ゲノム創薬研究所
Genome Pharmaceuticals Institute

Introduction of Novel antibiotic molecule “Kaikoshin” and the collaboration proposal of antibiotic drug discovery research using the silkworm screening and evaluation system.

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Genome Pharmaceuticals Institute

Company profile of Genome Pharmaceuticals Institute

Company name:	Genome Pharmaceuticals Institute Co. Ltd. <http://www.genome-pharm.jp/>
Established:	December 21st, 2000
Paid in capital:	31,370,000 JPY
Employees:	9 (as of August 31, 2010)
Management team:	
Representative Director	Norio Kobayashi
Board members	Kazunori Takeuchi, Kenji Miura
Inspector	Tetsuro Toriumi (TMI Associates)
Chief Financial Officer	Nobukazu Sekimizu
Advisor	Kazuhisa Sekimizu
Chief scientist	Satoshi Nishida

Research fields:

Anti-infectious agent,
Development of health food products
Establishment of disease model using silkworm for drug screening

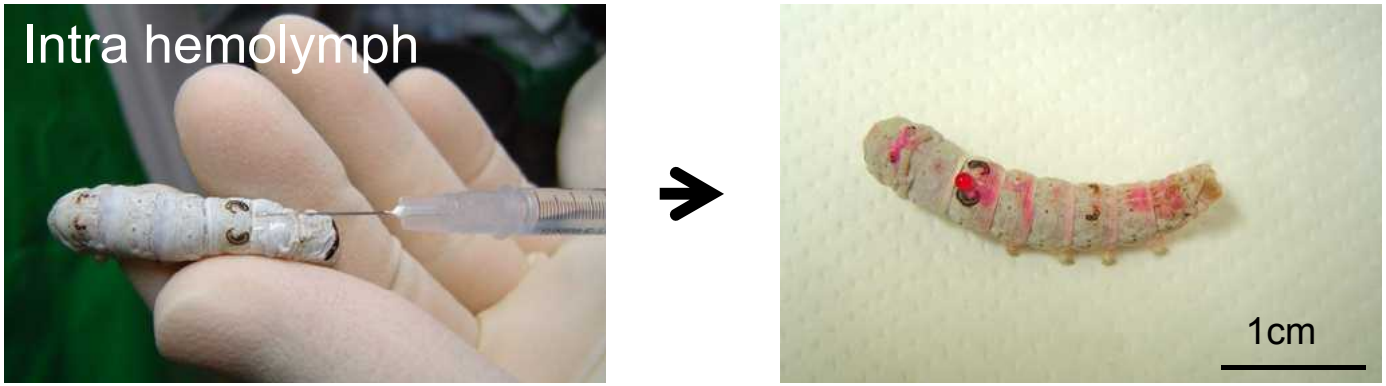
Advantages of silkworms for using discovery research of antibiotics

- Disease Model
  - Like mammal, silkworms are infected by bacteria and can be cured by antibiotics.
  - Similar therapeutic effects and safety profiles of antibiotics can be statistically monitored in silkworms as those observed in mouse models.
  - Silkworm genome completed
- Drug Discovery Screening Tools
  - High throughput screenings of antibiotics with therapeutic effects as primary index are feasible with silkworms
  - Typically, one technician can treat 400 silkworms/day
  - Low cost for breeding with artificial diet (without mulberry leaves)
  - Few ethical problems
  - Minimum biohazard potential

1. Advantage of silkworms for using disease models and for using discovery research of antibiotics

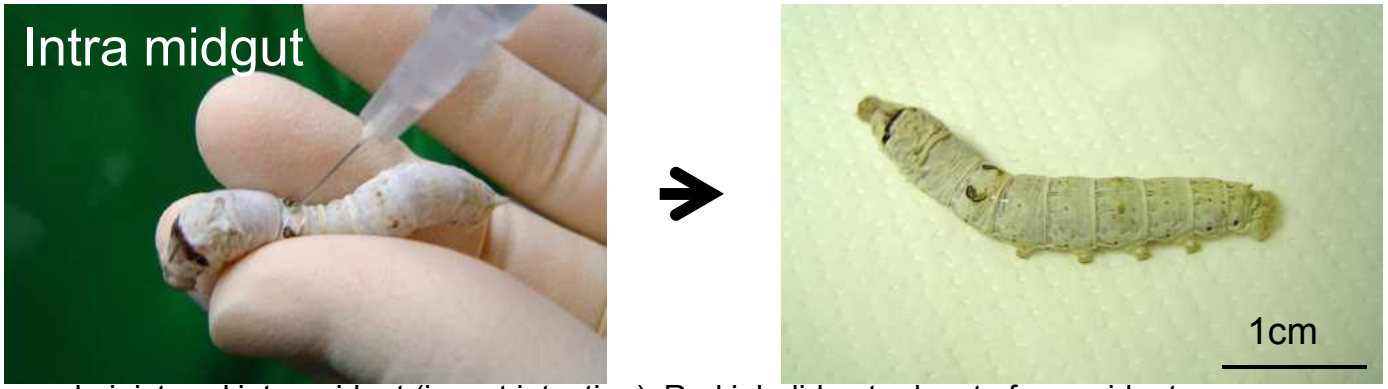
Route of drug administration can be well controlled in silkworms

- Intra hemolymph (i.h.) administration mimics mouse i.v. administration



Red ink was administered intra hemolymph (insect blood). Red ink was spread whole body immediately after injection

- Intra midgut administration mimics mouse p.o. administration



Red ink was administered intra midgut (insect intestine). Red ink did not relocate from midgut.

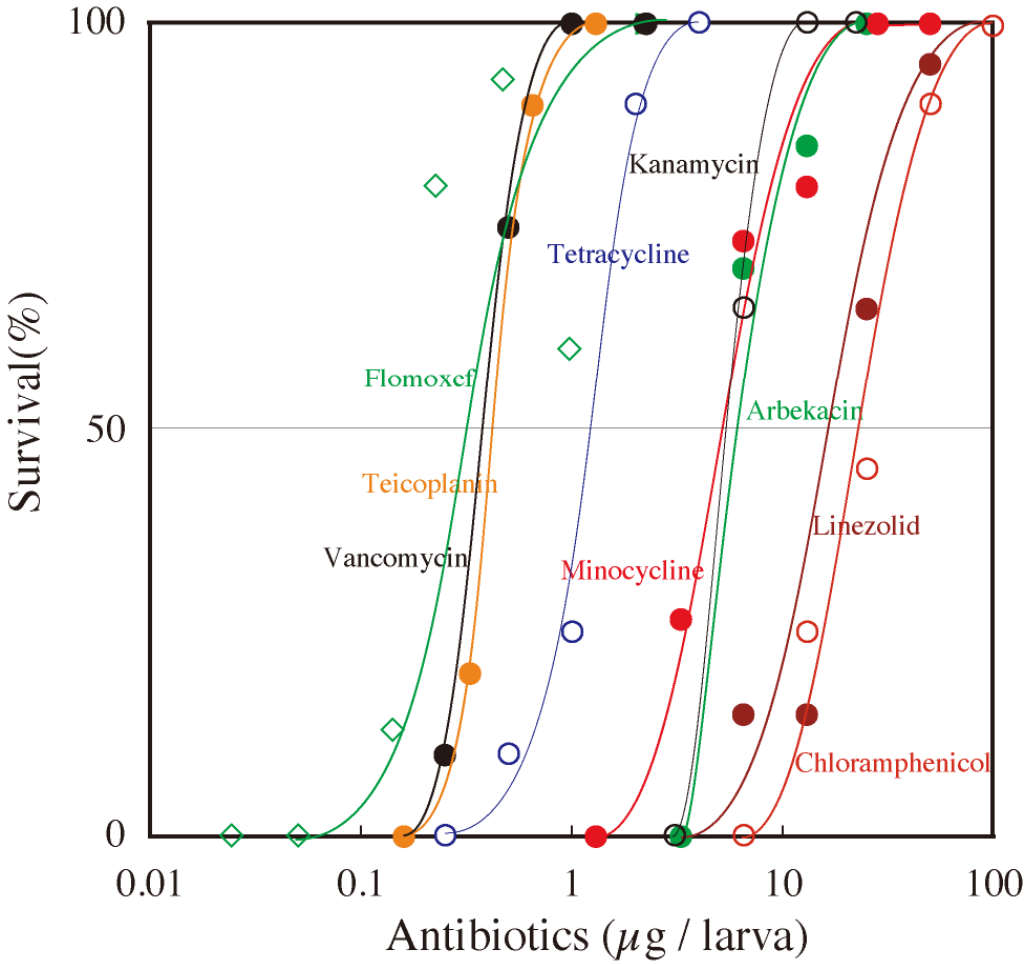
Silkworms are infected by bacteria and can be cured by antibiotics

Saline

S. aureus
(3.0x10^7 cells)

S. aureus
＋Chloramphenicol
(100μg i.h.)

Therapeutic effect of antibiotics in silkworm *S. aureus* infection model



S. aureus (3.0×10^7 cells) were injected into silkworm (i.h.), and each antibiotic was injected immediate after injection of *S. aureus*.

⇒ED_{50} of antibiotics can be calculated

Comparison of therapeutic effects of antibiotics between silkworm and mouse

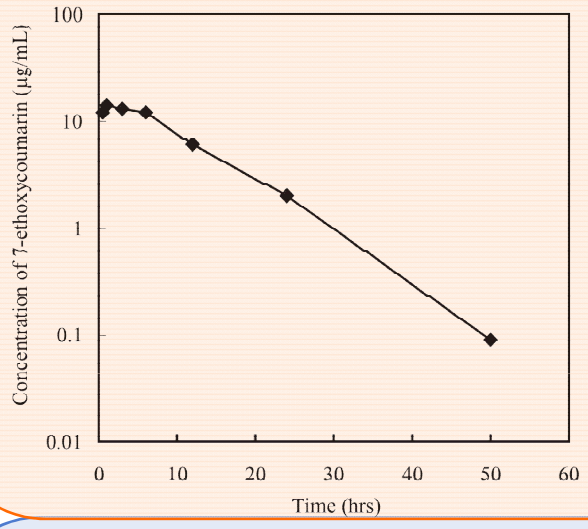
Drugs	ED_{50}(mg/g animal)	
	Silkworm	Mouse
Teicoplanin	0.3	0.1
Vancomycin	0.3	1
Minocycline	4	1
Flomoxicef	0.2	0.3
Linezolide	9	4
Katanosin B	0.1	0.7

Modified with Hamamoto et al., Antimicro Agents Chemother (2004) 48 774-779

⇒Similar ED_{50} values were obtained in silkworm and mouse

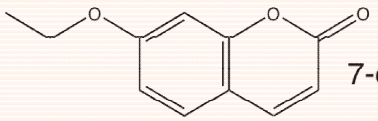
Typical drug metabolisms through P450 and conjugation reactions are observed in silkworms

Cytochrome P450 reaction



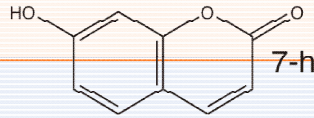
Hamamoto *et al.* Comp Biochem Physiol. 2009

Model



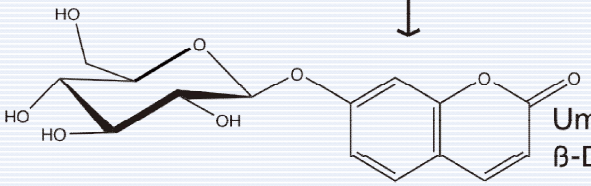
7-ethoxycoumarin

P450 (O-deethylation)



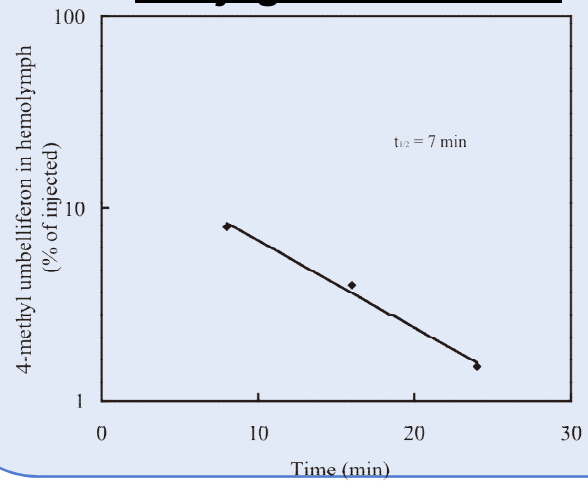
7-hydroxycoumarin

ß-glucosyltransferase

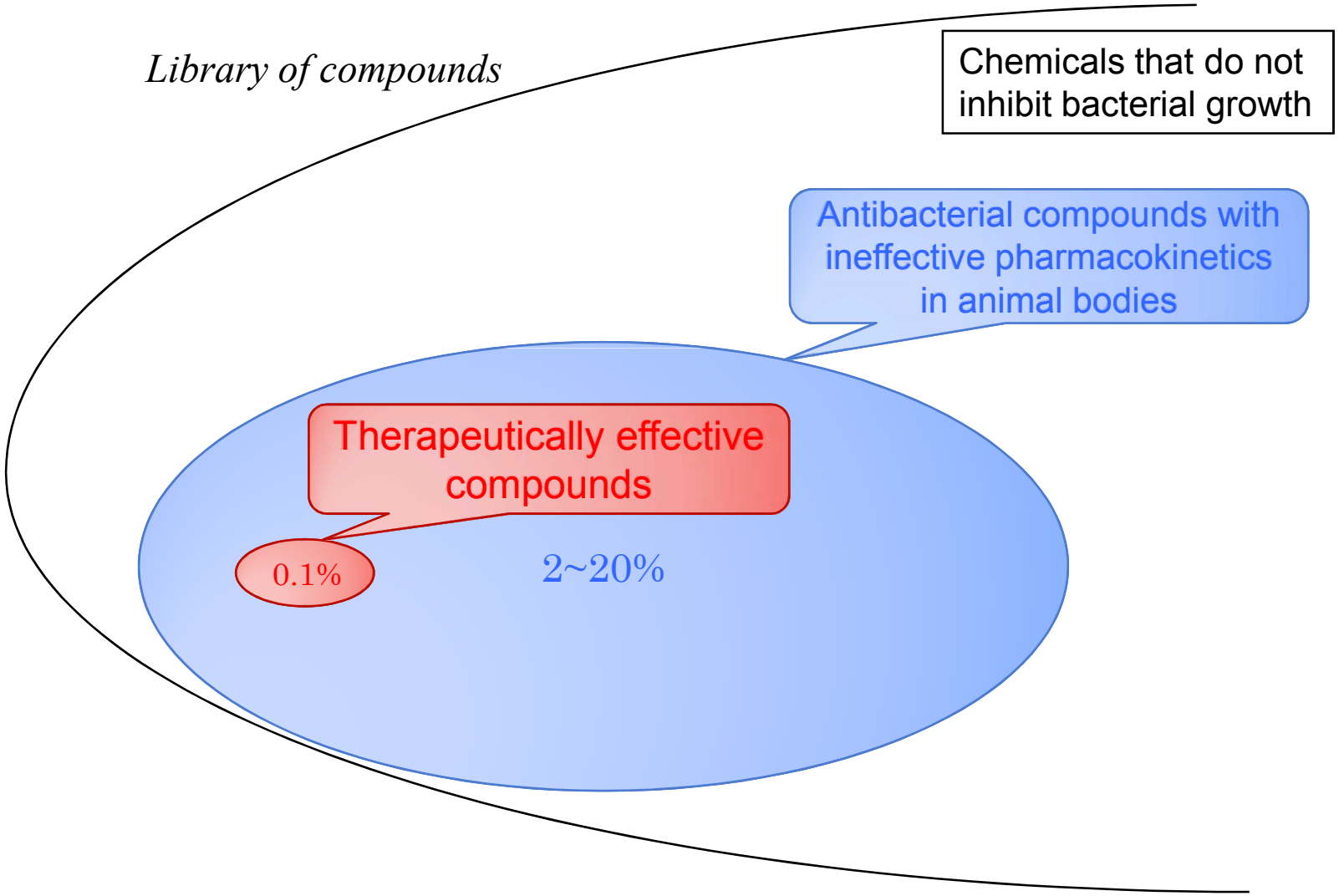


Umbelliferyl-
ß-D-glucopyranoside

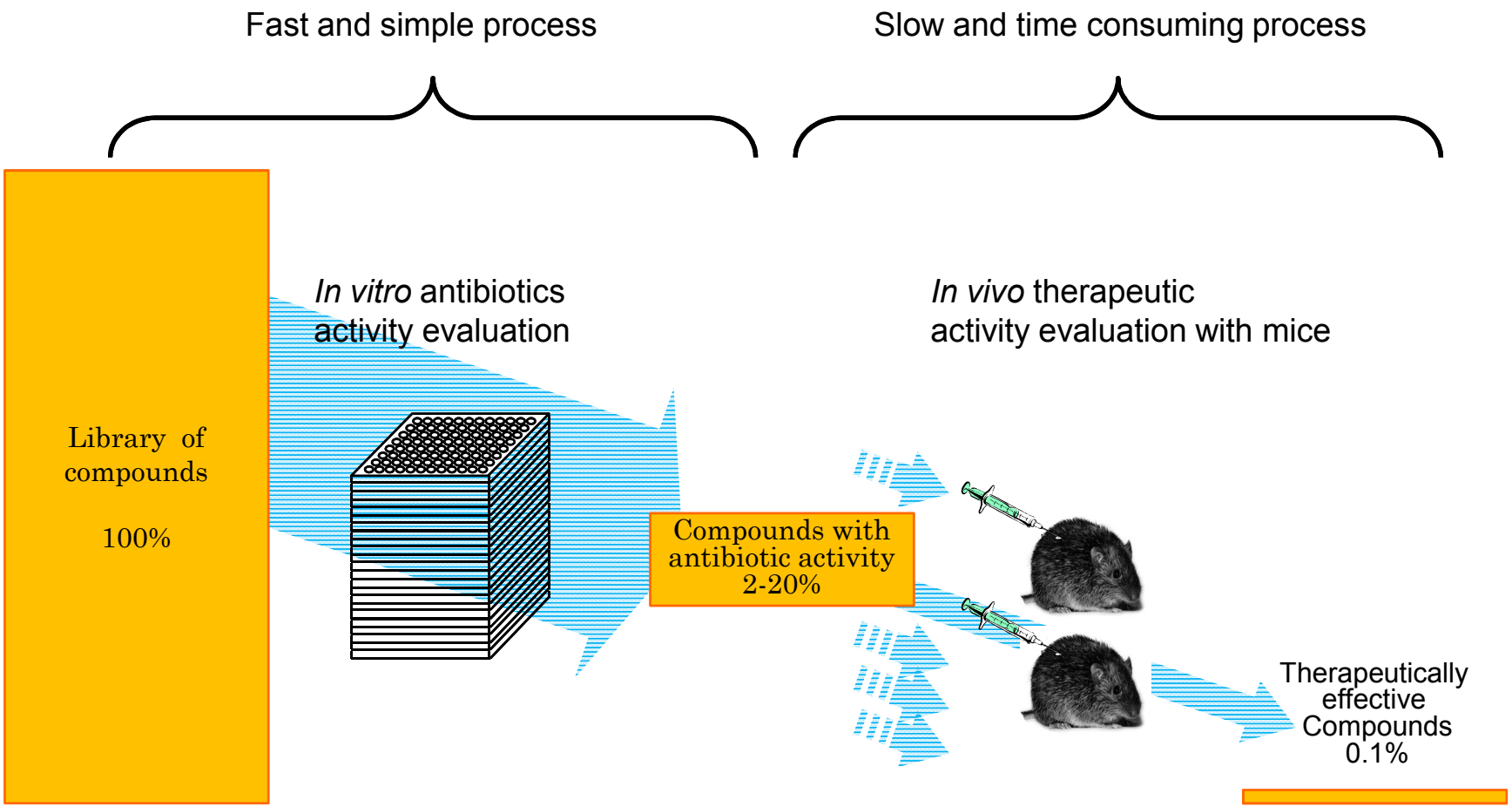
Conjugation reaction



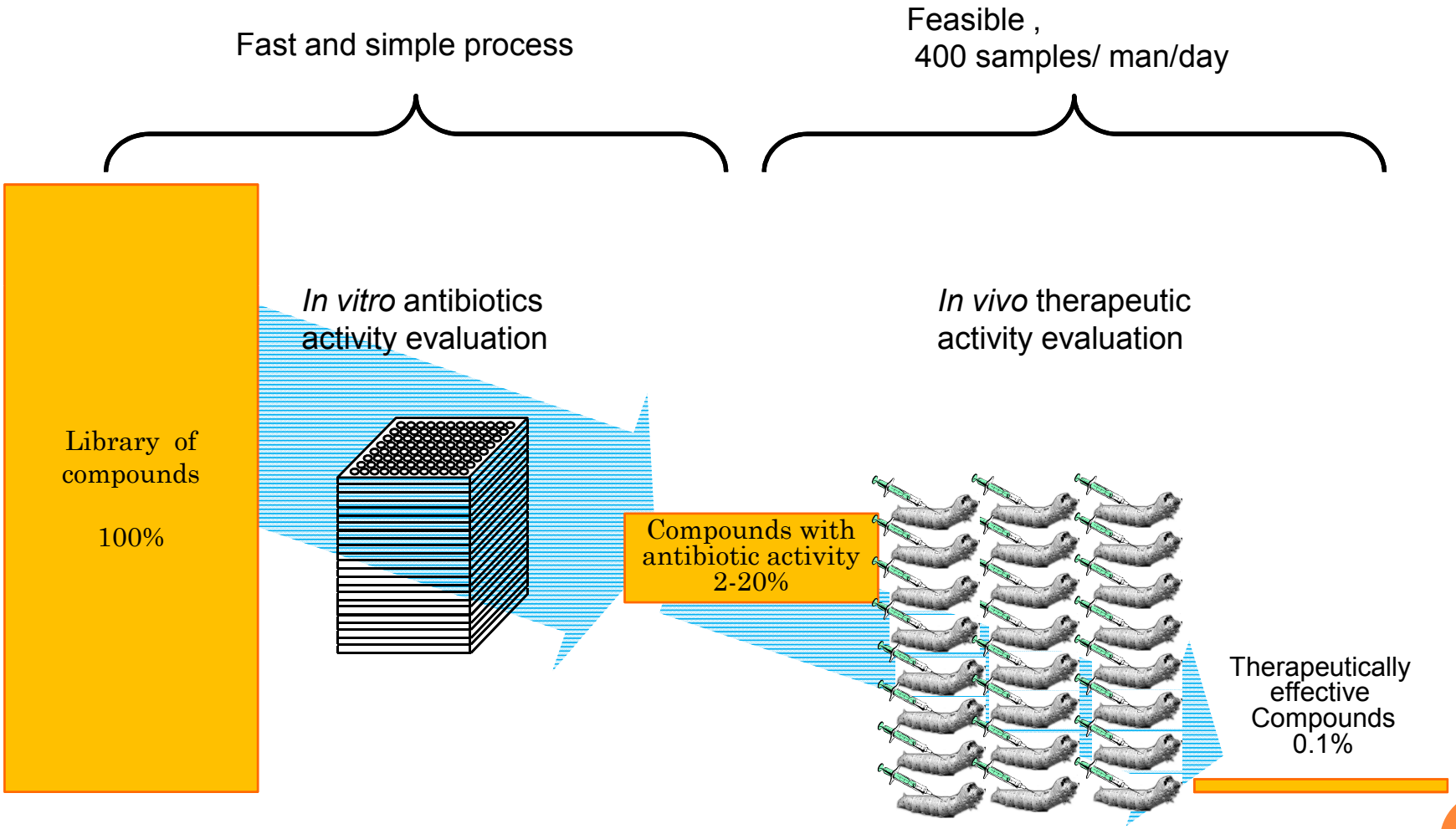
Typical screening process of antibiotics



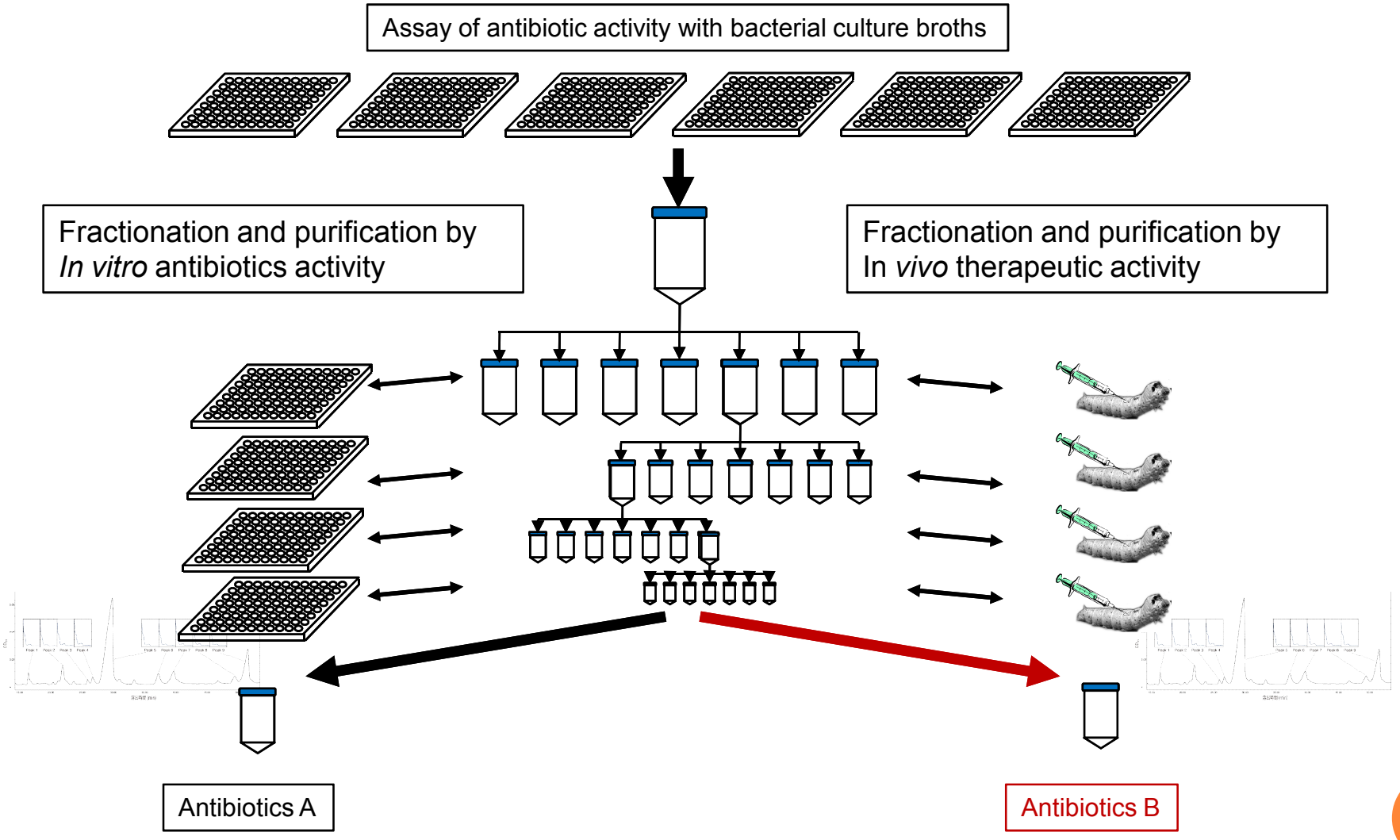
Therapeutic effect of antibiotics are not well evaluated in terms of therapeutic effects with mouse model due to time consuming processes



Therapeutic effect of antibiotics are WELL evaluated with silkworm model

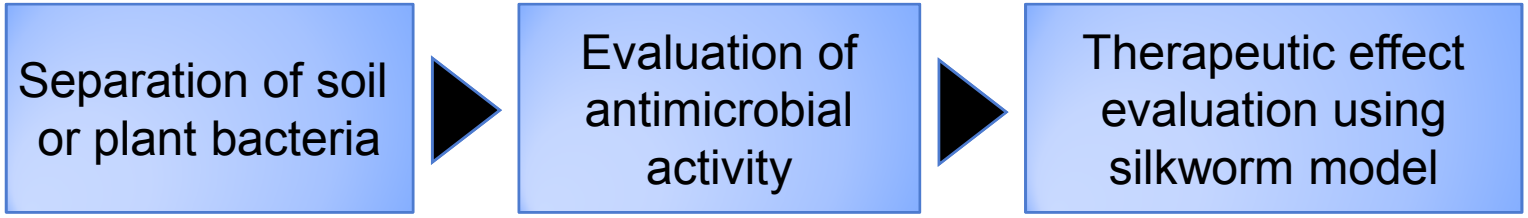


Screening of antibiotics *by in vitro* antibiotic activity and *in vivo* therapeutic effect may select different compounds



2. Screening and identification of novel antibiotics with therapeutic activity, “Kaikoshins”.

Screening of antibiotics with therapeutic activities from natural products using silkworms

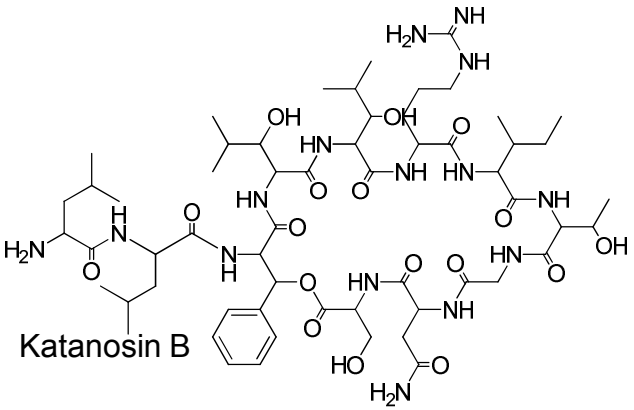


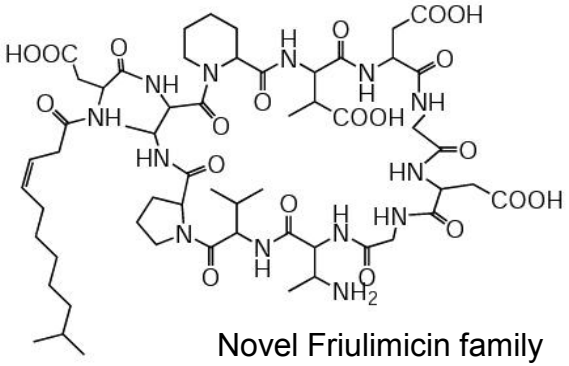
Soil bacteria were separated from Japan. Separated bacteria were cultured for 5d at 30˚C, and extracted with 50% acetone. Antimicrobial activities against MRSA *(S. aureus*) and PAO1 (*P. aeruginosa*) were tested by broth dilution method and positive samples were selected. Selected samples were injected into hemolymph of bacterial infected silkworms and measured survival rate of silkworm after 2 days incubation at 27˚C.

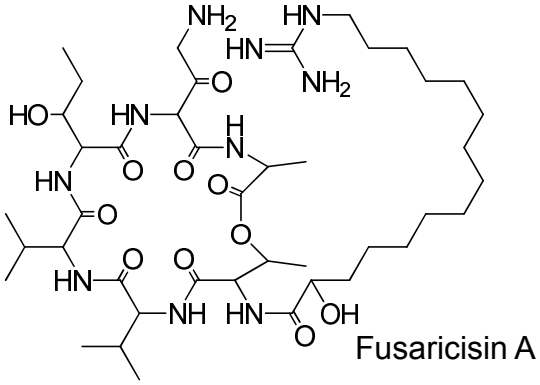
Separated bacteria	14,651
Antimicrobial activity	
S. aureus (MRSA)	2,794 (19 %)
P. aeruginosa	353 (2.4%)
Therapeutic effect	
S. aureus	23 (0.2%)
P. aeruginosa	17 (0.1%)

Indentified antibiotics

Strain No	16s rRNA analysis	Bacteria type	Producing antibitics
107-4	*Streptomyces sp.*	Streptomyces	Water soluble comp.
119-1	*Paenibacillus sp.*	Bacteria	Fusaricisin A
134-2	*Paenibacillus sp.*	Bacteria	Fusaricisin A
667-1	*Paenibacillus sp.*	Bacteria	LI-05b, LI-06b, LI-F08a, LI-07a
810-6	*Paenibacillus sp.*	Bacteria	
HV23-3	*Paenibacillus sp.*	Bacteria	Katanosin B
R243-1-1	*Aspergillus sp.*	filamentous fungi	
RH2175-11	*Lysobactor sp.*	Bacteria	Katanosins
RH2180-5	*Lysobactor sp.*	Bacteria, novel	Kaikoshins
RH2241-7	*Nocardia sp.*	Actinomyces, novel	
RG2435-1	*Paenibacillus sp.*	Bacteria	Fusaricisin A
RG2436-12	*Peanibacillus polymyxa*	Bacteria	
RG2742-6	*Paenibacillus sp.*	Bacteria	
H2305-9c	*Streptomyces sp.*	Streptomyces	
RG2749-10	*Penicillium sp.*	Fungus	


Katanosin B


Novel Friulimicin family


Fusaricisin A

Since MRSA was used for antimicrobial activity test, known antibiotics could be excluded

Intellectual properties covering “Kaikoshins”

- Patents covering series of Kaikoshin molecules and strains producing Kaikoshins are filed in 2010

Proposal for “Kaikoshins”

Feature of Kaikoshins

1. Novel structural antibiotics were found by screening for therapeutic effects in silkworm evaluation systems.
2. Kaikoshins are novel antibiotics with M.W. of more than 1600 dalton and consist of 12 L- and D- amino acids with fatty acid side chains.
3. Kaikoshin E shows rapid bactericidal activity.
4. Kaikoshin E shows therapeutic effects in a mouse *S. aureus* infection model with lower dose than vancomycin.
5. Kaikoshin is effective against MRSA and VRE.
6. The LD_{50} is 660-fold greater than the ED_{50}.

Genome Pharmaceuticals Institute would like to collaborate with a partner for further clinical development of Kaikoshins

3. Collaboration proposal of identifying novel antibiotics using silkworm model.

Collaboration scheme proposal (1)

1. Collaboration for lead-optimization of identified compounds.
   - GPI screened more than 100,000 compounds and identified several compounds with therapeutic activity by oral application.
   - GPI completed initial chemical synthesis approach for lead optimization and is planning to further conduct lead optimization with therapeutic effects as primary marker.
   - GPI and partner will collaborate and the partner will support GPI's activity for lead optimizations with the scheme of clearing pre-determined milestones and share the right for compounds.
   - Partner may have option of in-licensing the compounds and conducting further clinical development with normal up-front, milestones and running royalty payment schemes.

Collaboration scheme proposal (2)

2. Collaboration of screening partner's compound library and for further lead-optimization.

- Partner will select compound library with antibiotic activity and provide the library to GPI.
- GPI will further conduct screening of compounds with therapeutic activity as criteria
- GPI and partner will collaborate and the partner will support GPI's activity for lead optimizations with the scheme of clearing pre-determined milestones and share the right for compounds.
- Partner may have option of in-licensing the compounds and conducting further clinical development with normal up-front, milestones and running royalty payment schemes.