PO-18

In vitro and In vivo screening of potential probiotic activities of bacterial isolated strains from *Ceratitis capitata* guts for incorporation in larval diet based on SIT application

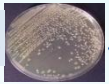
H. Hamdene [a], M.M. Guerfali[a], K. Charaabi[1], W. Djobbi[a], S. Fadhel[a], M. Mahjoubi[b], M. Saidi[a], C. Chevrier[c], A. Cherif[b]

[a] *Laboratory of biotechnology and nuclear technologies LR16CNSTN01, CNSTN, Technopole Sidi Thabet 2020, Ariana, Tunisia.*
[b] *ISBST, BVBGR-LR11ES31, University of Manouba, Technopole Sidi Thabet 2020, Ariana, Tunisia.;*
[c] *IRBI, University of Tours, UMR CNRS 7261, Tours, France.*

INTRODUCTION: The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is an important economic pest worldwide causing direct crop damage. One of the most successful control strategies for this pest is the application of the sterile insect technique. Several studies have shown that irradiation affects the Vienna8 Genetic Sexing Strain sterile males performance when competing with wild males for wild females. The exploitation of the intestinal microbiota by administration of members of the fly's community as probiotics in the larval diet have positively influenced the sterile insect performance. Hence, in the current study, we conducted a probiotic selection strategy to evaluate, "*in-vitro*" and "*in-vivo*", probiotic properties of each bacterial species isolated from Tunisian medfly'guts under conditions simulating the SIT application. Then, we realized a principal components analysis (PCA) to draw a final conclusion on the selection of potential probiotic candidates.

METHODS


Steril gut extraction


Colony isolation

ITS-PCR / PCR 16S / Sequencing

Colony identification

Probiotic properties of each isolated bacteria

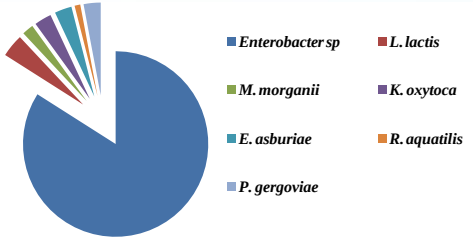
« In vitro »
- ✓ Tolerance to irradiation
- ✓ Cell surface hydrophobicity
- ✓ Autoaggregation
- ✓ Coaggregation
- ✓ Biofilm and EPS production

« In vivo »
- ✓ Eggs to adult recovery
- ✓ Pupal weight
- ✓ Fly capacity
- ✓ Longevity
- ✓ Mating competitiveness

Selection of potential probiotic candidates using PCA

RESULTS

1. Identification of intestinal bacterial strains



RESULTS

2. " *In vitro* " probiotic properties

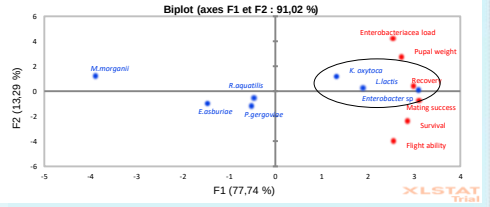
Isolates	Adhesion properties				
	Cell surface hydrophobicity (%)		Cellular autoaggregation (%)	Biofilm formation	EPS production (µg/ml)
	Chloroform	Xylene			
Enterobacter sp	65.9 ± 0.41[a]	90.07 ± 0.35[a]	40.35 ± 2.13[ab]	1.28 ± 0.03[a]	309.59 ±11.51[a]
E.asburiae	61.17 ± 1.86[ab]	89.01 ± 1.71[a]	31.25 ± 0.6[c]	0.34 ± 0.03[e]	147.33 ± 7.92[d]
K.oxytoca	62.79 ± 2.01[ab]	89.01 ± 1.71[a]	40.14 ± 0.35[ab]	1.13 ± 0.02[b]	227.18 ± 21.69[bc]
L.lactis	64.21[ab]	81.81± 1.73[b]	37.63 ± 1.64[b]	0.47 ± 0.05[d]	268.85 ± 17.73[ab]
R.aquatilis	65.43 ± 1.25[ab]	91.2 ± 0.63[a]	42.01 ± 0.34[a]	0.4 ± 0.04[de]	193.18 ± 8.61[c]
P.gergoviae	60.63[b]	92.98 ± 0.67[a]	24.37 ± 0.97[d]	0.9 ± 0.03[c]	146.51 ± 17.73[ab]
M.morganii	50.91 ± 3.03[c]	67.46 ± 4.6[c]	25.8 ± 1.16[d]	0.32 ± 0.02[e]	189.4 ± 14.54[cd]

3. "*In vivo* " probiotic properties

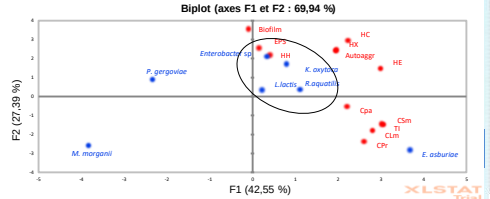
Bacterial strains	QC parameters			
	Eggs to adult recovery (%)	Pupal weight (g)	Flight capacity (%)	Longevity (%)
Enterobacter sp	42±1.15[a]	8.63±0.03[a]	92.83±0.6[a]	91.11±3.07[a]
E. asburiae	35.16±1.69[bc]	7.94±0.13[c]	91±0[a]	83.66±1.69[ab]
P. gergoviae	38.76±2.08[ab]	8.1±0.05[c]	90.29±0.7[a]	88.88±2.22[ab]
L. lactis	40.5±3.01[a]	8.44±0.04[ab]	90.83±0.72[a]	91.11±4.44[a]
R. aquatilis	35.16±1.3[bc]	8.4±0.05[b]	91.4±1.26[a]	84.42±0.51[ab]
K. oxytoca	40±1.52[ab]	8.52±0.08[ab]	90.29±0.91[a]	85.88±2.12[ab]
M. morganii	33.16±1.45[cd]	8.03±0.06[c]	83.83±0.72[b]	77.77±2.22[bc]
Control	29.33±0.92[d]	7.92±0.04[c]	79.8±0.62[b]	71.11±2.22[c]

4. Probiotic selection strains using PCA

4.1. *in vivo*



4.2. *in vitro*



CONCLUSION

In this study, a PCA was for characteriz and selection of bacteria isolated from *C. capitata* guts for studying their probiotic potential "*in vitro*" and "*in vivo*". The combination of the two PCAs allowed the selection of *Enterobacter sp.*, *Lactococcus lactis* and *Klebsiella oxytoca* as potential probiotic candidates to integrate in the larval diet.

FUNDING

This study was supported by the Ministry of Higher Education and Scientific Research under the Federated Research Project PRF "Gestion intégrée contre la Cératite pour la promotion d'une agriculture durable "**(PRF2019-D6P2)** and PRIMA Project "Innovative tools for Mediterranean crop protection" **(INTOMED).** *PRIMA* is supported under Horizon2020, The European Union Framework programme for research and innovation.