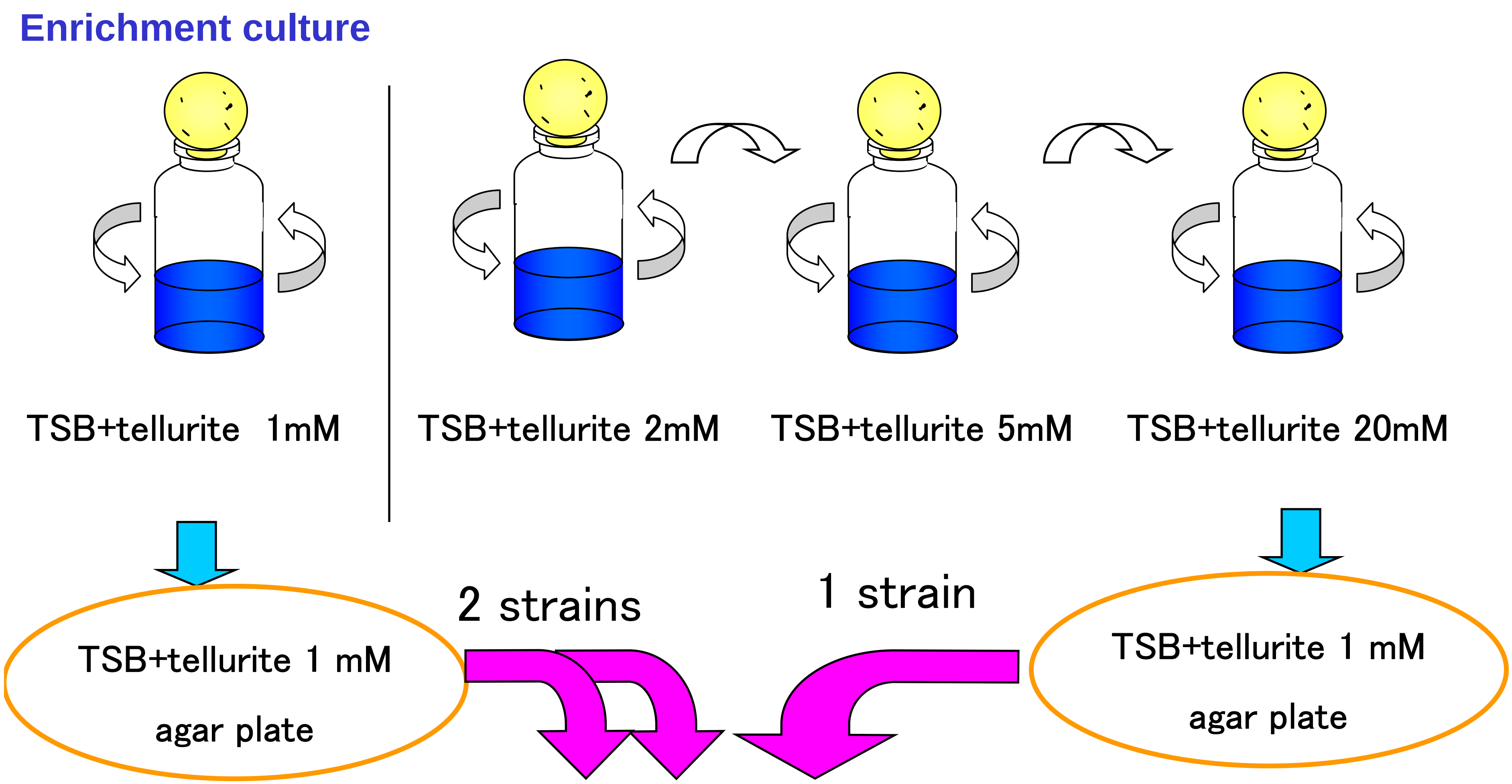
ZnTe green Light Emitting Diode (LED)

Te elemental mineral：native Te



Isolation of Tellurate Reducing bacteria

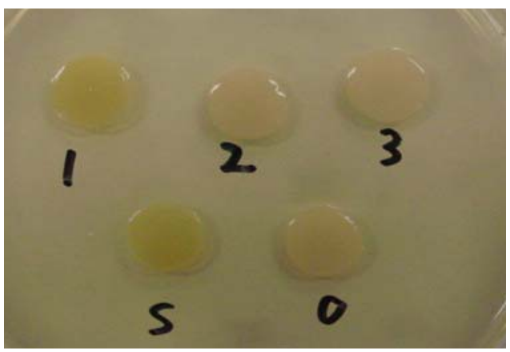
Three strains were isolated from enrichments with tellurite.



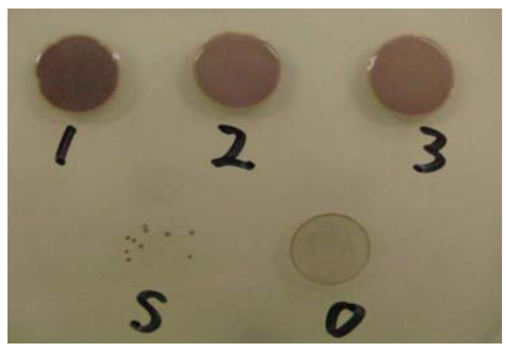
Tentative name as Ti-1,Ti-2,Ti-3

Physiology and Molecular Biology Test in Ti-1, 2, 3

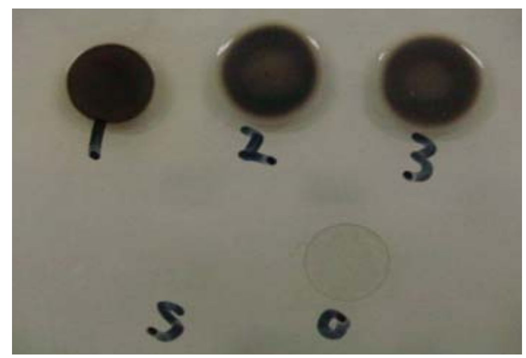
	Ti−1	Ti−2	Ti−3
Cell shape	rod	rod	rod
Gram stain	Negative	Negative	Negative
16S rDNA similarity	*Stenotorophomonas maltophilia* （100%）	*Ochrobactrum anthropi* （100%）	*Ochrobactrum anthropi* （100%）



TSB



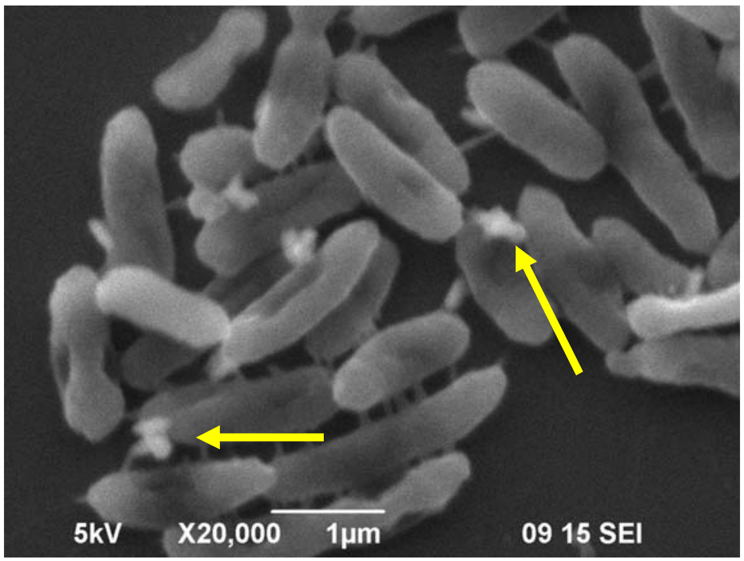
TSB+tellurate1mM



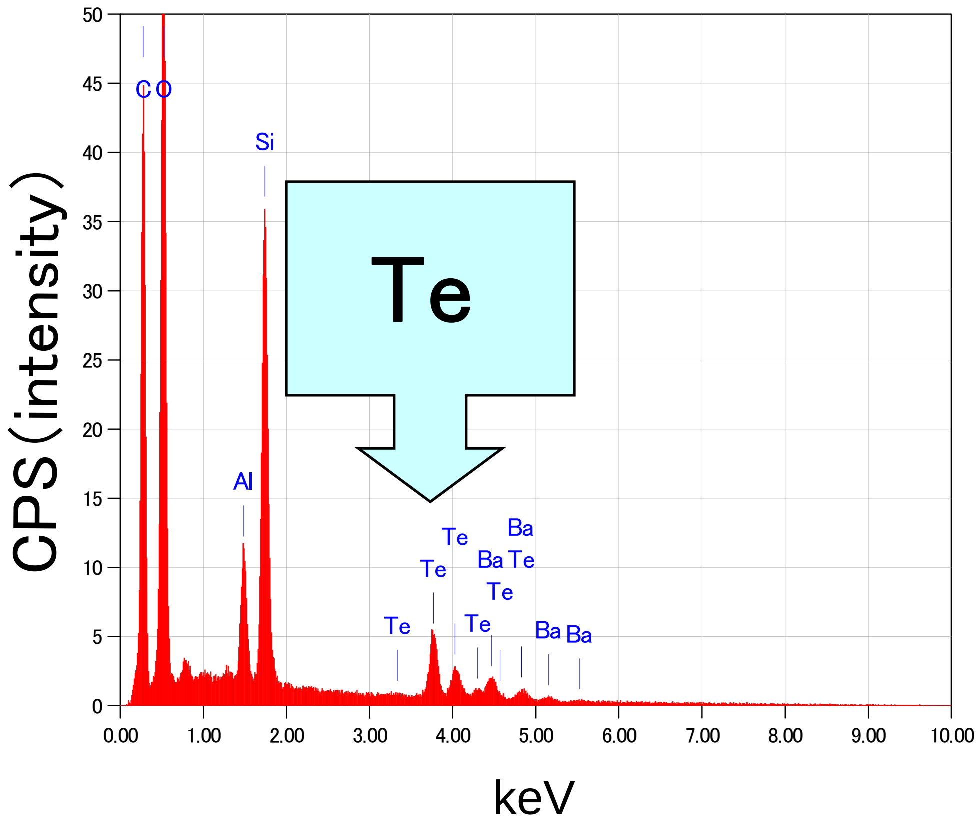
TSB+tellurite10mM

1：Ti−1, 2：Ti−2, 3：Ti−3、S： *Stenotorophomonas maltophilia* NBRC14161^{T}， O： *Ochrobactrum anthropi* NBRC15819^{T}

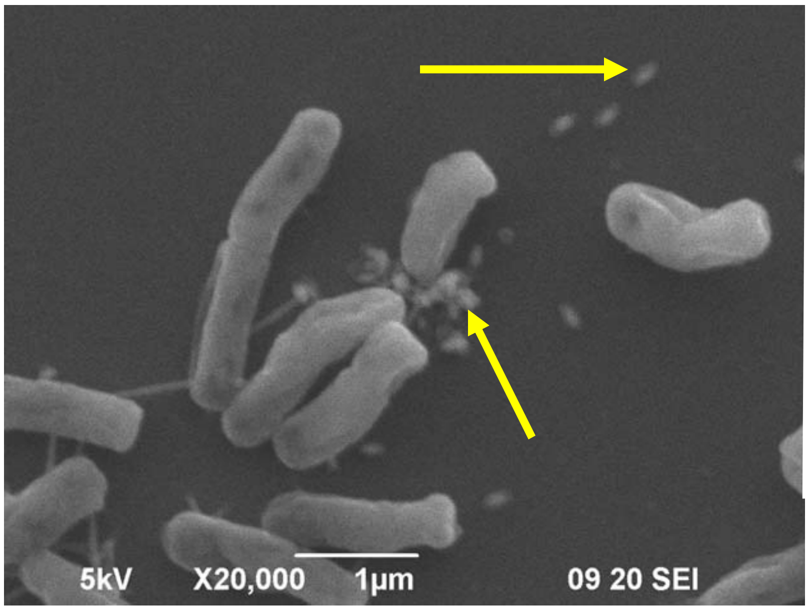
SEM (Scanning Electron Microscope) and elemental analysis in Ti-1



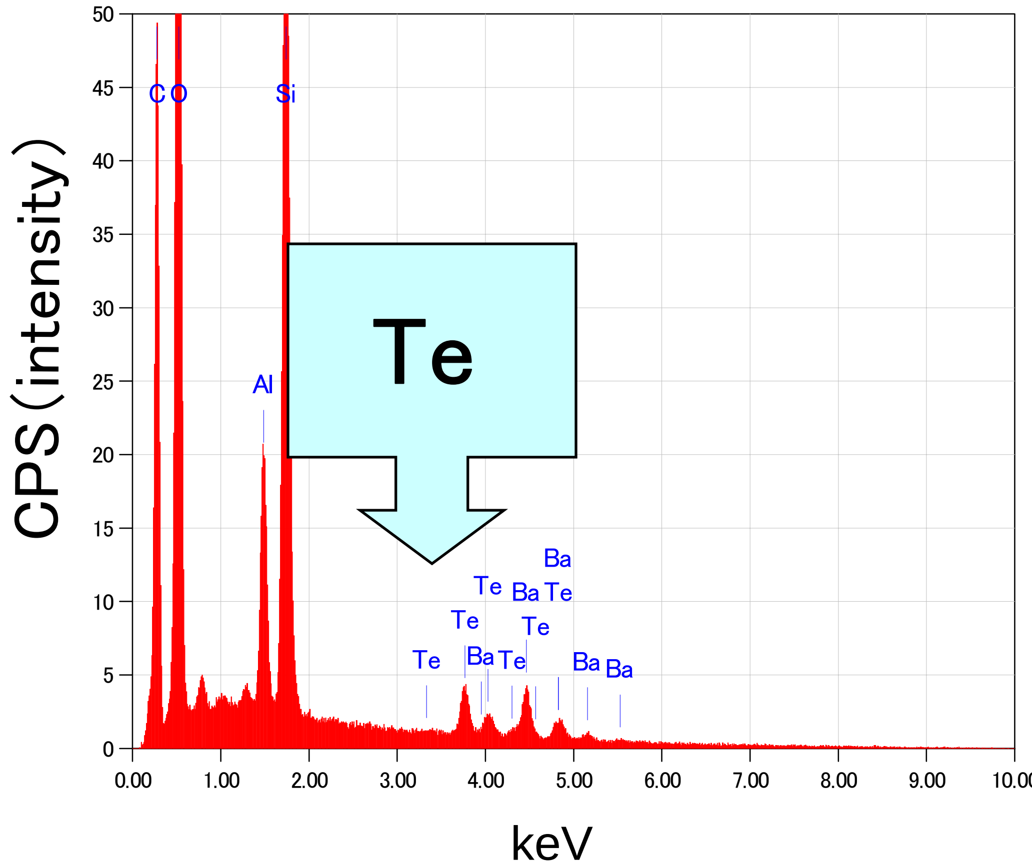
Arrows indicate elemental Te.



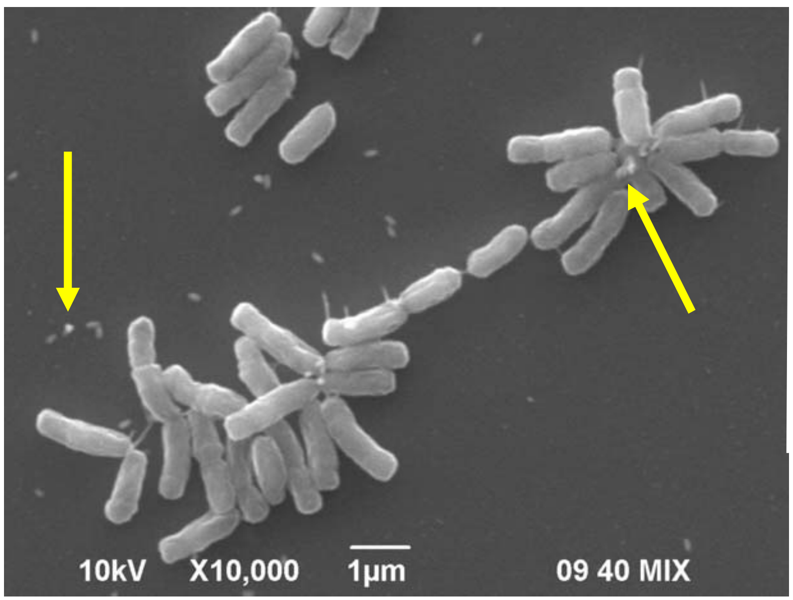
SEM and elemental analysis in Ti-2



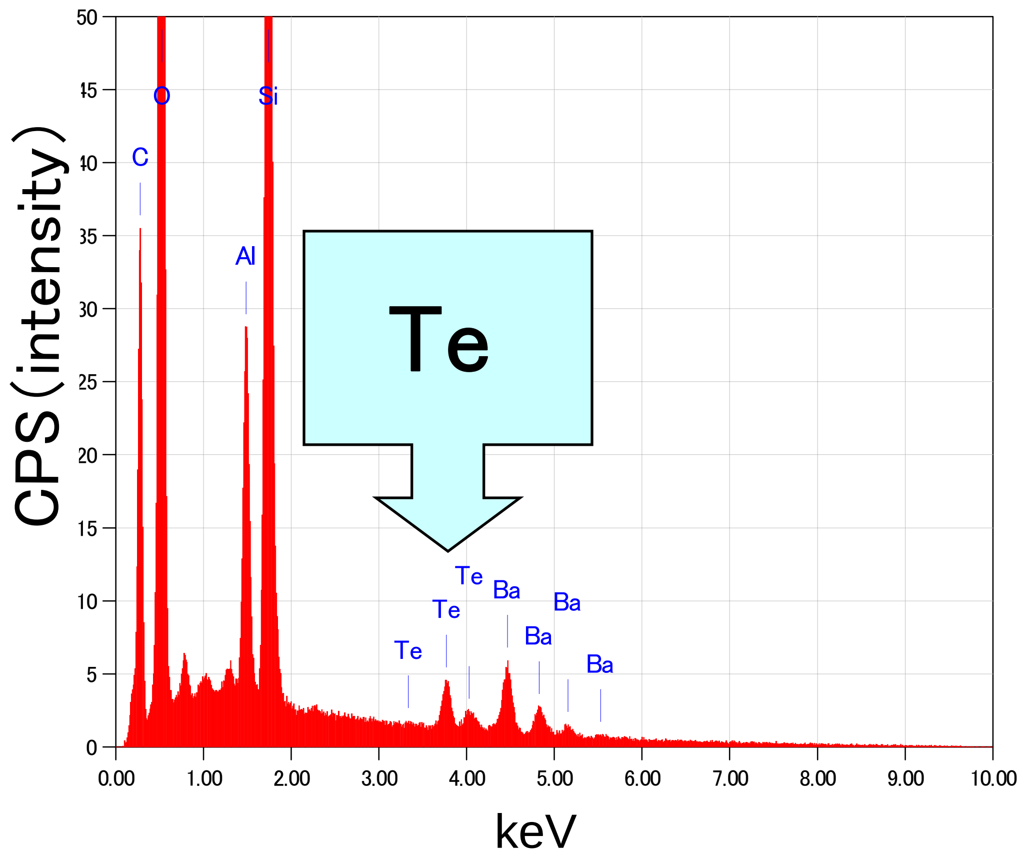
Arrows indicate elemental Te.



SEM and elemental analysis in Ti-3

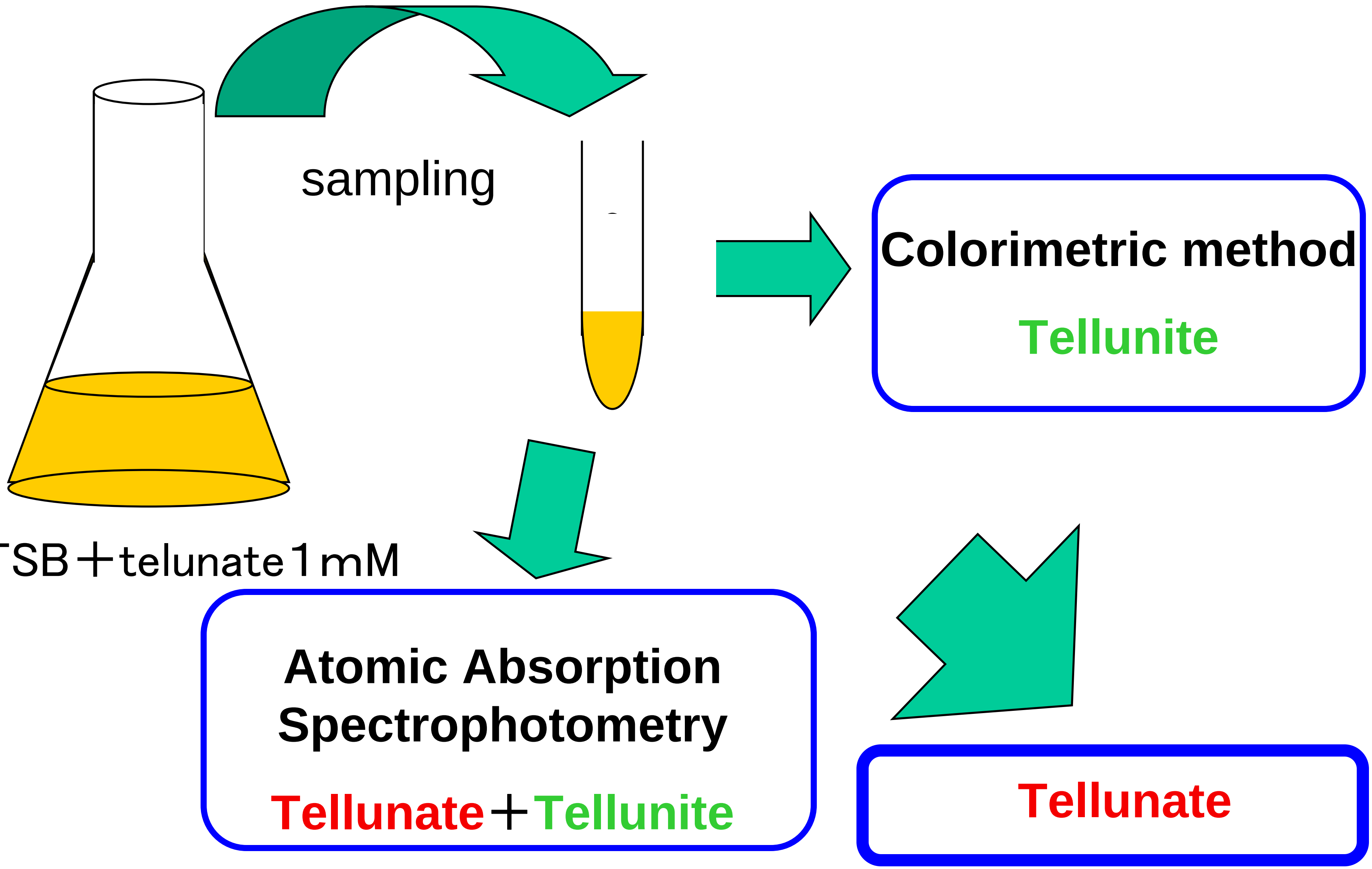


Arrows indicate elemental Te.

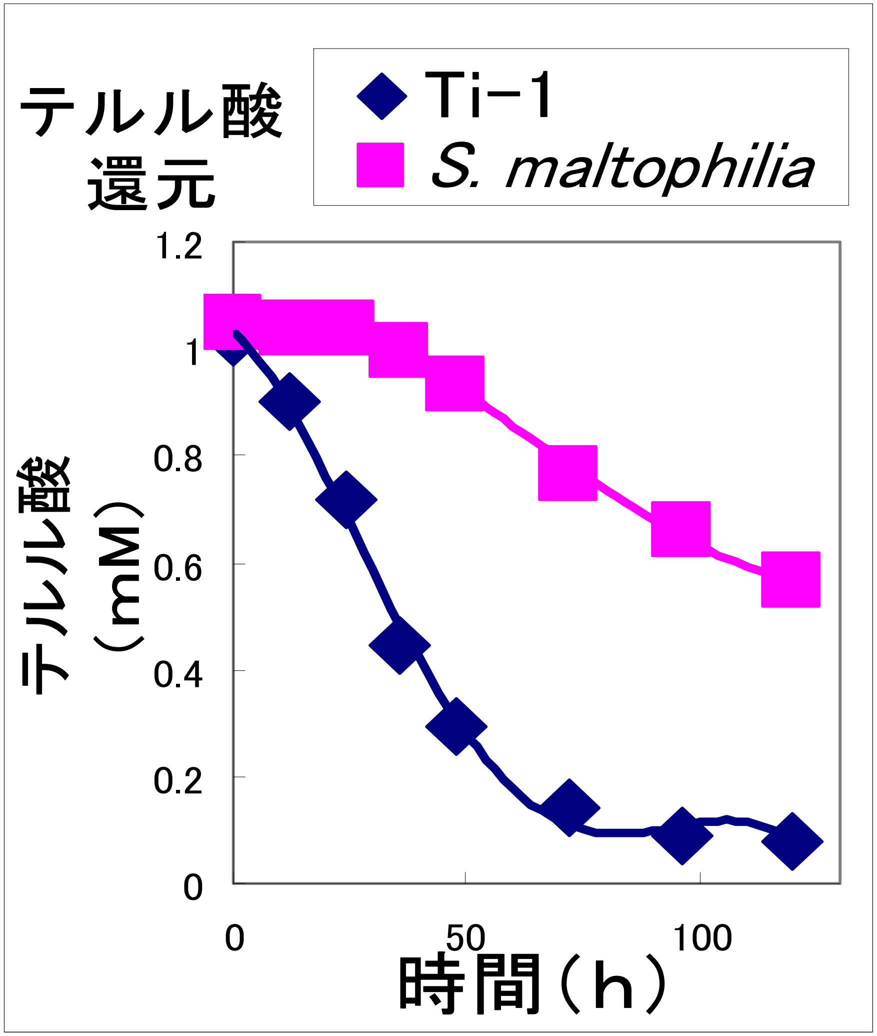


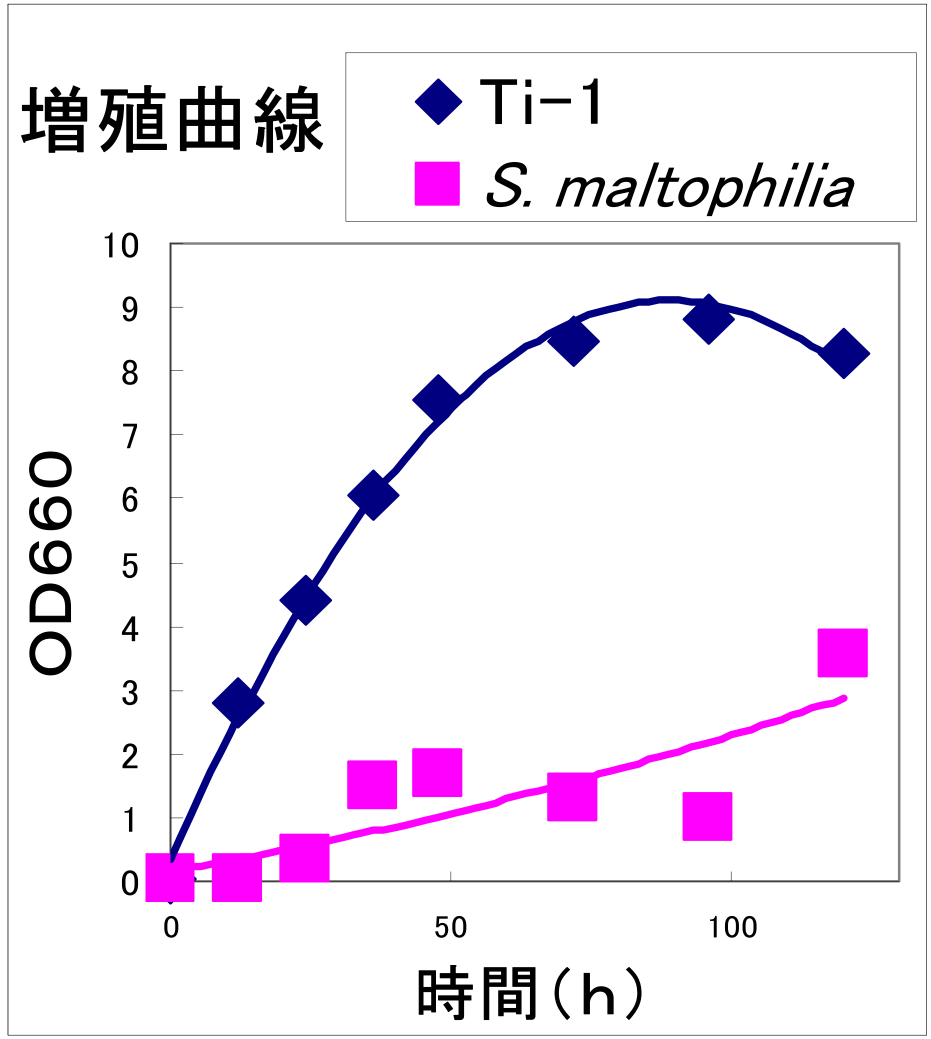
Te element synthesized from Ti-1 are bigger that that from Ti-2, 3.

Measurement of Tellunate reduction



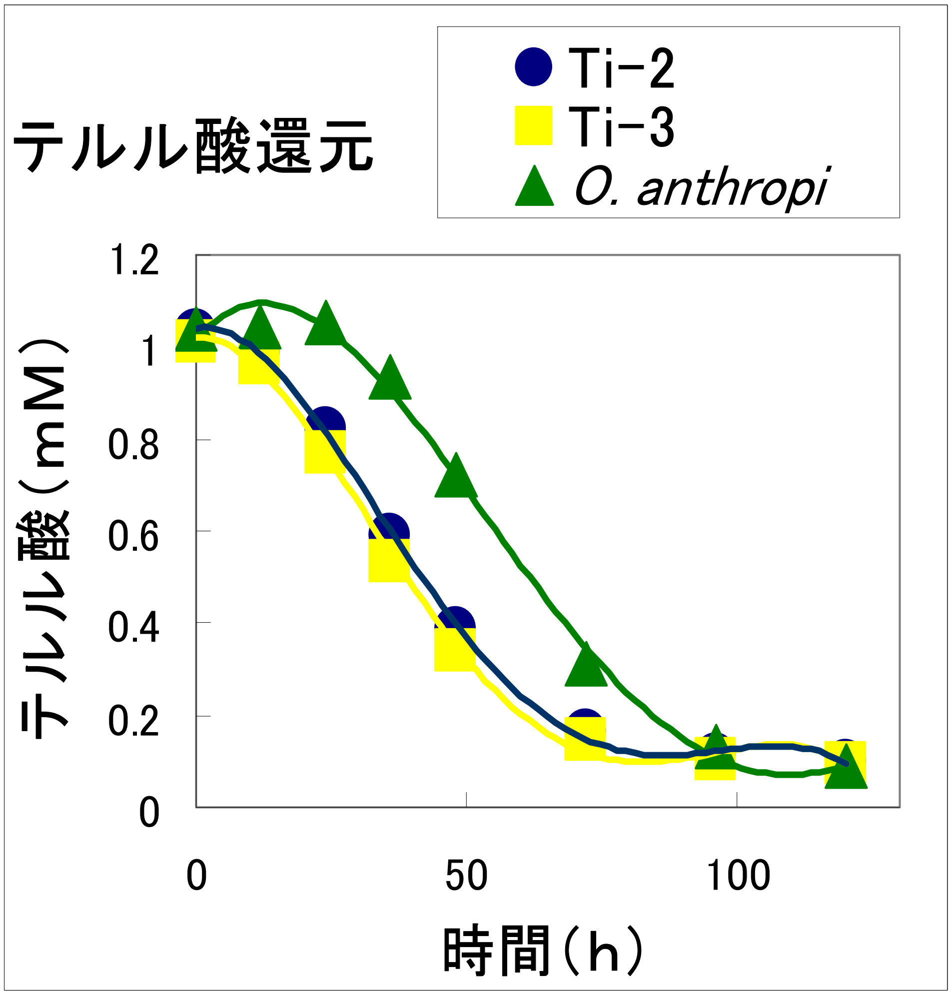
Tellunate reduction between Ti-1 and *S. maltophilia* NBRC14161^T

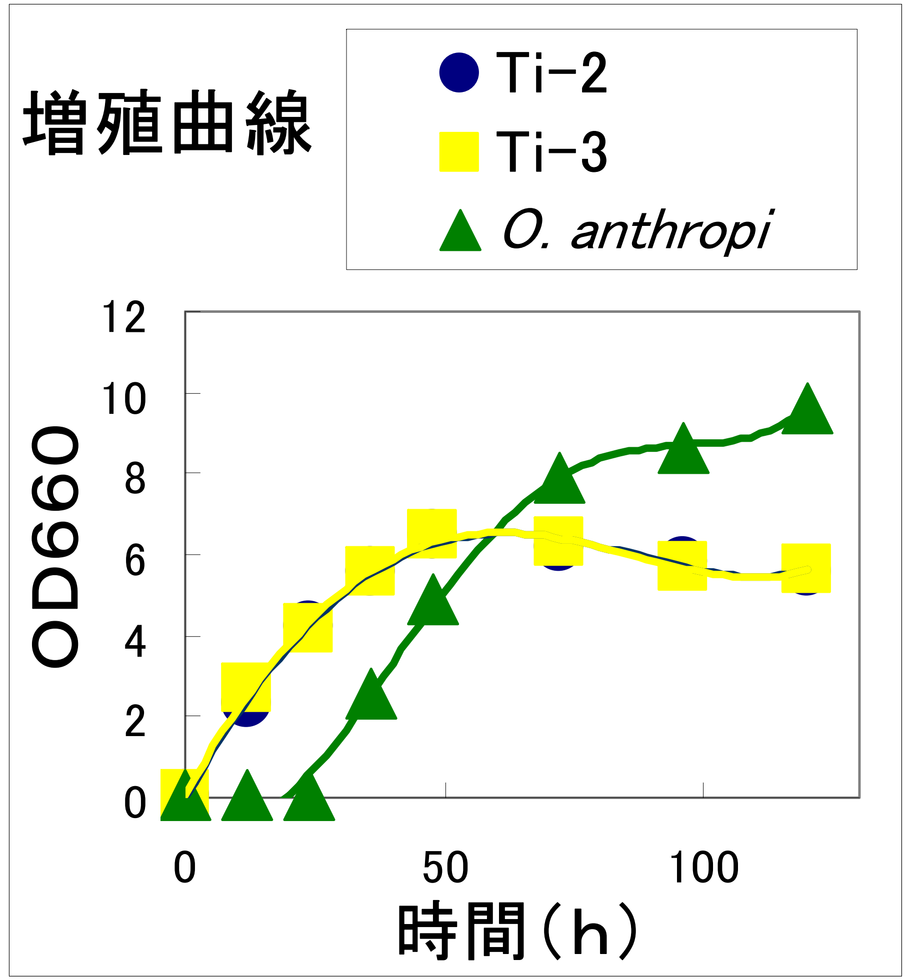




Ti-1 show high tellunate reducing activity.

Tellunate reduction between Ti-2, 3 and *O. anthropi* NBRC15819T





Ti-2, 3 show high tellunate reducing activity.

Summary

Selenium（Se）

Identification of SF-1

Cloning of selenate reducing genes

Isolation・Identification as *P. stutzeri* NT-I

High Selenate reducing activity

Tellunium（Te）

Isolation of Ti-1, 2, 3

High Tellunate reducing activity

→ Se and Te Remove and Recycle