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USE OF ANTISENSE RNA TO MODULATE *HSP* GENE EXPRESSION IN *OENOCOCCUS OENI*

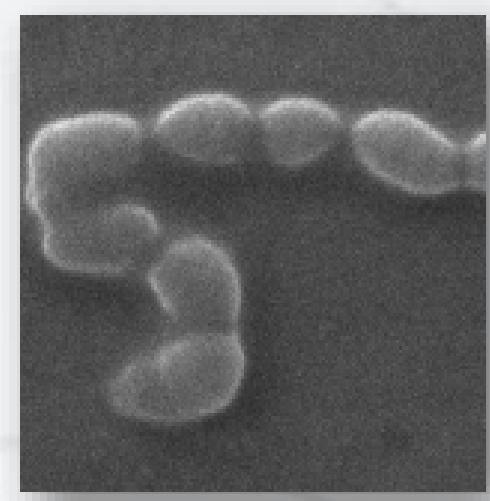
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ID *O. oeni*



Name: *Oenococcus oeni*, formerly *Leuconostoc oenos*

Description : Gram+, cocci in chains

Strain: ATCC BAA-1163

Family: Lactic Acid Bacteria (LAB), Firmicutes

Interest: Malolactic fermentation of wine → aromatic improvement of wine [1]

Environment: wine (low pH, low temperature, nutrient poor, ethanol)

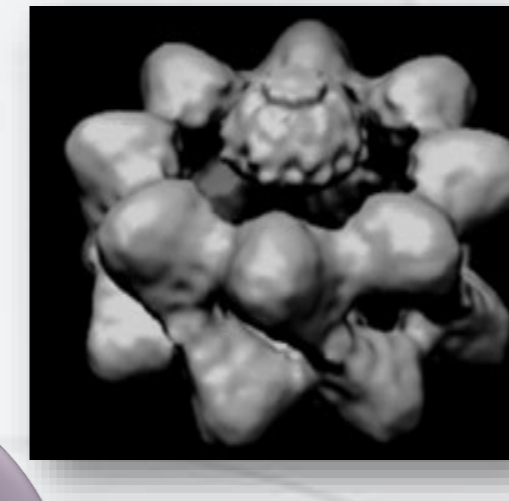
Genome ID and status: AAUV00000000.1, scaffold (62 contigs)

Genetic tools: few reliable tools, weak transformation efficiency

➔ Not easily manipulable bacterium,
Mutation not possible to investigate role of genes

Signature : *O. oeni*

ID *hsp18*



Gene and GI number: *hsp18*, OENOO_66120 (446 bp)

Protein: Lo18 (148 aa), GI: 118432051

Family: Small Heat Shock Protein (sHSP)

Regulation: Transcriptional repression by **CtsR** under optimal growth conditions [2]

Functions: • Molecular chaperone activity: prevent *in vitro* aggregation of damaged proteins [3]

• Possibly lipochaperone activity: *in vivo* membrane association [4] and *in vitro* regulation of liposome fluidity [5]

➔ In vivo function ?

Signature: *Lo18*

OBJECTIVES:

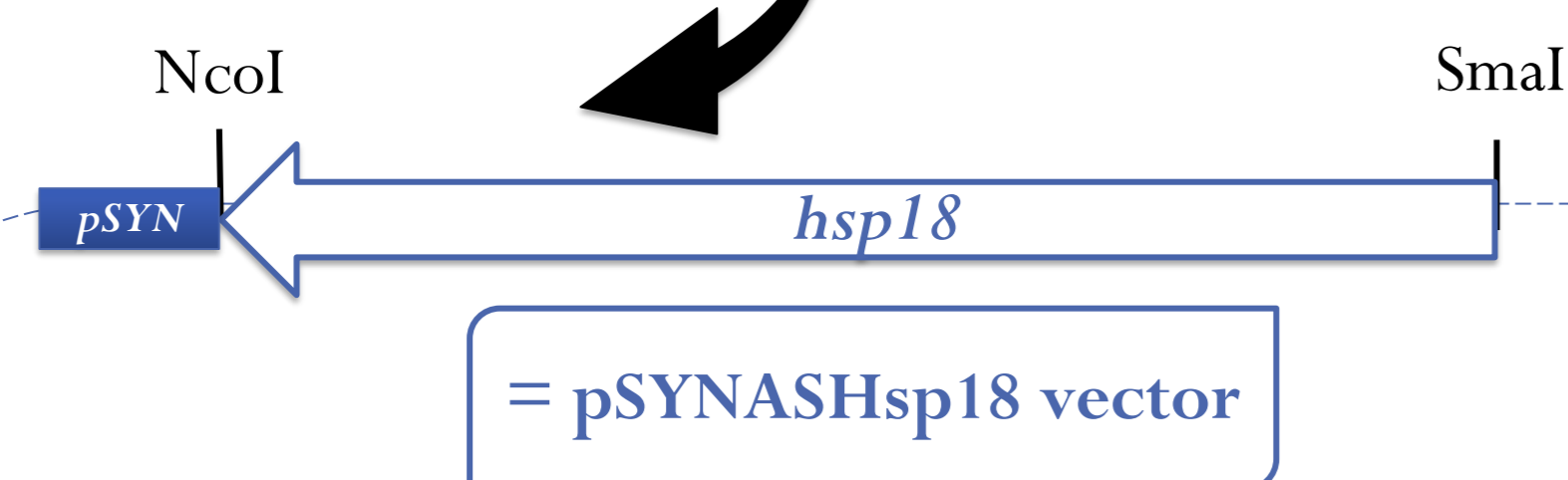
- To overcome the difficulties of manipulation of the genome of *O. oeni* due to the lack of genetic tools for gene replacement.
- To develop a gene inactivation method by using antisense RNA approach.
- To modulate *hsp18* gene expression in *O. oeni* and understand *in vivo* the Lo18 function.

Strategy

1: Targeting strategy: to target Open reading of the *hsp18* gene frame + PCR amplification

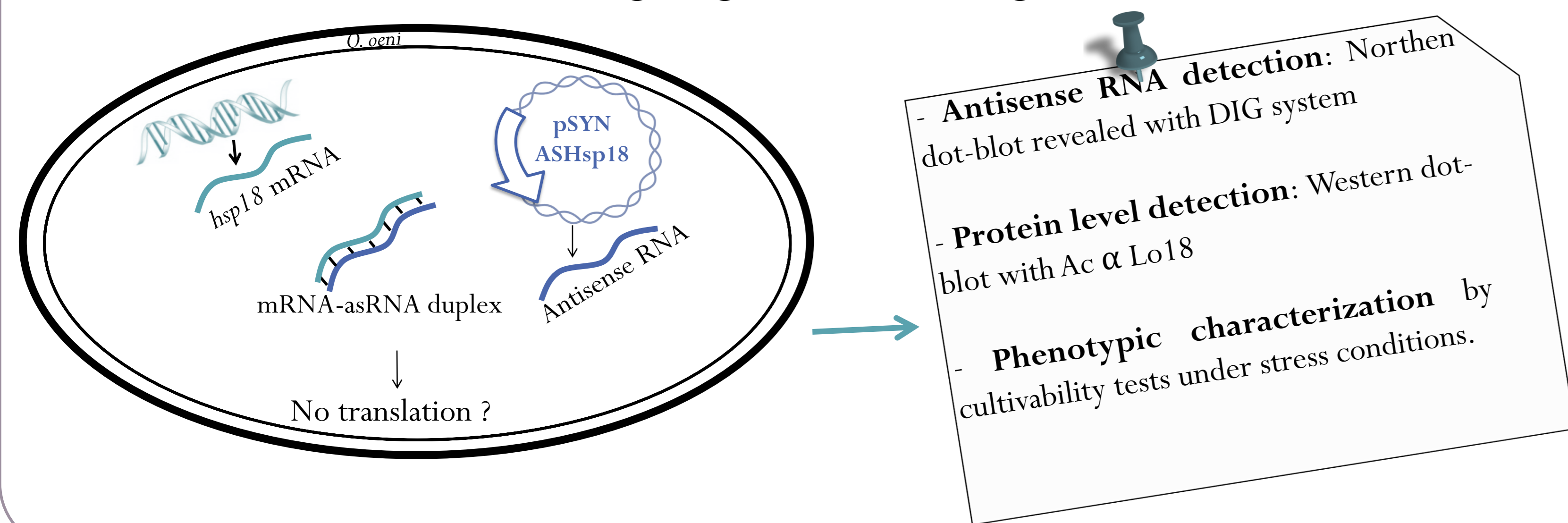


2: Cloning in antisense orientation into *E. coli*/LAB shuttle vector (pSIPSYN)



3: Transfert of pSYNASHsp18 in *O. oeni* ATCC BAA-1163

➔ Production of Antisense RNA targeting mRNA of *hsp18* gene in *O. oeni*



Results

1. Detection of *hsp18* messengers RNA and antisense RNA.

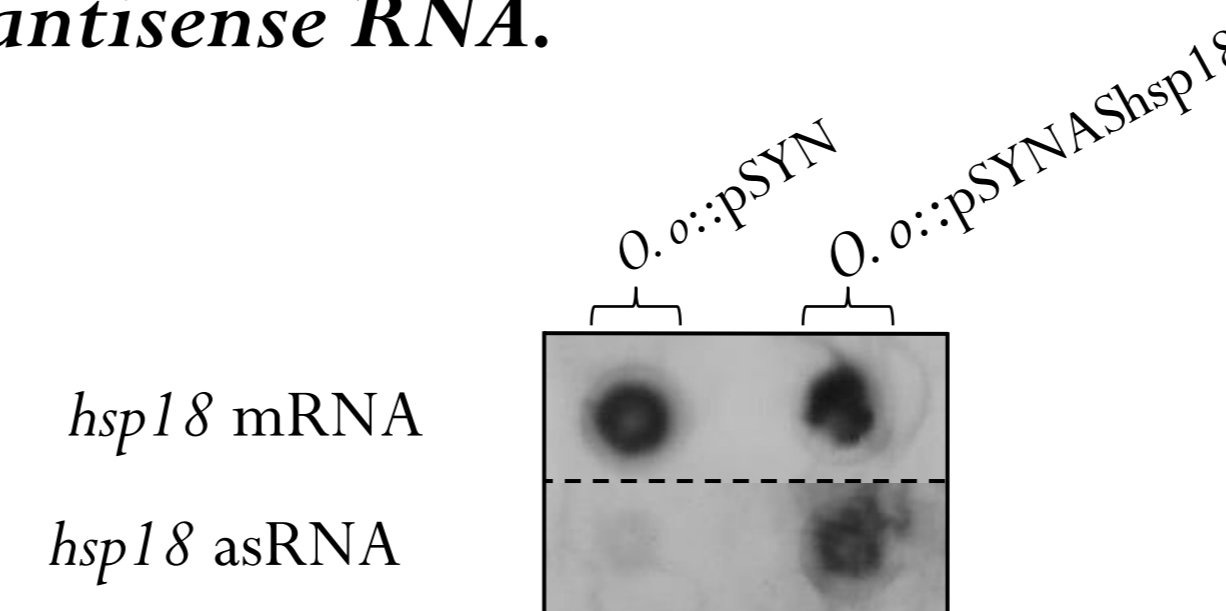


Fig. 1: Detection of asRNA and mRNA of *hsp18* in *O. oeni* recombinant strains by Northern dot-blot with DIG-probes. Total RNA were extracted from *O. oeni* carrying pSIPSYN (O.o.:pSYN) and *O. oeni* carrying pSIPSYNASHsp18 (O.o.:pSYNASHsp18) strains.

2. Detection of Lo18 protein

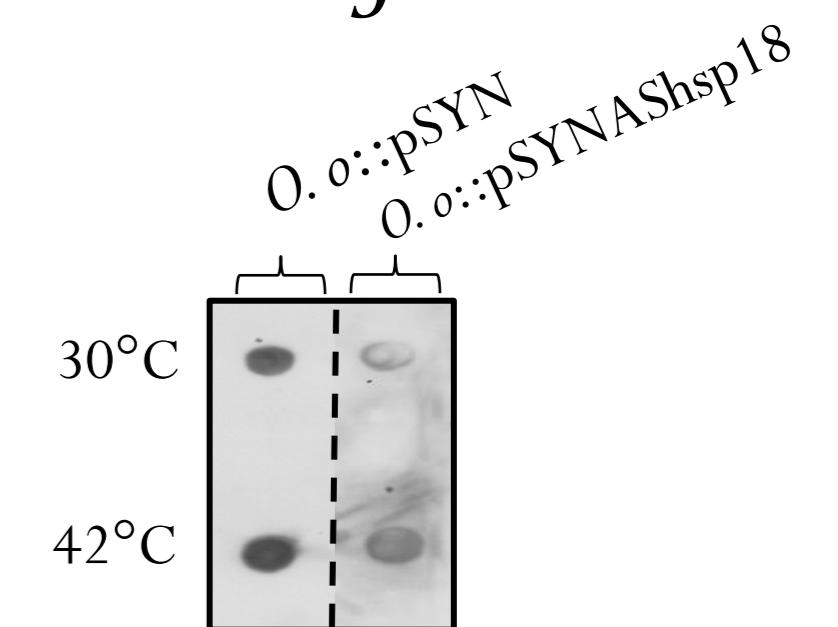


Fig. 2: Detection of Lo18 protein by western dot-blot with Ac α Lo18. O.o.:pSYN : *O. oeni* carrying pSIPSYN (O.o.:pSYN) and *O. oeni* carrying pSIPSYNASHsp18 (O.o.:pSYNASHsp18) strains were grown at 30°C or 42°C.

Validation of asRNA EXPRESSION

Validation of asRNA FUNCTION

2. Effects of *hsp18* asRNA expression on survival under stress conditions ?

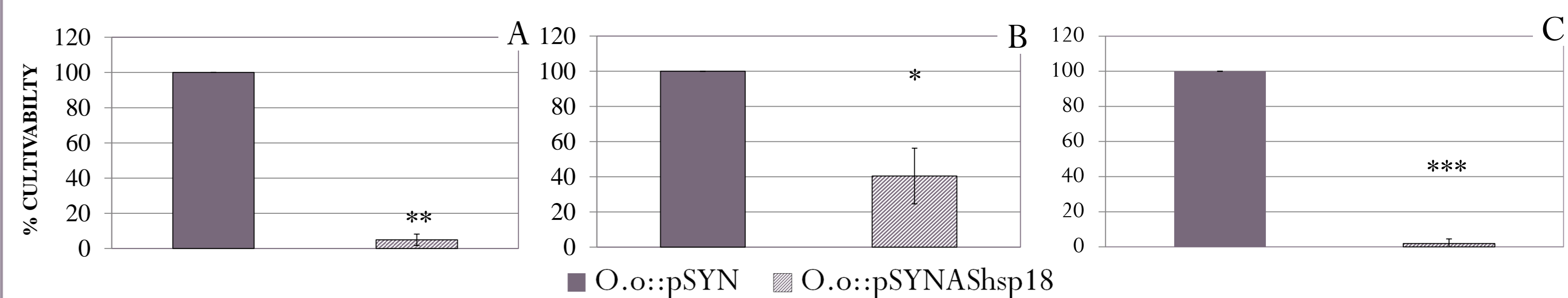


Fig. 3: Cultivability tests after heat shock (A), acid shock pH3.5 (B) or pH3 (C). Recombinant strains carrying native plasmid (O.o.:pSYN) or carrying plasmid expressing *hsp18* asRNA (O.o.:pSYNASHsp18) were grown at 30°C in FT80 medium until mid-exponential phase ($OD_{600} = 0.8$). Culture were incubated at 48°C (A) or cells were transferred into pH3.5 (B) or pH3 (C) FT80 medium and incubated at 30°C during 90 min. A numeration on agar plate was performed after decimal dilutions. Significant differences are based on an unilateral and paired T test. *** $P < 0.0005$, ** $P < 0.005$, * $P < 0.05$.

Significant loss of cultivability after heat shock (95%) and acid shock (60% at pH3.5 & 98% at pH3) → Lo18 plays a key role in *O. oeni* stress response

Validation of physiological effect of asRNA expression

Conclusions

- Expression of antisense RNA in *O. oeni* via *E. coli*/LAB shuttle vector developed in our laboratory.
- In vivo* inhibition of *hsp18* gene expression by using antisense RNA approach.
- Key role of Lo18 in survival of *O. oeni* under stress conditions.

➔ Development of the first efficient genetic tool for *O. oeni* by application of antisense technology to modulate gene expression in *O. oeni*

Perspectives

- Assess the effect of decreasing Lo18 level on membrane fluidity.
- Draw up our innovative asRNA method in *O. oeni* by exploring role of *hsp* genes.
- Draw up our new efficient expression tool to express genes involved in wine aroma and investigate their impact in wine aroma quality.

References:

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