COMPLEX NATURAL AMENDMENTS ENHANCE CELLULOLYTIC ACTIVITY OF BACTERIAL CONSORTIUM

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INTRODUCTION

Bioconversion of cellulose-containing biomass into fermentable sugars attracts great attention in many areas of biotechnology. Particular interest is focused on a higher efficiency of nutrient amendments (**Am**) and synchronously degraded carriers (e.g., sawdust, straw, etc.) used for biodegradation processes.

The objective of this study was to compare the kinetics of bacterial growth and their cellulolytic activity in the presence of molasses (**M**), cabbage leaf extract (**CLE**), yeast extract (**YE**) and glucose (**G**) added to carboxymethyl cellulose(**CMC**)-containing basic medium.

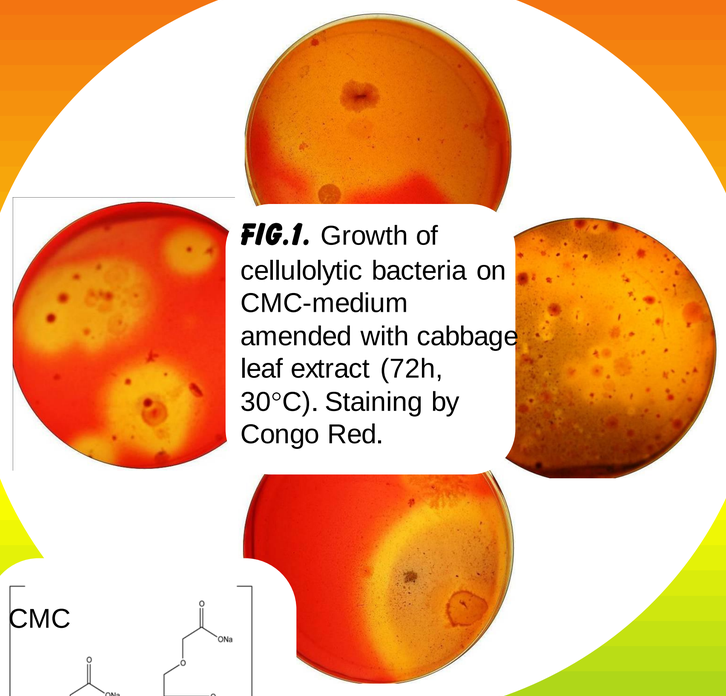
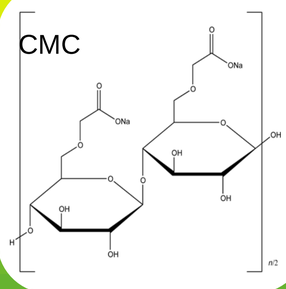


FIG.1. Growth of cellulolytic bacteria on CMC-medium amended with cabbage leaf extract (72h, 30°C). Staining by Congo Red.

CMC



MATERIALS & METHODS

Previously isolated bacterial consortium with cellulolytic activity was tested. CLE was prepared as indicated in [Muter et al., 2008] and added either to medium or water, in the concentration range from 1% to 30%, whereas M (sugar beet), YE and G - from 0.1% to 3%, respectively. CMC medium contained, g/L: $NaNO_3$ – 2; K_2HPO_4 – 1; $MgSO_4$ – 0.5; KCl – 0.5; CMC sodium salt– 2; peptone – 0.2. Two types of control were used, i.e., i) unamended CMC-containing basic medium and ii) distilled water with amendments. Cultivation was performed in microplates at 30 °C with periodic agitation, reading the OD_{620} in microplate reader ASYS Expert Plus (Biochrom, UK). For agar plates 1.7 % agar was added to CMC-medium. Cellulolytic activity of bacterial colonies was estimated by white zone diameter around colony after staining with Congo Red (Fig.1).

RESULTS

The effect of different nutrient amendments on the growth of cellulolytic bacterial consortium is shown in Figure 2. The CMC medium without Am did not support bacterial growth under tested conditions. The highest growth-promotnig effect was detected in batch culture cultivated with CLE in CMC medium (Fig. 2-I-A). Addition of molasses and yeast extract also stimulated the bacterial growth. In turn, addition of glucose to CMC-medium showed the lowest increase in growth activity, as compared to other Am tested (Fig.2-I-D).

Another part of experimental sets was performed with sterile destilled water instead of CMC medium (Fig.2-II). Among four Am tested, only CLE served as a sufficient growth medium, in a concentration-dependent manner (Fig. 3).

Stimulating effect of plant extracts Cha-poo (*Piper sarmentosum* Roxb.) for microbial (*Rhodococcus sp.* MI 2) growth and their cellulase activity was reported earler by Tanskul et al. (2013).

Thus, supplementing the cultivation medium with plant extracts could considerabely increase an efficiency of biotechnological processes via stimulating the microbial growth and activity, as well as reducing preparation costs.

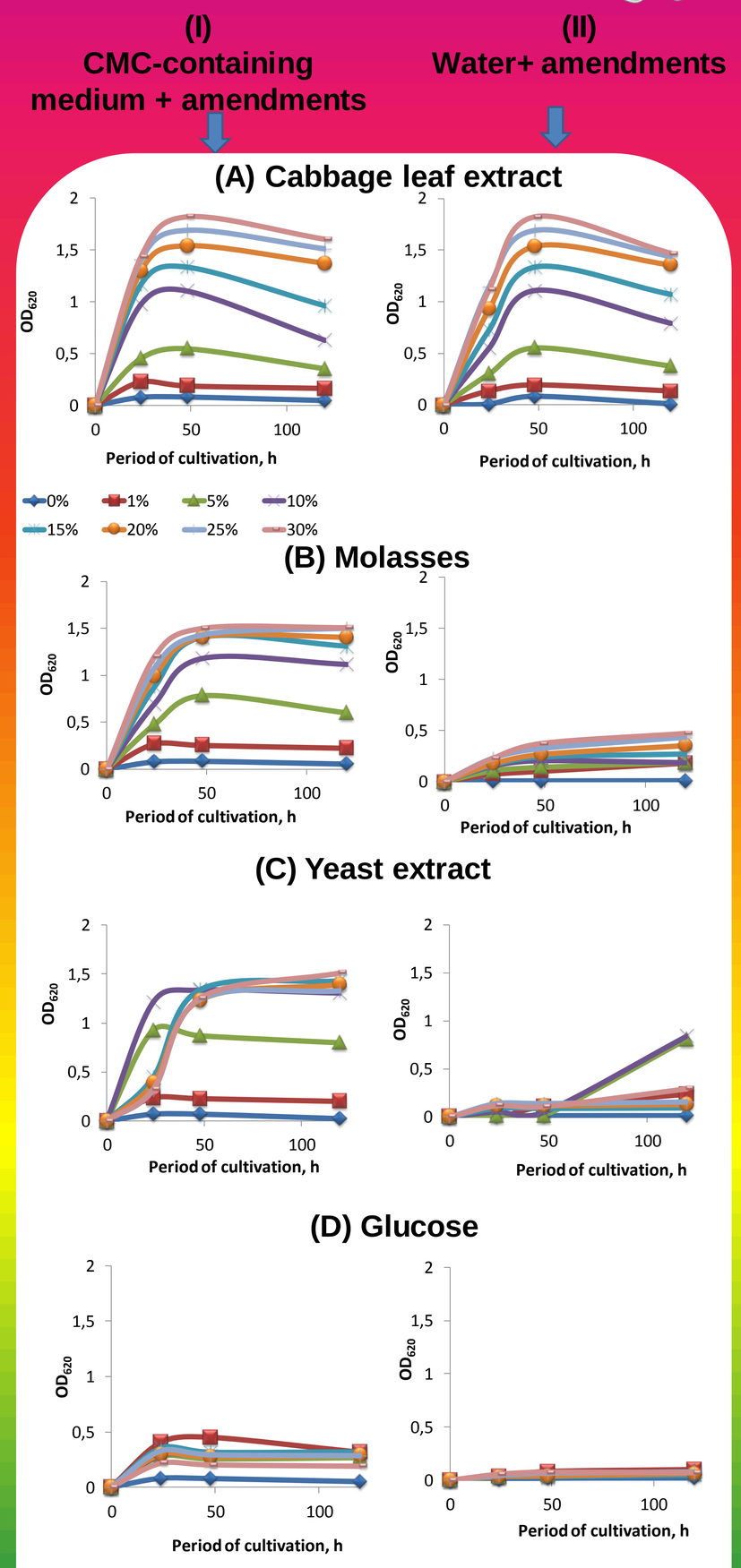


FIG.2. Growth of cellulolytic bacterial consortium in CMC-medium (I) and water (II) amended with CLE, M, YE and G. A – CMC-medium; B – water. CLE – cabbage leaf extract (1%÷30%); M – sugar beet molasses; YE – yeast extract; G – glucose.

CHARACTERIZATION OF DIFFERENT CABBAGE CULTIVARS

The growth-promoting effect of cabbage extract, in particular, *Streptococci, was reported in 1921* [Ayers and Mudge, 1921]. *The cabbage* juice medium prepared using Chinese cabbage (*Brassica campestris) was shown as a substrate for yeast biomass* production. Relatively high concentration of reducing sugars is suitable for yeast culture. As for lactic acid bacteria, opinions on CLE effect on these bacteria growth are different. Thus, fresh juice of Cecile cultivar cabbage (*Brassica oleracea) was inhibitory to the growth of lactic acid bacteria* [Fleming and Kyung, 1994]. Most probably, the effect of CLE on the microbial growth is microorganism and cabbage cultivar-dependent. In addition, the medium composition and the protocol for CLE preparing can noticeably infuence the above mentioned effect (Muter et al., 2008; Grube et al., 2008).

In our previous study, six cabbage cultivars used as nutrient amendments for bacteria cultivation, were compared by their C, N and reducing sugars content (Table 1) [Grube et al., 2008].

TABLE 1. The content of carbon, nitrogen and reducing sugars in different CLE (1-3 – White cabbage; 4 – Savoy cabbage; 5 – Chinese cabbage; 6 – Red cabbage). (Grube et al., 2008).

CLE sample[a]	Carbon (vol.%)	Total nitrogen (vol.%)	N/C	Sucrose (g l00 ml^{-1})	Glucose (g l00 ml^{-1})	Fructose (g l00 ml^{-1})	Total reducing sugars (g l00 ml^{-1})
1	0.555	0.38	0.68	0.57	7.08	6.03	13.68
2	1.186	0.29	0.24	1.33	13.41	10.24	24.98
3	1.221	0.57	0.47	1.04	11.01	8.74	20.79
4	1.186	1.00	0.84	1.38	6.06	4.97	12.41
5	0.823	0.38	0.46	0.64	6.38	5.51	12.53
6	1.251	0.22	0.18	2.09	10.79	8.30	21.18

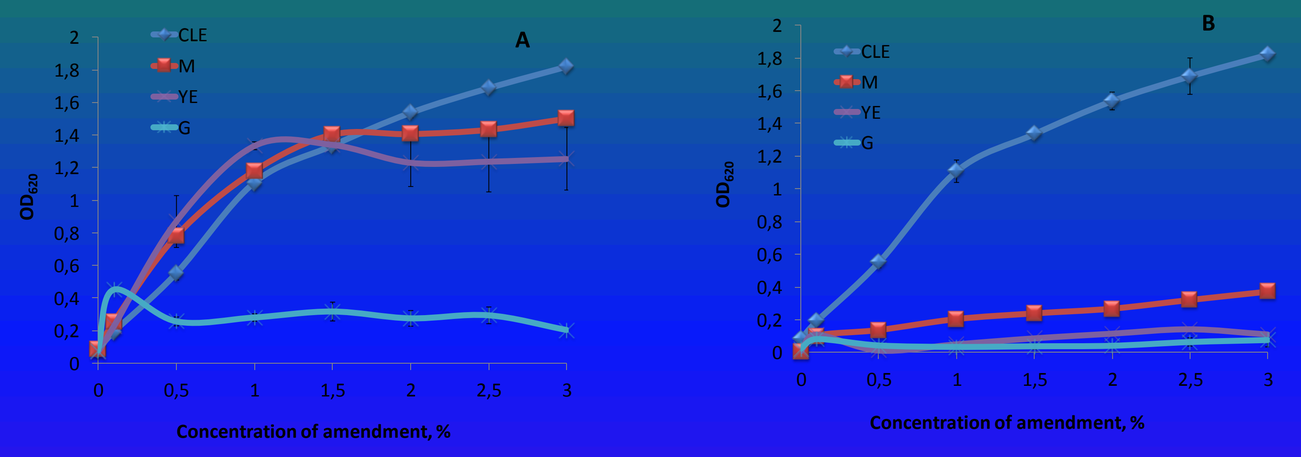


FIG.3. Dependence of bacterial growth on the concentration of amendments. A – CMC-medium; B – water. CLE – cabbage leaf extract (1%÷30%); M – sugar beet molasses; YE – yeast extract; G – glucose.

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