# Bacteria involved in the degradation of olive mill waste polyphenols: the tannic acid as case of study

Milva Pepi[1] pepim@unisi.it, Arianna Lobianco[1], Roberto Altieri[2], Alessandro Esposito[2], Guido Perra[1], Simone Gasperini[1], Antonio Feola[3] and Silvano E. Focardi[1]

1)Department of Environmental Sciences, University of Siena, Italy;
2)Institute for Agricultural and Forest Mediterranean Systems, National Research Council, Perugia, Italy;
3)National Park Agency Cilento and Vallo di Diano, Salerno, Italy.



Aerobic storage of olive mill waste (OMW) mixture: indoor a) and outdoor b).

Main characteristics of OMW mixtures used for inocula; T0 = starting of storage; Tf in. = final indoor storage; Tf out. = final outdoor storage (values on dry weight). Data are means of triplicate, with coefficient of variation (CV) < 5%.

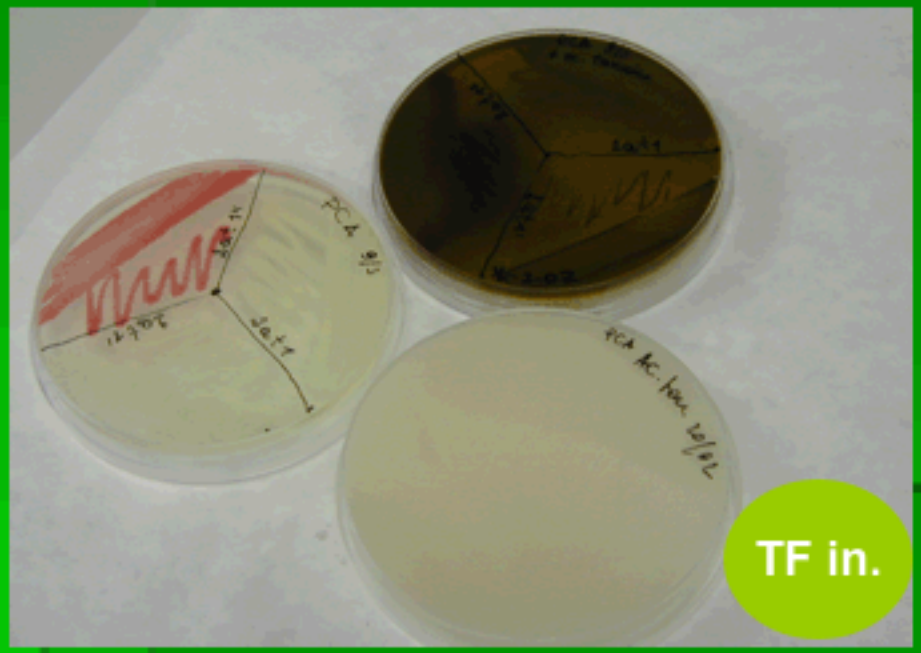
| | | $T_0$ | $T_f$ in. | $T_f$ out. |
|---|---|---|---|---|
| Moisture | % | 66.5 | 22.3 | 64.6 |
| pH* | | 6.18 | 6.96 | 7.12 |
| Electrical conductivity* | ds $m^{-1}$ | 1.59 | 2.52 | 1.35 |
| Ash | %. | 14.7 | 26.2 | 19.0 |
| Total organic carbon | % | 45.1 | 40.5 | 42.3 |
| Total nitrogen | % | 2.2 | 3.6 | 3.0 |
| Polyphenols | mg $kg^{-1}$ | 1007 | 251 | 342 |
| Germination index | % | 43.7 | 76.6 | 57.0 |
| OM-loss** | % | - | 51.5 | 26.5 |

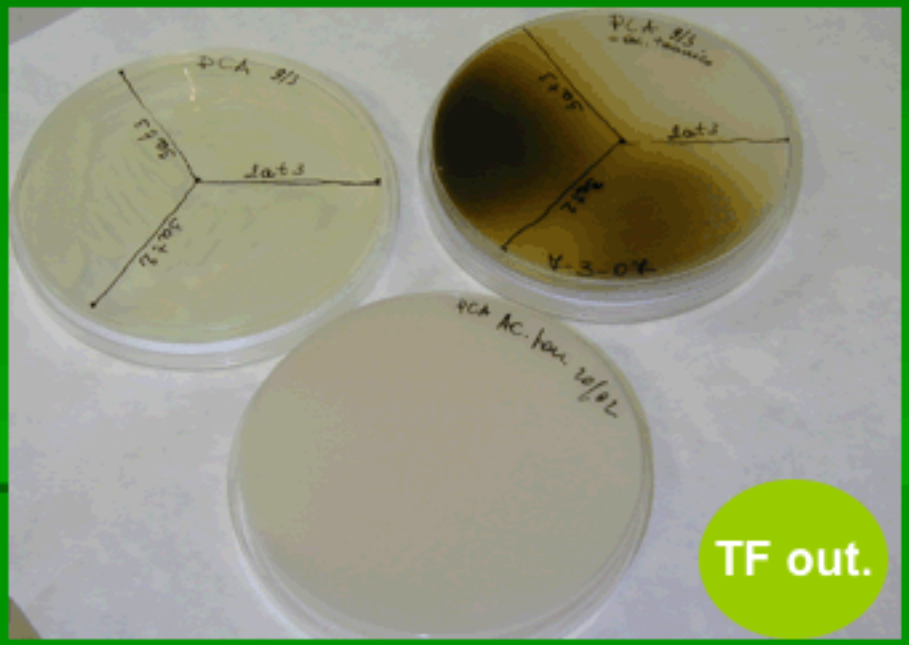
* detected in a water extract 1:10 (w/v)

** OM-loss % = 100-100[X1(100-X2)]/[X2(100-X1)] (Viel et al., 1987).


°C
60
50
40
30
20
10
0
0
10
20
30
40
Days
Outdoor air
Outdoor Stack Pile
Indoor air
Indoor Stack Pile

- Tannins are a group of naturally occurring polyphenols, abundant in leaves, fruits and seeds;
- Tannins have toxic effects on animals and also inhibit the growth of a number of microorganisms;
- When abundant in soil, tannins retard the rate of decomposition of orgnic matter, inhibiting microbial enzymes.

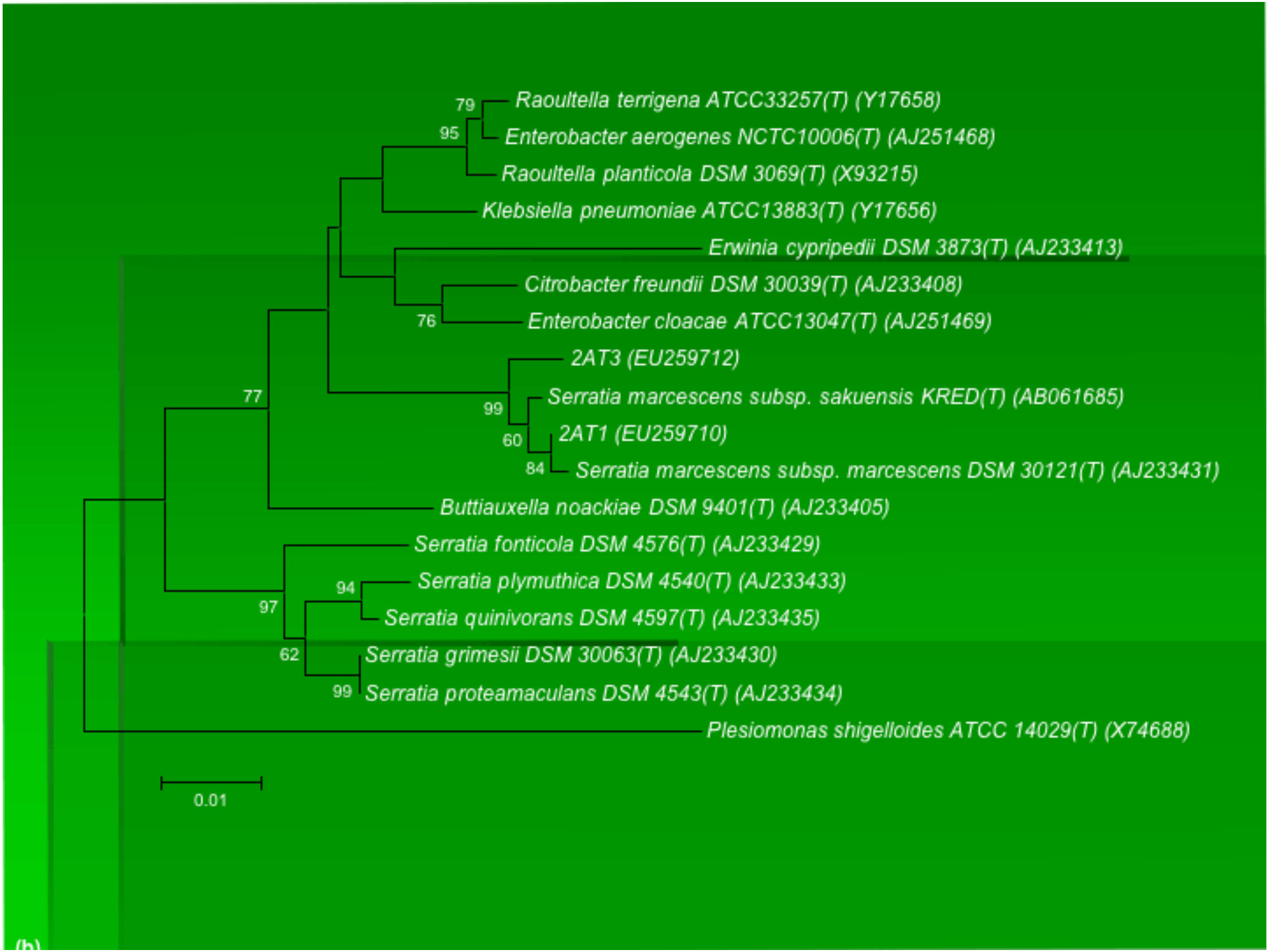


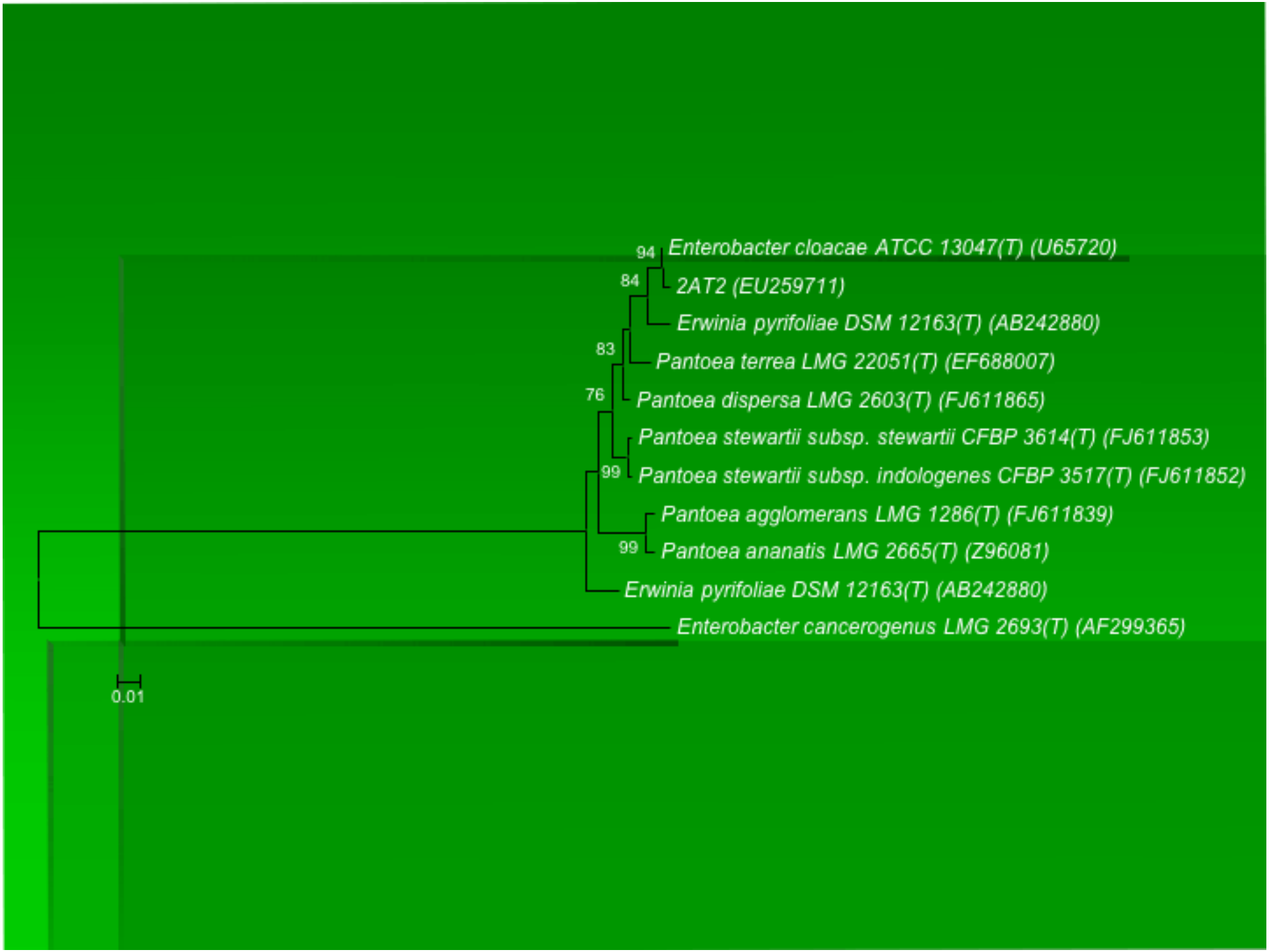



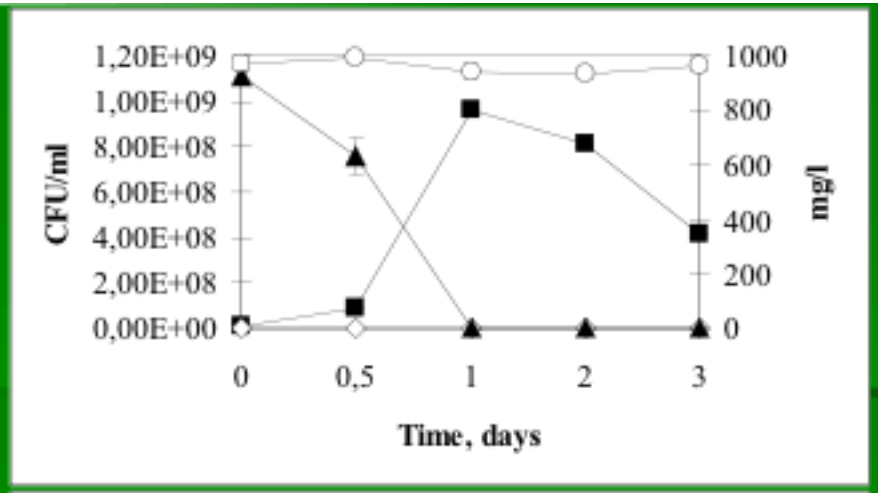

Cultures of the bacterial strains, arranged with or without tannic acid, isolated from OMW mixtures submitted to three months of aerobic storage.

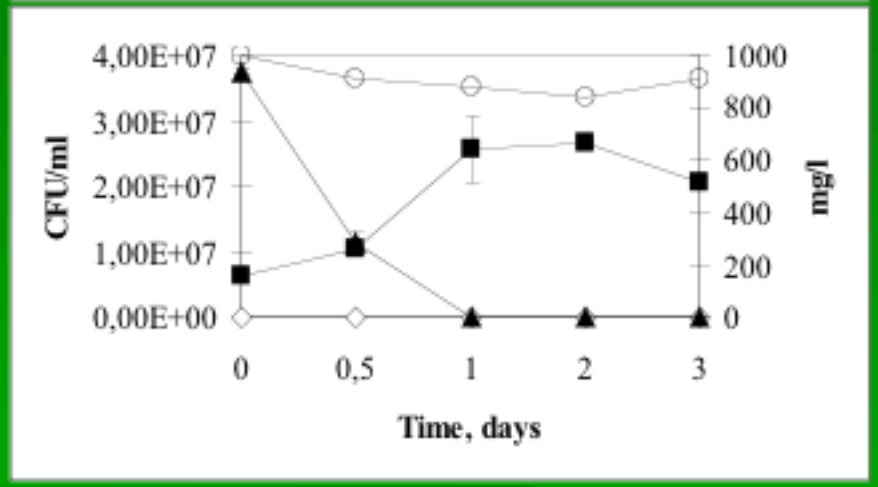
Tannic acid degrading bacterial strains isolated from OMW mixtures.

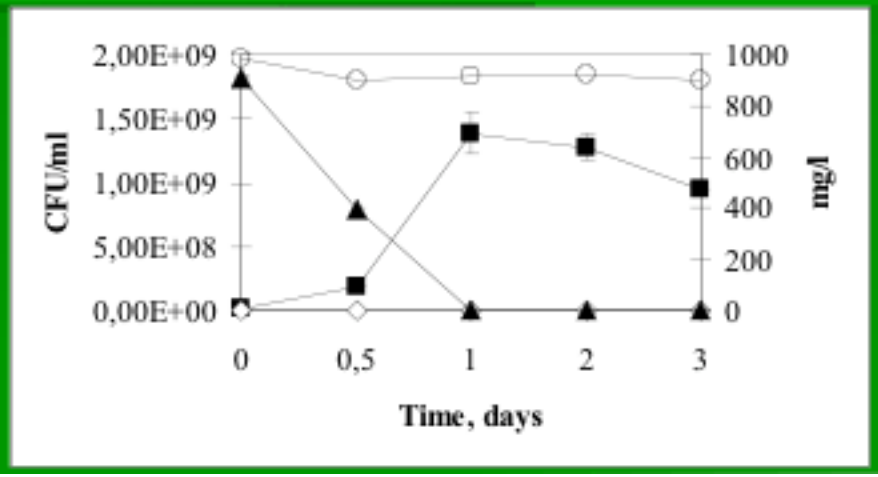
| Sample | Isolated strains | Morphologic study | 16S rRNA gene |
|---|---|---|---|
| $T_0$ | not found | - | - |
| $Tf_{in}$ | 2AT1 | Ø 2.0 mm; cream colour-beige; regular margins; flat; mat | *Serratia* sp. |
| | 2AT2 | Ø 1.5 mm; cream colour; regular margins; convex; moist | *Pantoea* sp. |
| | 2AT3 | Ø 1.8 mm; dark red; round; regular margins; flat | *Serratia* sp. |
| $Tf_{out}$ | 3AT2 | Ø 1.0 mm; cream colour; regular margins; convex; moist | n.d. |
| | 3AT3 | Ø 1.2 mm; cream colour; regular margins; convex; moist | n.d. |

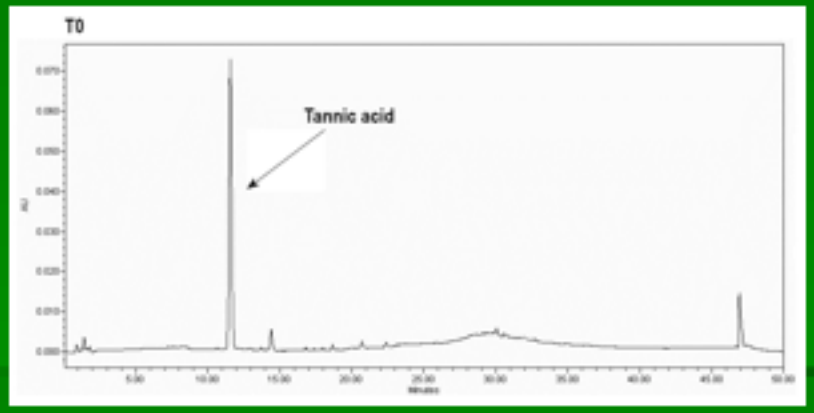

79
95
Raoultella terrigena ATCC33257(T) (Y17658)
Enterobacter aerogenes NCTC10006(T) (AJ251468)
Raoultella planticola DSM 3069(T) (X93215)
Klebsiella pneumoniae ATCC13883(T) (Y17656)
Erwinia cypripedii DSM 3873(T) (AJ233413)
Citrobacter freundii DSM 30039(T) (AJ233408)
76
Enterobacter cloacae ATCC13047(T) (AJ251469)
2AT3 (EU259712)
77
99
Serratia marcescens subsp. sakuensis KRED(T) (AB061685)
60
2AT1 (EU259710)
84
Serratia marcescens subsp. marcescens DSM 30121(T) (AJ233431)
Buttiauxella noackiae DSM 9401(T) (AJ233405)
Serratia fonticola DSM 4576(T) (AJ233429)
94
Serratia plymuthica DSM 4540(T) (AJ233433)
97
Serratia quinivorans DSM 4597(T) (AJ233435)
62
Serratia grimesii DSM 30063(T) (AJ233430)
99
Serratia proteamaculans DSM 4543(T) (AJ233434)
Plesiomonas shigelloides ATCC 14029(T) (X74688)
0.01
(b)

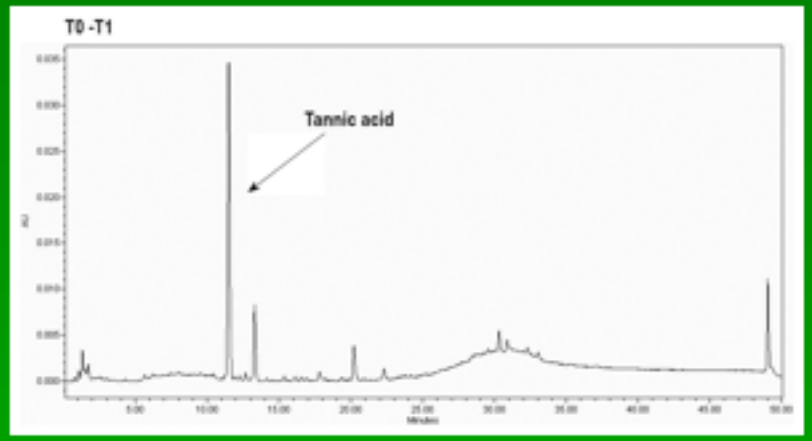

94
Enterobacter cloacae ATCC 13047(T) (U65720)
84
2AT2 (EU259711)
Erwinia pyrifoliae DSM 12163(T) (AB242880)
83
Pantoea terrea LMG 22051(T) (EF688007)
76
Pantoea dispersa LMG 2603(T) (FJ611865)
Pantoea stewartii subsp. stewartii CFBP 3614(T) (FJ611853)
99
Pantoea stewartii subsp. indologenes CFBP 3517(T) (FJ611852)
Pantoea agglomerans LMG 1286(T) (FJ611839)
99
Pantoea ananatis LMG 2665(T) (Z96081)
Erwinia pyrifoliae DSM 12163(T) (AB242880)
Enterobacter cancerogenus LMG 2693(T) (AF299365)
0.01

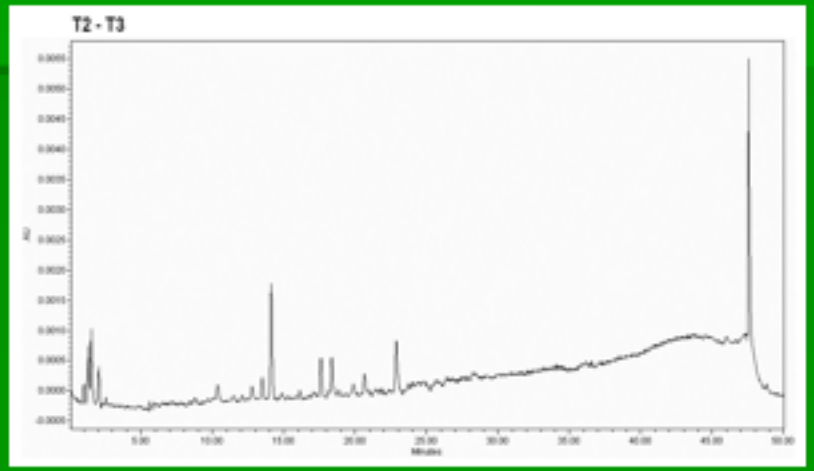

1,20E+09
1,00E+09
8,00E+08
6,00E+08
4,00E+08
2,00E+08
0,00E+00
CFU/ml
1000
800
600
400
200
0
mg/l
0
0,5
1
2
3
Time, days


4,00E+07
3,00E+07
2,00E+07
1,00E+07
0,00E+00
CFU/ml
1000
800
600
400
200
0
mg/l
0
0,5
1
2
3
Time, days


2,00E+09
1,50E+09
1,00E+09
5,00E+08
0,00E+00
CFU/ml
1000
800
600
400
200
0
mg/l
0
0,5
1
2
3
Time, days

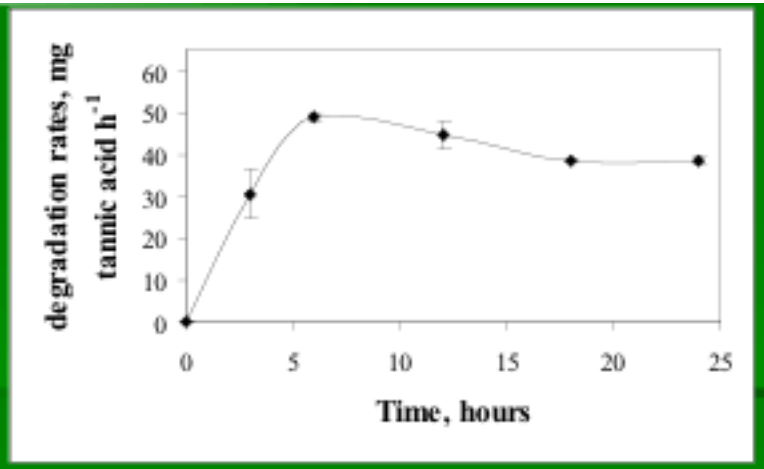

T0
Tannic acid

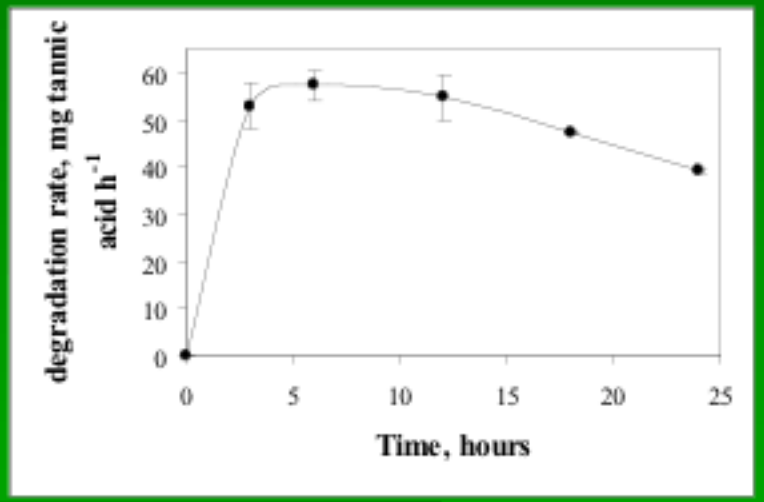

T0 -T1
Tannic acid

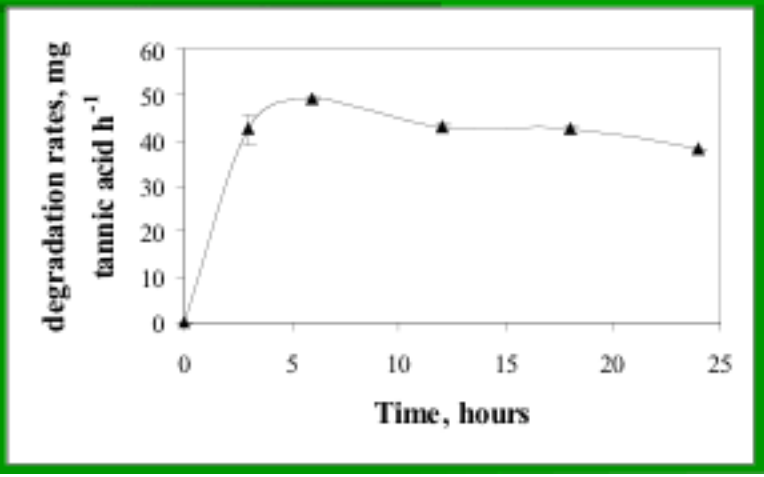

T2 - T3

Specific growth rates of the three bacterial strains isolated from olive mills wastes in the presence of tannic acid as the sole carbon and energy source. Data are means of triplicate ± standard deviation.

| Strain | growth rates (μ) |
|---|---|
| 2AT1 | 0.27 ± 0.03 |
| 2AT2 | 0.36 ± 0.01 |
| 2AT3 | 0.30 ± 0.04 |


degradation rates, mg tannic acid h-1
60
50
40
30
20
10
0
0
5
10
15
20
25
Time, hours


degradation rate, mg tannic acid h-1
60
50
40
30
20
10
0
0
5
10
15
20
25
Time, hours


degradation rates, mg tannic acid h-1
60
50
40
30
20
10
0
0
5
10
15
20
25
Time, hours

Tannase activity of the isolated bacterial strains evaluated by spectrophotometric and visual methods. Data are means of triplicate ± standard deviation.

| Bacterial strain | Tannase ($Abs_{440\ nm}$) | Colour change from green to brown |
|---|---|---|
| *Serratia* sp. 2AT1 | 0.902 ± 0.0205 | +++ |
| *Pantoea* sp. 2AT2 | 1.005 ± 0.0014 | +++ |
| *Serratia* sp. 2AT3 | 0.892 ± 0.0544 | +++ |
| Control | 0.319 ± 0.0021 | - |

## Conclusions

- Polyphenols-degrading bacterial strains were isolated from olive mill mixtures, on the model of the tannic acid;
- Identification of the isolated bacterial strains assigned them to the genera *Serratia* and *Pantoea*;
- Degradation of tannic acid was detected in the three bacterial strains;
- Tannase activity was evidenced in the three bacterial strains;
- For the first time tannic acid bacterial strains were isolated from olive mill mixtures;
- The role of these bacterial strains in the degradation of the polyphenol in the olive mill mixtures, is suggested for agronomic purposes.