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Supplementation to the MRS medium for the cultivation of fastidious beer spoilage bacteria

The rapid detection and cultivation of the specific beer spoilage bacteria has always been a problem and still unresolved for some of the most fastidious *Lactobacillus* and *Pediococcus* species. This paper presents a new medium based on a adaptation of the MRS medium proposed by DeMan, Rogosa & Sharpe in 1960. The new medium is compared to others in term of size of the colonies on plates and kinetic of growth in liquid media. The kinetic is followed by optical density as well as cfu enumerations. In conclusion, we can say that with the new medium, we can easily cultivate and count some of the most fastidious bacterial beer spoilers.

BC 43 Biological production control

(Descriptors: Culture media, MRS, NBB, beer spoilage bacteria, detection procedures, *Lactobacillus*, *Pediococcus*.)

Deskriptoren: Nährmedien, MRS, NBB, bierverderbende Bakterien, Nachweisverfahren, *Lactobacillus*, *Pediococcus*.)

1 Introduction

The rapid detection and cultivation of the specific beer spoilage bacteria has always been a problem and is still unresolved for some of the most fastidious *Lactobacillus* and *Pediococcus* species often strictly adapted to a particular product or environment.

In the last 30 years, many more or less sophisticated media have been proposed to resolve that problem. We mention, hereafter, the most important ones with their acronym and references: DeMan, Rogosa & Sharpe (MRS, *DeMan, Rogosa & Sharpe*, 1960); Universal Beer Agar (UBA, *Kozulis and Page*, 1968); VLB-S7 (VLB-S7, *Emeis*, 1972); Raka-Ray Lactic Acid Bacteria Medium (RR-3, *Saha, Sondag & Midlekauff*, 1974); NBB Medium (NBB, *Back*, 1980); Modified NBB Medium (NBBM1, *Nishikawa and Kohgo*, 1985); Modified NBB Medium (NBBM2, *Kindraka*, 1987) and KOT Medium with actidione and azide (KOT, *Taguchi et al.*, 1990).

All these media possess their own strong and weak points. All are results of compromises between selectivity, sensitivity and speed of growth. Some strains will grow better in some media and very slowly or not at all in some other media. At this time, no ideal medium can be recommended without restrictions for all strains.

The principal problems encountered with the most fastidious bacteria are the length of incubation, more than seven days in some cases, and the size of the colonies on solid media, causing difficulties for counting colonies.

It is the reason why efforts must continue to improve such media and the experience of the brewer will help him to select which medium is best suited for its own beer and its own contaminants detected in the past. But, at the present time, the original NBB medium becomes more and more the standard medium for breweries.

In our laboratory, to test the performances of disinfectant formulations, media giving rapid growth in liquid media and larger size of colonies in agar media are needed. Neither classic MRS medium nor NBB medium was satisfactory from that point of view. For that reason why we tried to develop a new culture medium, called in this paper MRS B+, easy to prepare, not too expensive, with shorter times of growing and giving larger colonies on agar, sufficiently universal for all kinds of beer lactobacilli and pediococci.

2 Materials and methods

2.1 Bacterial strains

The strains used for growing tests are *Lactobacillus brevis* DSM 1268, *Lactobacillus brevis* DSM 6235, *Lactobacillus lindneri* DSM 20691, *Lactobacillus parabuchneri* DSM 5708, *Lactobacillus sp.* DSM 6265, *Pediococcus damnosus* ATCC 43013, *Pediococcus inopinatus* ATCC 49902 and *Pediococcus inopinatus* DSM 20872. All strains come from official collections. These strains have been isolated from beer, spoiled beer or yeast (except *P. damnosus*, isolated from wine).

2.2 Culture media and conditions

For testing the potentials of this new medium, MRS B+, it was compared with two classic media, NBB and the ancestor of media for Lactobacilli, the MRS medium (*De Man, Rogosa and Sharpe*, 1960). All three media were tested in liquid and agar form (with addition of 30 gram per litre of agar).

Media Suppliers and Preparation:

MRS medium: Biokar Diagnostics, Beauvais, France

Agar: Biokar Diagnostics, Beauvais, France

NBB medium: Döhler Group, Darmstadt, Germany; reconstituted from the powder NBB-P and addition of a blend of 50% lager beer and 50% water.

MRS B+: prepared from the classic MRS medium supplemented by a blend of different sugars and selected growth factors with the addition of 10% lager beer.

Three sets of comparison have been performed:

- surface growth in aerobic conditions;
- surface growth in anaerobic conditions, in anaerobic jars fitted with Merck's Anaerocult® A devices, giving an anaerobic atmosphere with 18 – 19% CO₂;
- Kinetics of growth in liquid media.

3 Results

3.1 Size of the colonies

The table 1 shows the average diameter (mm) of surface colonies in Petri dishes observed after 4 days of incubation at 28 °C, in aerobic or anaerobic atmosphere.

The table 2 shows the average diameter (mm) of subsurface colonies in Petri dishes observed after 4 days of incubation at 28 °C, in aerobic or anaerobic atmosphere.

The classification of media regarding the size of colonies is always the same:

MRS B+ > MRS > NBB-P

3.2 Kinetics of growth in liquid media

3.2.1 Growth assessed by optical density

For the comparison of MRS with MRS B+ media, the growth curves have been observed by the variation of the optical density at the wavelength of 660 nm.

The figures 1 to 4 show the comparative growth curves obtained with the media MRS and MRS. B+ for different strains: *Lactobacillus brevis* DSM 1268, *Lactobacillus sp.* DSM 6265, *Pediococcus inopinatus* DSM 20872, *Lactobacillus lindneri* DSM 20691.

The differences of growing curves in liquid media show important variations among species and culture media media. To facilitate the comparisons, we put together in table 3 the O.D. after 10, 15, 20 and 25 hours of growth.

The advantages of MRS B+ over MRS can be classified in two classes, depending of the strain.

- Nearly identical curves for *L. brevis* DSM 6235, *P. damnosus* ATCC 43013 and *P. inopinatus* ATCC 49902.
- Very marked growth difference for *L. brevis* DSM 1268, *L. lindneri* DSM 20691, *L. parabuchneri* DSM 5708, *L. sp.* DSM 6265 and *P. inopinatus* DSM 20872.

In the second category, we found all the fastidious, hard to grow, strains of beer spoilers.

Table 1 Shows the average diameter (mm) of surface colonies in Petri dishes observed after 4 days of incubation at 28 °C in aerobic and anaerobic atmospheres

Strain	Aerobic conditions			Anaerobic conditions		
	MRS med.	NBB-P med.	MRS B+ med.	MRS med.	NBB-P med.	MRS B+ med.
<i>Lactobacillus brevis</i> DSM 1268	1.0	1.0	1.5	2.0	0.5	2.0
<i>Lactobacillus brevis</i> DSM 6235	1.5	0.5	2.0	1.6	0.5	1.6
<i>Lactobacillus lindneri</i> DSM 20691	0.5	1.0	1.5	1.5	1.0	2.0
<i>Lactobacillus parabuchneri</i> DSM 5708	1.0	0.2	2.0	2.0	0.5	2.0
<i>Lactobacillus sp.</i> DSM 6265	1.5	0.5	1.5	2.0	0.8	2.0
<i>Pediococcus damnosus</i> ATCC 43013	1.5	0.5	2.0	1.5	< 0.1	2.0
<i>Pediococcus inopinatus</i> ATCC 49902	0.5	< 0.1	0.5	1.0	< 0.1	1.5
<i>Pediococcus inopinatus</i> DSM 20872	0.7	0.2	1.0	1.0	0.5	1.5

Table 2 Shows the average diameter (mm) of colonies beneath the surface of the agar medium in Petri dishes observed after 4 days of incubation at 28 °C in aerobic and anaerobic atmospheres

Strain	Aerobic conditions			Anaerobic conditions		
	MRS med.	NBB-P med.	MRS B+ med.	MRS med.	NBB-P med.	MRS B+ med.
<i>Lactobacillus brevis</i> DSM 1268	1.0	0.5	1.0	1.2	0.5	1.0
<i>Lactobacillus brevis</i> DSM 6235	1.3	0.3	1.3	2.0	0.4	2.0
<i>Lactobacillus lindneri</i> DSM 20691	0.5	0.5	1.0	0.5	0.5	1.0
<i>Lactobacillus parabuchneri</i> DSM 5708	0.8	0.5	1.0	0.5	0.5	1.0
<i>Lactobacillus sp.</i> DSM 6265	1.0	0.5	1.2	1.0	1.2	1.0
<i>Pediococcus damnosus</i> ATCC 43013	1.3	0.3	1.3	1.8	0.3	1.8
<i>Pediococcus inopinatus</i> ATCC 49902	1.0	0.1	1.0	1.0	0.1	1.0
<i>Pediococcus inopinatus</i> DSM 20872	1.0	0.3	0.8	1.0	0.5	1.0

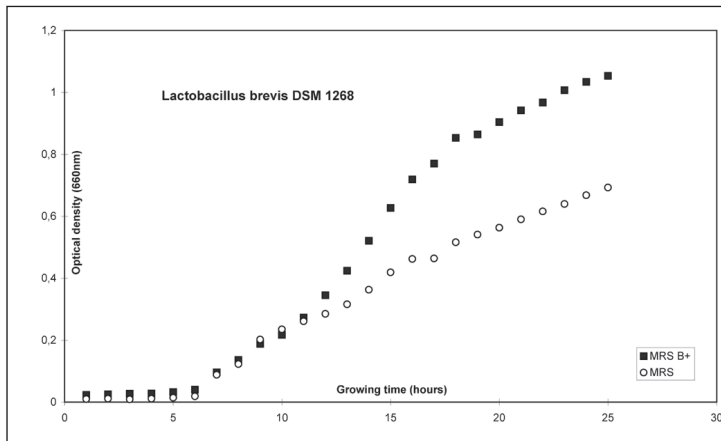


Figure 1 Growth curve of *Lactobacillus brevis* DSM 1268. Open circles are for the MRS medium and black squares are for the new medium developed

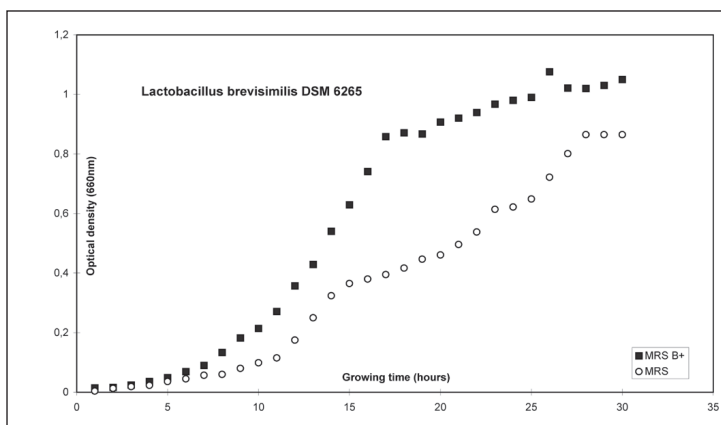


Figure 2 Growth curve of *Lactobacillus brevisimilis* DSM 6265. Open circles are for the MRS medium and black squares are for the new medium developed

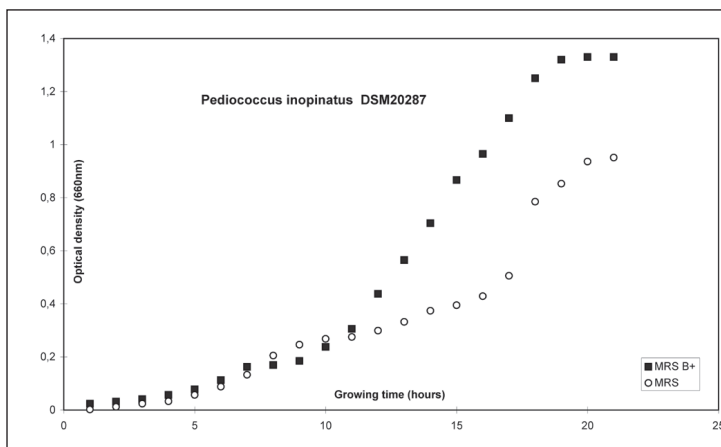


Figure 3 Growth curve of *Pediococcus inopinatus* DSM 20287. Open circles are for the MRS medium and black squares are for the new medium developed

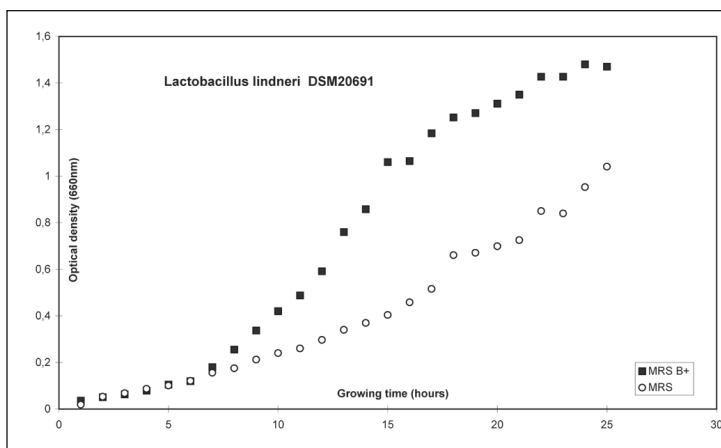


Figure 4 Growth curve of *Lactobacillus lindneri* DSM 20691. Open circles are for the MRS medium and black squares are for the new medium developed

Table 3 Variations of the optical density of the liquid culture media MRS and MRS B+ between 10 and 25 hours of incubation at 28 °C

Strain	Incub.h.	MRS	MRS B+
L. brevis DSM 1268	10	0.235	0.217
	15	0.419	0.627
	20	0.563	0.904
	25	0.693	1.053
L. brevis DSM 6235	10	1.335	1.402
	15	1.502	1.549
	20	1.607	1.576
	25	1.575	1.621
L. lindneri DSM 20691	10	0.240	0.420
	15	0.404	1.060
	20	0.699	1.311
	25	1.014	1.470
L. parabuchneri DSM 5708	10	0.243	0.380
	15	0.577	1.007
	20	0.992	1.446
	25	–	–
L. sp. DSM 6265	10	0.099	0.214
	15	0.365	0.629
	20	0.461	0.907
	25	0.649	0.990
P. damnosus ATCC 43013	10	1.305	1.392
	15	1.502	1.539
	20	1.547	1.616
	25	–	–
P. inopinatus ATCC 49902	10	1.167	1.280
	15	1.460	1.487
	20	1.647	1.596
	25	–	–
P. inopinatus DSM 20872	10	0.268	0.238
	15	0.395	0.867
	20	0.936	1.330
	25	–	–

Table 4 Evolution of the population (cfu) in liquid culture media MRS B+ and NBB between 0 and 72 hours of incubation at 28°C

Strain	Incub.h.	MRSB+	NBB
L. brevis DSM 1268	0	8.9 X 10 ⁶	8.9 X 10 ⁶
	20	1.6 X 10 ⁸	1.6 X 10 ⁷
	25	7.0 X 10 ⁸	3.3 X 10 ⁷
	30	8.0 X 10 ⁸	3.4 X 10 ⁷
	41	1.0 X 10 ⁹	3.7 X 10 ⁷
	72	–	4.2 X 10 ⁷
L. brevis DSM 6235	0	2.7 X 10 ⁷	2.7 X 10 ⁷
	10	1.2 X 10 ⁹	2.1 X 10 ⁸
	15	2.0 X 10 ⁹	3.0 X 10 ⁸
	20	7.0 X 10 ⁸	3.2 X 10 ⁸
	48	3.8 X 10 ⁹	3.4 X 10 ⁸
L. lindneri DSM 20691	0	4.1 X 10 ⁸	3.4 X 10 ⁸
	20	7.6 X 10 ⁸	3.7 X 10 ⁸
	25	9.1 X 10 ⁸	4.3 X 10 ⁸
	30	1.6 X 10 ⁹	4.6 X 10 ⁸
	41	3.5 X 10 ⁹	6.7 X 10 ⁸
	48	6.8 X 10 ⁹	6.7 X 10 ⁸
L. parabuchneri DSM 5708	0	7.6 X 10 ⁶	7.6 X 10 ⁶
	20	6.0 X 10 ⁸	6.2 X 10 ⁷
	25	1.8 X 10 ⁹	1.7 X 10 ⁸
	30	1.7 X 10 ⁹	2.0 X 10 ⁸
	41	2.9 X 10 ⁹	3.4 X 10 ⁸
	72	–	8.3 X 10 ⁸
L. sp. DSM 6265	0	8.9 X 10 ⁵	8.9 X 10 ⁵
	20	3.2 X 10 ⁷	8.2 X 10 ⁶
	25	1.1 X 10 ⁸	6.5 X 10 ⁷
	30	1.2 X 10 ⁸	7.0 X 10 ⁷
	41	1.5 X 10 ⁸	7.5 X 10 ⁷
	72	–	1.0 X 10 ⁸
P. damnosus ATCC 43013	0	1.3 X 10 ⁷	1.3 X 10 ⁷
	10	5.6 X 10 ⁷	3.0 X 10 ⁷
	15	2.3 X 10 ⁸	3.8 X 10 ⁷
	20	7.3 X 10 ⁸	1.1 X 10 ⁸
	48	2.6 X 10 ⁹	2.8 X 10 ⁸
P. inopinatus ATCC 49902	0	9.6 X 10 ⁵	9.6 X 10 ⁵
	10	1.1 X 10 ⁶	2.5 X 10 ⁶
	20	7.5 X 10 ⁷	7.7 X 10 ⁶
	30	1.3 X 10 ⁸	3.5 X 10 ⁷
	42	2.0 X 10 ⁸	6.6 X 10 ⁷
P. inopinatus DSM 20872	0	9.4 X 10 ⁵	9.4 X 10 ⁵
	10	4.2 X 10 ⁶	2.5 X 10 ⁶
	20	1.4 X 10 ⁷	8.0 X 10 ⁶
	30	6.8 X 10 ⁷	2.4 X 10 ⁷
	42	1.9 X 10 ⁸	8.3 X 10 ⁷

3.2.2 Growth assessed by cfu enumerations

For the NBB medium, it was not possible to follow the growth by optical density because the medium contains insoluble particles. The growth of the population has been established by direct enumeration of the cfu versus time of incubation for the media NBB and MRS B+

The results are presented in table 4.

In that case only living organisms are detected.

4 Conclusions

With the new medium, we can easily cultivate and count some of the most fastidious bacterial beer spoilers, at last strains coming from collections.

However, these results give us a hope to get good performances for the retrieving of the native strains from spoiled beer or yeast. In that case, it will be necessary to add an inhibitor for yeast overgrowing, with the hope that the supplemented medium will retain its good performances for the detection and the growing of spoiling bacteria.

5 Zusammenfassung

Gillet, A., Dupuche, M.-H., und Velings, N.: Ergänzung zum MRS Medium zur Kultivierung obligat bierverderbender Bakterien — Monatsschrift für Brauwissenschaft 56, Nr. 1/2, 10 – 14, 2003

BC 43 Biologische Betriebskontrolle

Der schnelle Nachweis und die Kultivierung der spezifisch Bier verderbenden Bakterien stellte schon immer ein Problem dar und ist noch immer für einige der am meisten obligaten Lactobacillus- und Pediococcuspezies ungelöst. Diese Abhandlung stellt ein neues Medium vor, welches auf einer Adaptation eines MRS Mediums, welches von DeMan, Rogosa & Sharpe 1960 vorgestellt wurde, basiert. Das neue Medium wird bezüglich der Koloniengröße auf Platten und der Wachstumskinetik in flüssigen Medien verglichen. Die Kinetik wird anhand der optischen Dichte so wie einer cfu Zählung verfolgt. Abschließend können wir sagen, dass man mit dem neuen Medium leicht einige der am meisten bierverderbenden Bakterien kultivieren und zählen können.

Gillet, A., Dupuche, M.-H., et Velings, N.: Enrichissement du milieu MRS pour la culture des bactéries nuisibles à la bière et de croissance difficile — Monatsschrift für Brauwissenschaft 56, No 1/2, 10 – 14, 2003

BC 43 Contrôle de qualité microbiologique

La détection ainsi que la mise en culture des bactéries responsables de la contamination de la bière a toujours été et reste un problème ardu et ce particulièrement pour les espèces Lactobacillus et Pediococcus. Cet article présente un nouveau milieu développé à partir d'une adaptation du milieu MRS proposé par DeMan, Rogosa & Sharpe en 1960. Ce nouveau milieu est comparé à d'autres en terme de dimension de colonies sur boîtes ainsi que du point de vue cinétique de croissance en milieu liquide. La cinétique a été suivie aussi bien par l'évolution de densités optiques que par le comptage des ufc. Nous pouvons conclure qu'avec ce nouveau milieu, il est possible de mettre en culture et de compter de manière plus aisée beaucoup des bactéries responsables de la contamination de la bière.

6 References

Back, W.: Brauwelt **120**(43), 1562 – 1569, 1980.
 DeMan, J. D. , Rogosa, M. & Sharpe, M. E.: Journal of Applied Bacteriology **23**, 130 – 135, 1960.
 Emeis, C.-C.: German Federal Republic Patent Application, (1972) 1 673 280.
 Kindraka, J. A.: Technical Quarterly, MBAA, **24**(4), 146-151, 1987.
 Kozulis, J. A. & Page, H. E.: Proceedings, ASBC, 52 – 58, 1968.
 Nishikawa, N. & Kohgo, M.: Technical Quarterly, MBAA, 22, 61, 1985.
 Saha, R. B., Sondag, R.J. & Middlekauff, J.E.: Proceedings, ASBC, **5**(1), 9 – 10, 1974
 Taguchi, H., Ohkochi, M., Uehara, H., Kojima, K. & Mawatari, M.: Journal of the ASBC, **48**(2) 72 – 75, 1990.

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