# INHIBITORY ACTIVITY OF LACTIC ACID BACTERIA AGAINST SPOILAGE YEAST *RHODOTORULA MUCILAGINOSA*

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# ABSTRAK

*Rhodotorula* spp. yang terlibat dengan kerosakan potongan sayuran menghasilkan permukaan berlendir dan berbau. Kajian ini bertujuan menyelidik aktiviti antiyis oleh bakteria asid laktik (LAB) yang diasingkan daripada sumber-sumber yang berbeza terhadap dua strain tempatan *Rhodotorula mucilaginosa* CBR dan *R. mucilaginosa* CRT diasingkan daripada lobak merah dan timun. Sebanyak 38 pencilan LAB telah disaring untuk aktiviti antiyis menggunakan dua kaedah tindihan agar terhadap yis sasaran. Tiga pencilan yang dikenal pasti oleh API 50CH kit dan 16S rDNA sebagai *Lactobacillus casei* DF22, *L. casei* YG1 dan *L. plantarum* C16 menunjukkan aktiviti perencatan tertinggi (> 13mm) selepas 48 jam simpanan pada suhu 30 °C. Supernatant bebas sel (CFS) bagi 3 pencilan LAB mengurangkan pertumbuhan yis sasaran sebanyak 96% hingga 99% dan menyebabkan 6 $\log_{10}$ pengurangan dalam tempoh 24 jam simpanan pada suhu 30 °C. Pelarasan pH CFS hingga 3, 5, 6 dan 7 mengakibatkan perubahan pada aktiviti antiyis antara 23.4% ke 54.8%. CFS *L. casei* DF22 menunjukkan aktiviti tertinggi pada pH 3, 5, 6, dan 7 berbanding LAB lain. Pemanasan LAB CFS pada 90°C dan 120°C selama 30 minit dengan ketaranya ($P < 0.05$) mengurangkan aktiviti antiyis daripada LAB berbeza. Pemanasan CFS pada 90°C selama 30 minit mengurangkan aktiviti antiyis *L. casei* YG1 kepada 59.2%, manakala aktiviti *L. plantarum* C16 dikekalkan 50.2% pada 121 °C selama 30 minit. Rawatan CFS dengan pepsin, papain dan proteinase K ($P < 0.05$) mengurangkan aktiviti antiyis dengan ketara sebanyak 26.8% kepada 52.5% menunjukkan kehadiran protin. Rawatan dengan papain mengurangkang aktiviti antiyis dengan ketara ($P < 0.05$) daripada CFS *L. casei* YG1. *L. casei* YG1, *L. casei* DF22 dan *L. plantarum* C16 mempunyai keupayaan untuk merencat satu sama lain dengan kaedah dwi agar bertindih menunjukkan kehadiran bakteriosin. CFS daripada LAB menunjukkan aktiviti anti lekat terhadap lapisan yang dihasilkan oleh *R. mucilaginosa* CBR melalui kaedah pra-lapisan dan kaedah co-inkubator. Melaraskan pH CFS menyebabkan aktiviti berkurangan antara 50.4% ke 69.7%. Pemanasan LAB CFS pada 90°C selama 30 minit dan 120°C selama 30 minit dengan ketaranya ($P < 0.05$) mengurangkan aktiviti anti-lekatan LAB yang berbeza antara 40.5% ke 66.4%. CFS *L. plantarum* C16 kekal aktivitinya sebanyak 66.4% pada 90°C. Rawatan CFS dengan pepsin, papain dan proteinase K dengan ketaranya ($P<0.05$) mengurangkan aktiviti anti lekat sebanyak 26.2% ke 60.6%. CFS telah terjejas dengan ketara oleh enzim papain dengan *L. casei* YG1 kehilangan aktiviti dengan ketara ($P<0.05$) sebanyak 26.2%. Kajian ini menunjukkan bahawa LAB-CFS menghasilkan komponen dalam supernatan yang mempunyai keupayaan sebagai antiyis dan anti-lekatan terhadap *R. mucilaginosa* dan mempunyai potensi untuk digunakan sebagai pengawetan bio dalam industri makanan.

## ABSTRACT

*Rhodotorula* spp. are implicated for spoilage of cut vegetables producing slimy surfaces and off odours. This study evaluated the antiyeast activity of lactic acid bacteria (LAB) isolated from different sources against two local strains of R. *mucilaginosa* CBR and *R. mucilaginosa* CRT isolated from carrot and cucumber, respectively. A total of 38 LAB isolates were screened for antiyeast activity using dual agar overlay method against the target yeast. Three isolates identified by API 50CH kit and 16S rDNA as *Lactobacillus casei* DF22, *L, casei* YG1 and *L. plantarum* C16 showed highest inhibition activity (>13mm) after 48 h incubation at 30°C. Cell free supernatant (CFS) of all LAB reduced growth of target yeast by 96 to 99% and resulted in 6 $\log_{10}$ reduction within 24h incubation at 30°C. Adjusting pH of CFS to 3, 5,6 and 7 resulted in variable antiyeast activity ranging from 23.4% to 54.8%. CFS of L. *casei* DF22 showed highest activity at pH 3, 5, 6, and 7 compared to other LAB. Heating the CFS LAB at 90°C for 30 rnin and 120°C for 30 min significantly ($P<0.05$) reduced the antiyeast activity of different LAB. Heating of CFS (pH 3.8 to 3.9) at 90°C for 30 min reduced the antiyeast activity of *L. casei* YG1 by 59.2%, while the activity L. *plantarum* C16 was maintained 50.2% at 121°C for 30 min. Treatment of CFS with pepsin, papain and proteinase K significantly ($P<0.05$) reduced the antiyeast activity by 26.8% to 52.5% indicating the presence of protein. Treatment with papain significantly reduced ($P<0.05$) the inhibitory activity of CFS L. *casei* YG1. *L. casei* YG1, *L. casei* DF22 and *L. plantarum* C16 have the ability to inhibit each other by dual agar ovelay method indicating the presence of bacteriocin. CFS of LAB showed anti-adhesion activity against biofilm produces by *R. mucilaginosa* CBR by pre-coating and co-incubation method. Adjusting pH of CFS resulted in significantly reduced activity ranging from 50.4% to 69.7%. Heating the CFS LAB at 90°C for 30 min and 120°C for 30 min significantly ($P<0.05$) reduced the anti-adhesion activity of different LAB ranging from 40.5% to 66.4%. CFS of L. *plantarum* C16 was maintained by 66.4% at 90°C. Treatment of CFS with pepsin, papain and proteinase K significantly ($P<0.05$) reduced the anti-adhesion activity by 26.2% to 60.6%. CFS were significantly ($P<0.05$) affected by enzyme papain with L. *casei* YG1 resulted in significantly ($P<0.05$) lost of activity by 26.2%. This study demonstrated that the selected LAB-CFS produced compounds in the supernatant that have the ability as antiyeast and anti-adhesion against R. *mucilaginosa* and have potential to be used as biopreservative in food industry.

# الملخص

تعتبر الخمائر جنس *Rhodotorula* spp المسببة لفساد الخضروات المقطعة وذلك بأنتاج طبقة رقيقة عديمة اللون على السطح. في هذه الدراسة تم تقدير مضادات الخمائر عن طريق استخدام بكتيريا حمض الاكتيك والتي عزلت من مصادر مختلفة حيث استخدمت هذه العزلات ضد نوعان من الخمائر *R. mucilaginosa* CRT and *R. mucilaginosa* CBR والتي عزلت من الجزر والخيار. 38 عزلة من بكتيريا حمض الاكتيك جميعها اختبرت كمضادات للخمائر بأستخدام طريقة حفر الاجار. 3 عزلات من بكتيريا حمض الاكتيك وقد عرفت بالطريقة التخمرات الكربوهيدراتية API 50CH kit واستخلاص الحامض النووي 16S rDNA على انها *Lactobacillus casei* DF22, *L. casei* YG1 and *L. plantarum* C16 حيث اظهرت اعلى نشاط ضد الخمائر المستهدفة في هذه الدراسة بعد فترة تحضين لمدة 48 ساعة. وعند استخدام السائل البكتيري لبكتيريا حمض الاكتيك لمدة كانت نسبة التثبيط من 96- 99% وفي هذه الدراسة كذلك تم ضبط درجة الحموضة الpH الى درجات حموضة مختلفة 3 و5 و 6 و7 كانت النتيجة اختلاف نسبة نشاط التثبيط للخمائر في مدى يتراوح مابين 23.4% الى 54.8%. السائل البكتيري لبكتيريا *L. casei* DF22 اظهر اعلى نشاط ضد الخمائر التي في هذه الدراسة عند درجة حموضة 3 و5 و 6 و 7 مقارنة مع البكتيريا الاخرى. عملية تسخين السائل البكتيري المنتج من بكتيريا حمض الاكتيك الى درجة حرارة 90°C و 120°C لمدة 30 دقيقة كان له تأثير معنوي عند مستوى ($P<0.05$) تثبيط الخمائر وكذلك تسخين السائل البكتيري *L. casei* YG1 ذو درجة حموضة 3.8 pH-3.9 الى 90 درجة مئوية لمدة 30 دقيقة نتجه عنه تثبيط الخمائر الى 59.2% بينما البكتيريا نوع *L. plantarum* C16 كانت نسبة التثبيط 50.2% وذلك عند تسخين السائل الى درجة حرارة 120 لمدة 30 دقيقة. معاملة السائل بالانزيمات مثل الببسين والبابين و البروتينيز ك كان له تأثير معنوي ($P<0.05$) في تخفيض نشاط الخمائر وكذلك عند تسخسن السائل الى درجة حرارة 90 120 درجة مئوية كان له تأثير معنوي عند مستوى ($P\leq0.05$) ضد anti-adhesion من 40.5% الى 66.4%. والسائل المنتج بواسطة بكتيريا *L. plantarum* C16 كان له تأثير بنسبة 66.4% عند درجة حرارة 90 درجة مئوية في هذه الدراسة اظهرت ان بكتيريا حمض الاكتيك لها القدرة على تثبيط الخمائر وكذلك لها القدرة على anti-adhesion ويمكن استخدامها كمواد حافظة حيوية في مصانع الاغذية.

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| | |
|---|---|
| $CaCO_3$ | calcium carbonate |
| NaOH | sodium hydroxide |
| HCl | hydrochloric acid |
| DNA | deoxyribonucleic acid |
| MRS | Man, Rogosa and sharpe agar |
| PDA | potato dextrose agar |
| PDB | potato dextrose broth |
| PCR | polymerase chain reaction |
| rDNA | ribosomal deoxyribonucleic acid |
| rpm | revolution per minute |
| LAB | lactic acid bacteria |
| CFS | cell free supernatant |
| µl | microlitre |
| min | minutes |
| h | hour |
| sec | second |
| w | weight |
| v | volume |
| N | normality |
| M | molarity |