Efflux Pumps Mediate Reduced Sensitivity to Fluazinam in *Phytophthora infestans*

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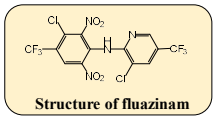
< Introduction >



Fluazinam was developed by Ishihara Sangyo Kaisha, Ltd.

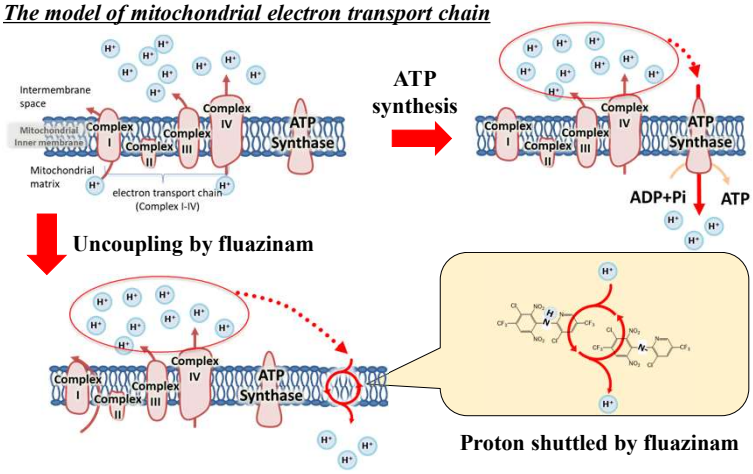
It demonstrates high efficacy against a broad spectrum of pathogens, particularly *Phytophthora infestans*, the causal agent of potato late blight.

Fluazinam belongs to the FRAC code 29 (uncouples oxidative phosphorylation in mitochondria) and is classified as having **a low risk of resistance development**.

It is hypothesized that fluazinam function as a proton carrier in the mitochondrial inner membrane (right), **acting independently of any specific protein target**.
Since its launch in 1988, fluazinam has played an important role in controlling late blight.

However, an EU_37 strain of *P. infestans* exhibiting reduced sensitivity to fluazinam was identified in Europe in 2018.
We attempted to identify the mechanism involved in this decrease in sensitivity.

The model of mitochondrial electron transport chain



< Results & Discussion>

I. *Genotypes and sensitivity to fluazinam*

The field-isolated strains were tested for sensitivity to fluazinam, and the results are shown in table.

Genotype	Isolate	The lowest concentration that completely inhibited zoospore motility (ppb)	Inhibition of cystospore germination EC_{50} (ppb)	Inhibition of lesion development on leaf disc EC_{50} (g a.i./ha)
EU_13	18FR07	10 < X ≤ 50	51	20.1
EU_36	17BE08	10 < X ≤ 50	32	37.4
EU_37	16-02	> 100	135	—
	18BE02	> 100	106	> 200
	19BE01	> 100	118	> 200
	19FR01	> 100	208	> 200
EU_41	18SE01	10 < X ≤ 50	45	18.5

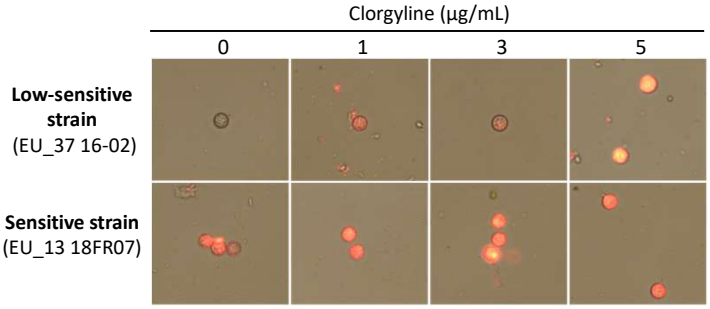
Genotype EU_37 was the least sensitive to fluazinam.

II. *The function of efflux pump in low-sensitive strain*

・Exclusion of fluorescent dye

To confirm the mechanism of reduced sensitivity beyond the target-site mutations, the efflux ability of the EU_37 and EU_13 strains was compared using fluorescent dye, Rhodamine 6G (70 mM).

✓ Visualization under a fluorescent microscope revealed that the fluorescence intensity of Rhodamine 6G increased in a dose-dependent manner when clorgyline, an efflux pump inhibitor, was applied. The sensitive strain (EU_13) showed higher fluorescence than the low-sensitive strain (EU_37).



・Efficacy of fluazinam on cystospore germination 【EC_{50} (ppb)】

✓ The EC_{50} analysis revealed that clorgyline treatment restored the sensitivity of the low-sensitive strain EU_37 to fluazinam, matching the level observed in the sensitive strain EU_13.

	Isolate	Clorgyline (μg/mL) 0	1	3	5
Low-sensitive	EU_37 18BE02	170	96	33	12
Sensitive	EU_13 18FR07	52	27	17	10

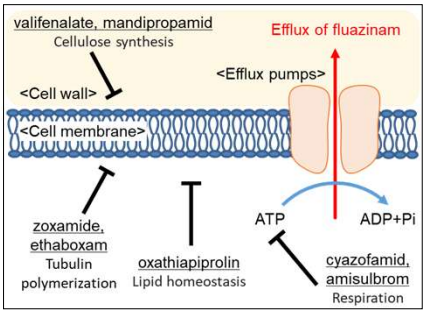
These data strongly suggest that efflux pumps are constitutively active in EU_37 strain, leading to the exclusion of fluazinam.

III. *Efflux pumps and strategy for disease management*

Studies on multidrug resistance have identified two efflux pump classes involved in resistance: ATP Binding Cassette (ABC) transporter and Major Facilitator Superfamily (MFS) transporter.

It has been suggested that certain fungicides, which disrupt membrane function or inhibit ATP synthesis, may block the efflux of fluazinam, thereby restoring its antifungal activity.

- ABC transporter:
  use energy from ATP hydrolysis.
- MFS transporter:
  rely on the electrochemical gradient across the membrane.



・Rate of fluorescence retained

To confirm our hypothesis, cystospores (EU_37 18BE02) were treated with fluazinam alone or in combination with fungicides targeting different transporter classes, and Rhodamine 6G uptake was measured by flow cytometry to evaluate their synergistic effect with fluazinam.

✓ At 100 ppb fluazinam alone, the average fluorescence retention rate was 12.1%.
✓ The combination with other fungicides increased the fluorescence retention rates.

Combination fungicide	Concentration	Rate of fluorescence retained: fluazinam concentration 0 ppb	100 ppb
cyazofamid	5 ppb	5.8 %	19.8 %
amisulbrom	50 ppb	6.9 %	22.8 %
valifenalate	500 ppb	8.4 %	32.9 %
mandipropamid	25 ppb	7.1 %	41.0 %
oxathiapiprolin	12.5 ppb	9.1 %	37.0 %
zoxamide	100 ppb	9.7 %	30.5%
ethaboxam	500 ppb	6.0 %	25.0 %

・Inhibition of lesion development

Cystospores (EU_37 18BE02) were inoculated onto fungicide-treated leaves. The data shows lesion control value, with values in parentheses representing the theoretical synergistic effects calculated using Colby's method.

✓ The addition of combination fungicides improved efficacy of fluazinam against the infection.

Combination fungicide g a.i./ ha		fluazinam g a.i./ha 60	40	30	0
cyazofamid	9	84 (74)	83 (72)	84 (70)	68
	6	92 (59)	71 (55)	69 (53)	50
valifenalate	5	95 (76)	92 (73)	83 (72)	70
	3	99 (47)	58 (42)	58 (40)	35
	0	19	11	6	

These results indicate that increased fluazinam efflux contributes to reduced sensitivity in EU_37, and that this can be restored by certain fungicides. They highlight efflux pump modulation as a valuable strategy for disease management.

Our findings confirm fluazinam's status as a low-risk fungicide and highlight its continued potential in managing *P. infestans* outbreaks.