Optimization of fermentation and stabilization conditions for the production of a functional bio-ingredient based on *C. divergens* M35

Laurent Dallaire ✉ [a,b], Allison Vimont[a,b], Benoit Fernandez[a,b], Denis Groleau[c] and Ismail Fliss[a,b]

[a] STELA Dairy Research Center, Institute of Nutrition and Functional Foods, Laval University, Québec, Canada

[b] Department of Food Science, Laval University, Québec, Qc, Canada

[C] Chemical and biotechnological engineering, Sherbrooke University, Sherbrooke, Qc, Canada

laurent.dallaire.1@ulaval.ca

Introduction

Context: In previous works, a bio-ingredient allowing the bioconservation of cold-smoked salmon with a strong anti-*Listeria* activity was developed and characterized. This bio-ingredient consists of a culture medium fermented by *Carnobacterium divergens* M35 and containing the bacteriocin produced by the strain, namely divergicin M35[1].

Problematic: The current production conditions do not allow an efficient and profitable use of this bio-ingredient.

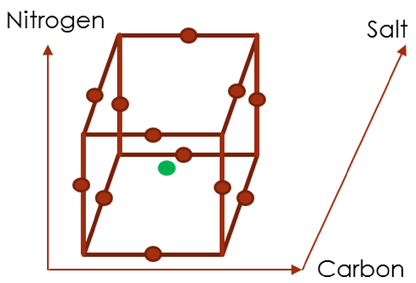
Goal of the study: To develop a low cost process to produce and stabilize the bio-ingredient and allow its usage in the sea-product transformation industry. This is possible by creating a new culture medium that is cheaper, but promotes a better growth of the strain and its production of divergicin, and by drying the fermented medium by spray-drying which is approximately 10 times cheaper than freeze drying[2].

Methods

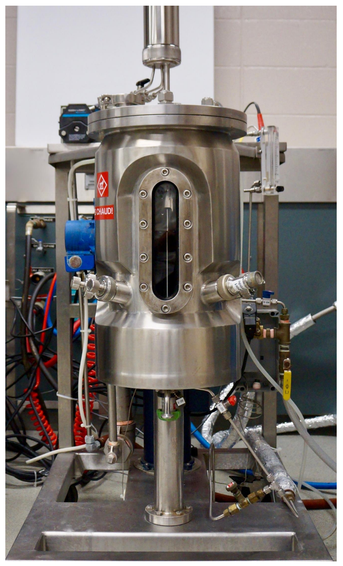
1. Culture medium components screening

Carbon	Nitrogen	Salts
Glucose	Yeast Extract	Sodium acetate
Sucrose	Pea peptones	
Sugarcane Molasse	Potato extract	Ammonium Citrate
	Meat extract	
	Fish extract	
Malt extract	Whey	None
	Buttermilk	

2. Concentration optimization by Surface Response Method (SRM)



3. Fermentation scaling-up to 30L



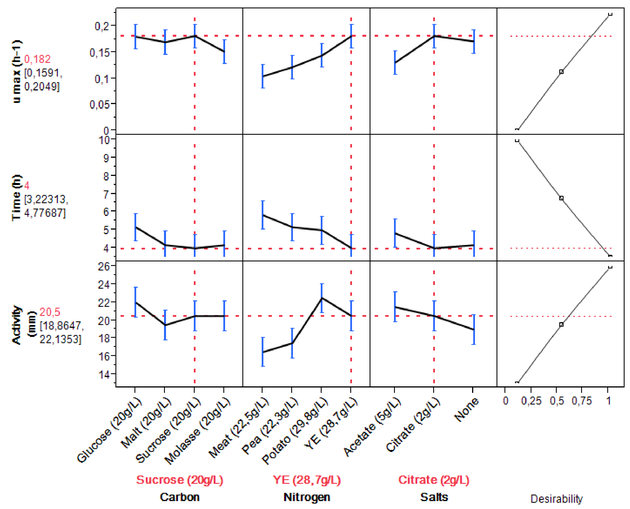
4. Spray-dryer operating conditions optimization by SRM

- Anti-*Listeria* activity: critical dilution microtitration method and agar diffusion assay
- A_c : Centrifuged supernatant, A_{nc} : No centrifugation
- Microbial growth : Optical density and microbial count

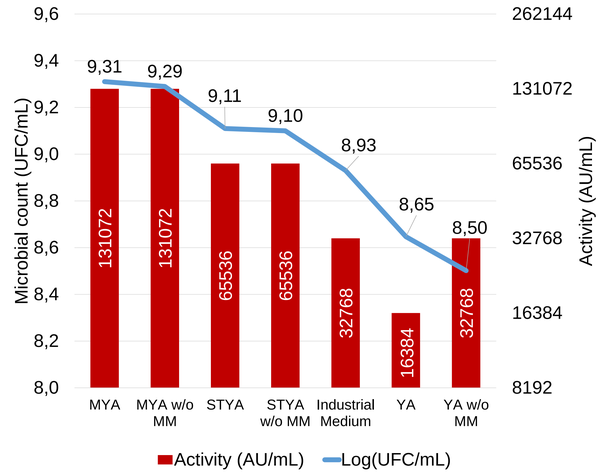
Results & Discussion

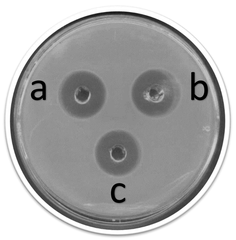
1. Best composition: Acetate, sucrose, molasse, yeast extract and tween 80.
   Sucrose was replaced by table sugar (-0.36$/L) and doesn't change the response from the strain.
2. RSM: Concentration optimization: CONFIDENTIAL.
   STYA medium (table sugar) and MYA (molasse) both cost 0.87$/L. MRS cost 8$/L
3. 30L fermentation: STYA: 8.9 Log(UFC/mL), A_c:1.5X10^5 AU/mL - MYA: 9.0 Log(UFC/mL), A_c: 2.6X10^6 AU/mL
4. Spray-drying: Addition of a drying-aid agent that resolve stickiness problems and protects the strain from heat.
   Optimization of the operating conditions: CONFIDENTIAL

Component screening



Culture media comparison in 1L fermentor



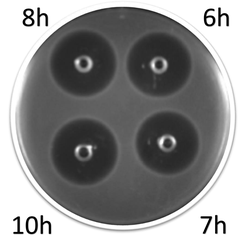


Before optimization

a: Pediocin

b: Divergicin in MRS

c: Divergicin in industrial medium



After medium optimization

Divergicin produced in 30L fermentor with STYA medium (Fermentation time)

Dried bio-ingredient

Conclusion

Culture medium: Two culture media (STYA and MYA) were developed at a relatively low cost (0,87$/L). They respectively promote *C. divergens* M35 growth up-to 8.90 and 9.01 Log(UFC/mL) and an anti-*Listeria* activity A_c of 1.6X10^5 and 2.6X10^5 AU/mL. This activity is 4 times higher than any other medium referenced in the literature for this strain.

Spray-drying: Spray-drying doesn't affect significantly the viability of the strain, nor its anti-*Listeria* activity. This work demonstrated that spray-drying is the appropriate drying technique for this bio-ingredient and that it can be an cost-saving alternative to freeze-drying.

Dried bio-ingredient: Microbial count: 9.85 UFC/g – Anti-*Listeria* A_{nc}: 1.6X10^6 AU/mL

Application: 140mg of bio-ingredient / kg of cold-smoked salmon

Acknowledgment

1. Tahiri, I., et al., *Comparison of different application strategies of divergicin M35 for inactivation of Listeria monocytogenes in cold-smoked wild salmon.* Food Microbiology, 2009. **26**(8): p. 783-793.
2. Huang, S., et al., *Double use of highly concentrated sweet whey to improve the biomass production and viability of spray-dried probiotic bacteria.* Journal of Functional Foods, 2016. **23**: p. 453-463.