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Influence of extracts from *Mentha aquatica* L. and *Mentha longifolia* L. on the activity of glycosyltransferases from *Leuconostoc mesenteroides* strains

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ABSTRACT

A study of the influence of different extracts from *Mentha aquatica* L. and *Mentha longifolia* L. on the activity of glycosyltransferase complex from *Leuconstoc nesenteroides* URE 13 and dextransucrase from *Leuconostoc mesenteroides* Lm 28 was carried out. The extracts from two plant species showed an inhibitory effect on studied glycosyltransferases at minimal concentration of the total extract 1.87 mg/ml in the reaction mixtures. At this concentration the enzyme complex from URE 13 strain lost about 18% (*M. longifolia* L.extract) and 12% (*M. aquatica* L.extract). For dextransucrase from Lm 28 strain a higher inhibition varying from 22% (*M. longifolia* extract) to 30% (*M. aquatic* extract) was detected. When the concentration of the extracts was raised to 9.35 mg/ml, the inhibitory effect on the enzyme complex from URE 13 and dextransucrase from Lm 28 strain increase. At this concentration of the extracts a higher percent of inhibition was detected for the enzyme from Lm 28 strain – 100% for the extract from both *M. longifolia* L and 98% for this from *M. aquatica* L. For the enzyme from *L. mesenteroides* URE 13 strain, at concentration 9.35 mg/ml was determined 97% inhibition for both plant extracts. The inhibitory effect of both extracts on the studied enzymes was compared with equivalent concentrations of essential oil from *M. piperita* L. where an inhibitory effect ranging from 63% (URE 13 enzyme complex) to 76% (Lm 28 dextransucrase) was observed. The effect of the extracts from both *Mentha* sp. on transferase to hydrolase activity ratio was also studied and showed predominance of transferase activity with more than 90% at 24 h from the start of the reactions.

Key words: glycosyltransferases, *Leuconostoc mesenteroides*, *Mentha aquatica* L., *Mentha longifolia* L.

Introduction

Mentha sp. are aromatic perennial herbs belonging to a family Lamiaceae which are distributed on several continents including Europe, Asia, Africa, Australia (Lange & Croteau, 1999; Sharapov et al., 2012) From more than 25 species comprising the family, only *M. piperita* L. (peppermint), *M. arvensis* L. (cornmint) and *M. spicata* L. (spearmint) are traditionally cultivated in the world for the extraction of mentha essential oil from the leaves (Gulluce et al., 2007). The aerial parts of *Mentha* sp. (leaves, flowers, stem) are traditionally used for preparation of herbal teas and as supplements to various commercial products including cosmetics, foods and drugs because of their characteristic aroma and flavor (Moreno et al., 2002). Preparations from *Mentha* sp. are used in tradition medicine for treatment of

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morbid conditions like bronchitis, nausea, flatulence, colitis, liver complaints, gum and teeth diseases, due to their anti-inflammatory, antiemetic, diaphoretic, antispasmodic, anticatharrhal, analgesic, antibacterial and antioxidant properties (Cowan, 1999; Iscan et al., 2002; Chaiya et al., 2013). *Streptococcus mutans* is primary causative agent of the dental caries due its ability to produce glucose polymer which is the main compound of dental plaque. The polymer production is catalyzed by glycosyltansferase enzymes. (Hamada & Slade, 1980). The characteristic smell and flavor of *Mentha* sp. is due to the presence of cyclic terpene alcohol menthol which is present in menthe essential oil. In addition, the different *Mentha* sp. contains a large number of other terpenoids and flavonoids with potential biomedical properties and applications: methanol, menthone, isomenthone, 1,8-cineole, menthyl acetate, menthofuran, limonene, β-myrcene, β-carophyllene, pulegone, carvone, linalool, camphor, thymol, carvacrol, luteolin-7-O-glycoside and etc (Henley-Smith et al., 2013; Kumar & Pandey, 2013).The composite nature of mentha extracts corresponds to the significant number of biological activities reported for this plant and many more are waiting to be elucidated and confirmed especially for its immunomodulating and anticancer effects (Cosentino et al., 2009; Abirami & Nirmala, 2014**).**

The flavonoids and terpenoids available in preparations and extracts from *Mentha* spp. are all characterized as a substances with poor water solubility which limits their absorption and medical effectiveness. A potential strategy for increase of water solubility of these aromatic compounds (e.g. catechin, luteolin, myricetin) includes their modification by glycosilation. At this point of view, the enzyme approaches using specific glycosyltransferases seems promising (Andre et al., 2010). It is known that glycosyltransferases produced by several genera lactic acid bacteria (*Leuconostoc*, *Lactobacillus*, *Weissella*, *Streptococcus*) are able to catalyze the transfer of glycosyl residues (glucose or fructose) from sucrose to a growing polymer chain (glucan or fructan production), or to suitable acceptor molecules – acceptor reaction (Monsan et al., 2010). In most cases the best acceptor molecules are carbohydrates such as maltose, isomaltose and raffinose, but despite of comparatively low effectiveness, some non-carbohydrate molecules such as plant flavonoids could be also glycosylated (Bertrand et al., 2006).

In the present work, we studied the influence of extracts from *M. aquatica* L. and *M. longifolia* L. on the activity of glycosyltransferases from *Leuconstoc nesenteroides* URE 13 and *Leuconstoc nesenteroides* Lm 28. In addition the effect of the studied extracts on transferase reaction catalyzed by glycosyltransferases from both strains was compared.

Materials and Methods

Bacterial strains and culture media

Leuconostoc mesenteroides URE 13 and *Leuconostoc mesenteroides* Lm 28 were obtained from the bacterial culture collection of the Department of General and Industrial Microbiology, Sofia University. For the production of glycosyltransferases the strains were cultivated 6-8 h in culture media containing 4% (w/v) sucrose at 27°C on a rotary shaker (200 rpm) (Iliev et al., 2008).

Biomass measurements

Bacterial growth was measured by a turbidimetric method at 620 nm and calibrated against cell dry-weight measurements as previously described (Iliev et al., 2003).

Preparation of extracts from M. longifolia L. and M. aquatica L.

Extracts from *M. longifolia* L. and *M. aquatica* L. (harvested from south-east of Rodopa mountain) were prepared from 500 g dried and finely powdered aerial parts by acetone extraction at room temperature for a week. The acetonic solution was concentrated by evaporation under vacuum and low temperature (45°C) to dryness. The extracts from both *Mentha* sp. were dissolved in 100% dimethyl sulfoxide (DMSO) (Calbiochem). The essential oil from *M. piperita*. was purchased from Rozaimpex Ltd., Plovdiv.

Concentration of glycosyltransferases

The culture medium after sucrose cultivation was first centrifuged for 20 min at 7000 g and 4°C for cell separation. The supernatant was then filtered with a Sartorius membrane (0.2 μm cut-off) to ensure the total absence of cells in the supernatant. The glycosyltransferases were separated from the supernatants and concentrated by using of PEG 1500 (polyethylene glycol with molecular weight 1500) to final concentration of 20% (w/v) (Goyal & Katiyar, 1994). The glycosyltransferases were separated by centrifugation at 7000 g for 20 min at 4°C, collected in the pellet, and diluted in 20 mmol sodium acetate buffer, pH 5.4.

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Enzyme activity assays

One unit of glycosyltransferase activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of fructose or glucose per 1 min at 30°C in 20 mmol/l sodium acetate buffer (pH 5.3), 0.05 g/l $CaCl_2$ and 100 g/l sucrose (Dols et al., 1998). Additionally, D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Cat. No. K-FRUGL, Megazyme International Ireland ltd, Wicklow, Ireland). All activities were assayed at least in triplicate and average values are given.

Determination of inhibitory effect

The inhibitory effect of the studied *Mentha* sp. extracts was determined at 1.87 mg, 3.74 mg, 5.61 mg, 7.48 mg and 9.35 mg per ml end concentration in the reaction mixtures. The inhibitory effect of *M. piprita* essential oil was tested at 13.3%, 26.6%, 39.9, 53.2% and 66.5% end concentration in the reaction mixtures. The reactions were performed with 1 U/ml enzyme in reaction mixtures containing 20 mmol/l sodium acetate buffer (pH 5.3), 0.05 g/l $CaCl_2$, 100 g/l sucrose at 30°C. D-fructose and D-glucose were determined with hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase (commercially available kit, Cat. No. K-FRUGL, Megazyme International Ireland ltd, Wicklow, Ireland). All the reactions were performed at least in triplicate and average values are given.

Protein determination

Protein concentration was determined according to the procedure of Bradford (Bradford, 1976) using bovine serum albumin as a standard.

Statistical analysis

In all the cases the software Programmable scientific calculation "CITIZEN" SRP-45N and Excel 2007 (Microsoft) were used for data analysis.

Results and Discussion

Study of inhibitory effect of Mentha sp. extracts on the activity of glycosyltransferases from L. mesenteroides URE 13 and L. mesenteroides Lm 28

In order to determine the inhibitory effect of the extracts from *M. longifolia* L., *M. aquatica* L. and essential oil from *M. piperita* on the activity of glycosyltransferase complex from URE 13 strain and dextransucrase (EC 2.4.1.5) from Lm 28 strain, we performed enzyme reactions in the presence of different concentrations of the substances (Figure 1, Figure 2). In previous studies, we showed that strain *L. mesenteroides* Lm 28 secretes a single dextransucrase, and *L. mesenteroides* URE 13 produces a glycosyltransferase complex including dextransucrase, glucosyltransferase and fructosyltransferase (EC 2.4.1) (Iliev et al., 2008; Vasileva et al., 2012). Most of the components in *Mentha* sp. extracts (terpenoids and flavonoids) have poor water solubility so we used DMSO as a compatible organic solvent to dissolve the extracts. The lowest inhibitory effect for enzyme complex and dextransucrase was observed in the presence of 20% DMSO – about 10% for both strains (data not shown).

The extracts from *M. longifolia* L. and *M. aquatica* L. showed an inhibitory effect at minimal studied concentration of 1.87 mg/ml - 18% and 12% for the enzyme complex from URE 13, and 22% and 30% for dextransucrase from Lm 28 strain, respectively (Figure 1 A, B). With increase of the concentration of the extracts, the observed inhibitory effect gradually increase. At the highest studied concentration of the extract from *M. longifolia* L. (9.35 mg/ml) no activity was detected for dextransucrase from Lm 28, and glycosyltransferase complex from URE 13 retains only 3% of its initial enzyme activity (Figure 1 A). When the extract from *M. aquatica* L. was tested, at 9.35 mg/ml concentration in the reaction mixtures the enzymes from both strains retain 3% and 2% of their activity, respectively (Figure 1 B). In all the cases, the inhibitory effect on dextransucrase activity from Lm 28 strain is stronger than observed values for the enzyme complex from URE 13 strain. Possible explanation for this difference is that the complex from URE 13 strain contains not only dextransucrase but also additional glucosyltransferase and fructosyltransferase, which are affected differently by the compounds of the extracts. No significant differences between the inhibitory effects of the extracts from both *Mentha* sp. were observed. Higher concentrations of the extracts were not tested because of their low solubility at 20% DMSO, presented in the reaction mixtures.

When the inhibitory effect of essential oil from *M. piperita* was tested, the same difference between the enzymes from both studied strains was observed, as the glycosyltransferase complex from URE 13 showed 63% of

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inhibition and dextransucrase from Lm 28 – 76% of inhibition at the highest tested concentration of the oil in the reaction mixtures (Figure 2). In both cases a full inhibition by the essential oil from *M. piperita* was not reached at the tested concentrations. Higher concentrations than 66.5% oil lead to not reliable changes in the enzyme activities because of the phase partitioning between the oil and water in the reaction mixtures on one hand, and changes of its buffering capacity on the other.

The received results showed a significant degree of inhibition by total acetone extracts from *M. longifolia* L. and *M. aquatic* L.

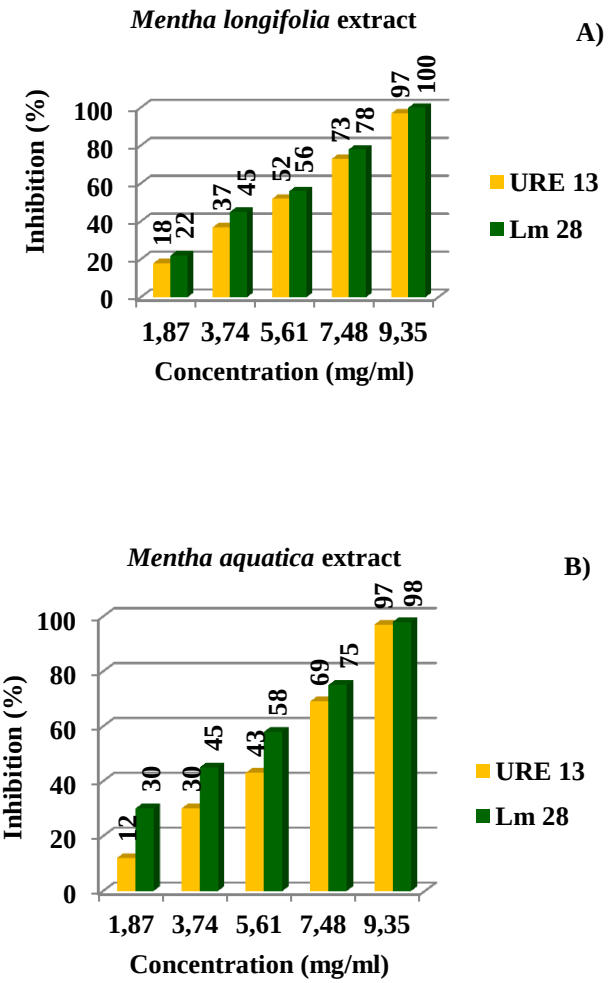

Figure 1. *Inhibitory effect of extracts from M. longifolia L. (A) and M. aquatic (B) on glycosyltransferase complex from L. mesenteroides URE 13 and dextransucrase from L. mesenteroides Lm 28.*

This is in correlation with data from other studies reporting good antimicrobial activity of extracts and essential oils from different *Mentha* sp (Pramila et al., 2012). *Mentha* sp. extracts and essential oils show antimicrobial effects against oral pathogens, including the primary causative agent of dental caries *Streptococcus mutans* which produces glucosyltransferases responsible for teeth plaque formation. Thus the *Mentha* sp. preparations seems to inflict not only the growth of this bacterium but also the activity of the produced glucosyltransferases by their inhibition (Chaiya et al., 2013; Henley-Smith et al., 2013).

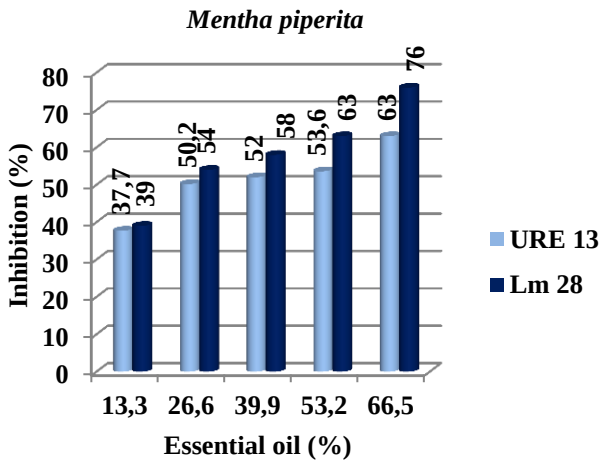

Figure 2. *Inhibitory effect of essential oil from M. piperita L. on glycosyltransferase complex from L. mesenteroides URE 13 and dextransucrase from L. mesenteroides Lm 28.*

Transferase and hydrolase activity of glycosyltransferases from URE 13 and Lm 28 strains in the presence of Mentha sp. extracts

On the basis of the amounts of released glucose and fructose, measured during the reactions catalyzed by studied glycosyltransferases, in the presence of 10% of sucrose and 5.61 mg/ml of *M. longifolia* L. or *M. aquatica* L. extracts the ratio between transferase and hydrolase activity was determined (Table 1, Table 2). For the enzymes of both studied strains the transferase activity is predominant. The highest transferase activity for glycosyltransferase complex from URE 13 strain was determined at 3 h – above 98% in the presence of *M. longifolia* L. or *M. aquatica* L., respectively (Table 1). For dextransucrase from Lm 28 strain, the highest value of transferase activity, in the presence of *M.*

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longifolia L. extract was determined at 3 h from the start of the reaction, while in the presence of *M. aquatica* L. extract, the highest transferase activity was observed at 1 h (Table 2). At the next hours the ratio of hydrolase activity increase gradually to 24 h and this change is more significant for the reactions performed in the presence of extract from *M. longifolia* L (Table 1, Table 2.).

The lack of significant change of transferase to hydrolase activity ratio during the reactions catalyzed by studied enzymes, in the presence of average of the tested concentrations of mentha extracts is a good basis for possible application of glycosyltransferase enzymes in the glycosylation of the low-soluble flavonoids and terpenoids found in these plant preparations. Examples for such an application of glycosyltransferases in modification of bioactive compounds exist in the literature. Successful enzyme glycosylation of the flavonoids catechin, naringin, rutin, quercetin, myricetin, luteolin is already achieved. Bertrand et al. (2006) performed glucosylation at C3' and C4' of B-ring in the flavonoid structure by dextransucrase from *L. mesenteroides* NRRL B-512F. The obtained glycosylated compounds have improved water solubility and increased biological activity. (Bertrand et al., 2006).

Conclusions

In the presence of extracts from *M. longigolia* L. or *M. aquatica* L. the glycosyltransferase complex from *L. mesenteroides* URE 13 and dextransucrase from Lm 28 are inhibited in a manner depending on inhibitory concentration. At 9,35 mg/ml extract from *M. longigolia* L., dextransucrase from Lm 28 strain is completely inhibited, and URE 13 gycosyltransferase complex retains only 3% of its initial activity. The inhibitory effect of both *Mentha* sp. extracts on the activity of dextransucrase from Lm 28 strain is stronger than this one on glycosyltransferase complex from *L. mesenteroides* URE 13.

Table 1. *Transferase and hydrolase activity of glycosyltransferase complex from L. mesenteroides URE 13 in the presence of 5.61 mg/ml extracts from M. longifolia L. and M. aquatica L.*

Time (h)	Transferase : hydeolase activity (%)	
	***M. longifolia* L.**	***M. aquatic* L.**
0.5	94.62 : 5.38	95.20 : 4.80
1	98.26 : 1.74	97.82 : 2.18
3	98.75 : 1,25	98.12 : 1.88
5	92.21 : 7.79	96.32 : 3.68
10	91.81 : 8.19	96.60 : 4.40
24	92.12 : 7.88	94.10 : 5.90

Table 2. *Transferase and hydrolase activity of glycosyltransferase complex from L. mesenteroides Lm 28 in the presence of 5.61 mg/ml extracts from M. longifolia L. and M. aquatica L.*

Time (h)	Transferase : hydeolase activity (%)	
	***M. longifolia* L.**	***M. aquatica* L.**
0.5	93.18 : 6.82	92.41 : 7.59
1	97.33 : 2.67	98.10 : 1.90
3	98.14 : 1.86	97.83 : 2.17
5	94.11 : 5.89	95.41 : 4.59
10	90,64 : 9.36	95,86 : 4.14
24	90.16 : 9.84	93.22 : 6.78

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This could be due to the production of dextransucrase, additional glucosyltransferase and fructosyltransferase by URE 13 strain having a different degree of inhibition. In the presence of 66.5% of essential oil from *M. piperita* L. the enzymes from URE 13 and Lm 28 strains retain 37% and 24% of their activity. The higher concentrations of essential oil do not affect the enzyme activity due to phase partitioning between the oil and water content of the reaction mixtures or lead to drastic lose of activity due to disruption of the buffer and concentration of the compounds in the reaction mixture. In the presence of average concentrations of the extracts from *M. longifolia* L. or *M. aquatica* L., glycosyltransferase complex from URE 13 and Lm 28 strains retain predominant transferase activity, while the hydrolase activity of the enzymes slowly increase to the end of the reactions (24 h). The results presented in the current study give a perspective for future application of glycosyltransferases from the studied strains as an effective catalytic agent for glycosylation of *Mentha* sp. compounds.

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