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TOLERANCE OF YEASTS ISOLATED FROM PICKLED CUCUMBERS TO STRESS FACTORS

The aim *of this work was to study yeast microbiota composition of market pickled cucumbers, its tolerance to various stress factors (salt and sugar stress, food preservative tolerance) and hydrolytic activities that could contribute to the product spoilage.* ***Methods.*** *Yeast strains from pickled cucumbers were isolated by serial dilution method and identified according to phenotypic characteristics. Stress tolerance and hydrolytic properties of the isolated strains have been studied.* ***Results.*** *23 yeast strains were isolated from nine samples of pickled cucumbers and brine. The yeast numbers isolated from the samples varied from zero to $9.3x10^5$ CFU/ml. Most of isolated yeasts belonged to species Debaryomyces hansenii (26,1%), Wickerhamomyces anomalus (30,4%) and to genus Pichia (26,1%). The isolated strains demonstrated various levels of tolerance to such food preservatives as weak organic acids - relatively sensitive to sorbic and acetic acids, sorbic acid being most inhibitory of all the studied substances, but exhibiting a high tolerance to benzoate and propionate. Most isolated strains were osmo- and halotolerant while some exhibited high sodium chloride tolerance (MIC higher than 15% NaCl). MIC of NaCl for some isolated strains were 1.5-2-fold higher on agar medium compared to broth medium. All but one of isolated strains possessed lipolytic properties and none exhibited cellulolytic activity, 13% isolates were proteolytic. 56.5% of the isolated yeasts fermented glucose.* ***Conclusions.*** *This work provides characterization of yeast microbiota of pickled cucumbers demonstrating their tolerance to salt and osmotic stress and sensitivity to such food preservatives as sorbic and acetic acids. The isolated yeasts could potentially cause the spoilage by gas formation or lipid hydrolysis but mostly lack hydrolytic activity that could affect the final quality of the product.*

Keywords: pickled cucumbers, yeasts, tolerance to stress factors, hydrolytic properties

Fermentation is a method of food preservation well-known from ancient times. Pickled cucumbers are thought to be first produced in the Middle Yeast around 2000 BC [1]. Fermented vegetables are one of the most favourite food products in Ukraine [2]. Many people prefer home-made pickled products, however the problem of quality monitoring arises.

The quality of pickled products is preserved due to high salt content and low pH which prevents the development of undesirable microbiota [1]. Sodium chloride content in brine used for the production of pickled cucumbers on the industrial scale usually comprises up to 10% [3] thus permitting the growth of halotolerant microorganisms. The yeasts are known to be present in pickled foods in comparatively high numbers, e.g 10^4-10^5 cells/ml in pickled cucumbers [4], up to 10^5 cells/ml in fermented olives [5]. Halotolerant yeasts are capable of causing spoilage of fermented vegetables due to gas formation (bloating) and film formation [6] and also off-flavours and off-odours [7], although spoilage caused by yeasts

does not usually results in human diseases as yeasts are rarely pathogenic and do not produce toxins [7]. However yeast tolerance to low pH, high salt concentrations and common food preservatives, including weak organic acids, are the factors which determine the survival and multiplication of these microorganisms in foods with high acidity and salt content [8]. Therefore, the monitoring of yeast microbiota in fermented products especially in home-made products is an important aspect of food production.

The aim of this work was to study yeast microbiota composition of market pickled cucumbers, its tolerance to various stress factors (salt and sugar stress, food preservative tolerance) and hydrolytic activities that could contribute to the product spoilage.

Materials and methods. *Yeast enumeration and isolation from pickled cucumbers.* Four samples of pickled cucumber brine and five samples of pickled cucumbers were obtained at the local markets in Kiev and Bila Tserkva. Samples were analyzed the same day on arrival. pH of the samples was determined using pH meter I-160 MI. Prior to analysis the surface of the cucumbers was removed using the sterilized knife. 9 ml of 0.9% NaCl solution was added to 1g sample of cucumbers and was homogenized in the homogenizer MPW-302. Appropriate decimal dilutions of the samples (cucumbers and brine) were made in 0.9% NaCl solution and 0.1ml of each dilution was inoculated in triplicate on agar plates containing YPD medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, 20 g/l agar) and YPD medium containing 6% NaCl, final pH=5.0, supplemented with 100 mg/l chloramphenicol and 1.5 g/l sodium propionate to inhibit bacterial and fungal growth. The plates were incubated at 25-26^{0}C for 1 week. Each morphologically distinct colony was microscopically examined and colonies belonging to yeasts were counted and selected for further research. Each strain was purified by streaking on YPD agar at least 3 times.

Phenotypic identification of isolated yeasts. Isolated yeast strains were characterized based on their morphology, spore and pseudomycelium formation, assimilation of carbon and nitrogen sources, sugar fermentation and other physiological tests performed according to Kurtzman et al [9].

Determination of osmo- and halotolerance of isolated yeasts. Halorolerance of the isolated yeasts was tested in YPD broth containing 10-20% sodium chloride and on YPD agar medium containing 7-15% sodium chloride. The ability of yeast strains to grow at high sugar concentrations was tested in YPD medium containing 50-80% glucose. Suspensions of 48-hour yeast culture grown on YPD agar slants at 25-26^{0}C were placed in duplicate on agar plates using a multipoint inoculators. The plates containing NaCl agar medium were incubated at 25-26^{0}C for 2 weeks, while those with glucose agar – for 4 weeks.

The initial yeast concentration in broth media comprised 3-4x10^4 CFU/ml. The tubes were cultivated at 25-26^{0}C for up to a month. Yeast growth at high glucose concentration was observed by serial dilutions on YPD agar plates due to high viscosity of the medium while yeast growth at high NaCl concentration was measured by optical density at 540 nm [10].

Determination of weak acids tolerance of isolated yeasts. The ability of yeast strains to grow in medium containing weak acid preservatives was tested

on YPD agar medium containing sorbic acid, benzoic acid, propionic acid, acetic acid. The preservatives were added to YPD medium after sterilization from stock solutions and pH of media was adjusted to pH 3.5 and 5.5 with HCl or KOH. Acetic acid was added directly to the medium. Benzoic acid stock solution was prepared using sodium benzoate, propionic acid stock solution – from sodium propionate. Sorbic acid was dissolved in ethanol before addition to the medium. Suspensions of 48-hour yeast culture grown on YPD agar slants at 25-26^{0}C were placed in duplicate on agar plates using a multipoint inoculator. The plates were incubated at 25-26^{0}C for 1 week. Minimal inhibitory concentration (MIC) of the studied substances was considered the lowest concentration to completely inhibit yeast growth [10].

Determination of hydrolytic activity of isolated yeasts. The ability of the isolated yeasts to hydrolyze lipids or long-chain esters (lipolytic and esterase activity, respectively) was determined on two different agar medium. Plates were incubated at 25-26^{0}C for 1 week. Lipolytic activity was determined on tributyrin agar (5 g/l peptone, 3 g/l yeast extract, 10g/l tributyrin, 15 g/l agar, final pH 6.0). The appearance of the clear zone around yeast colonies in the opaque tributyrin agar indicated the presence of lipolytic activity. Esterase activity was determined on tween-80 agar (10g/l peptone, 5g/l NaCl, 0.1 g/l $CaCl_2x2H_2O$, 10g/l tween-80, 20 g/l agar). The formation of precipitate around yeast colonies demonstrated positive esterase reaction. Plates were incubated at 25-26^{0}C for 1 week [11].

Cellulolytic activity was determined on agar YPD medium supplemented with 0.5% carboxymethylcellulose. Plates were incubated at 25-26^{0}C for 5 days. Yeast colonies were removed from the plates by rinsing with distilled water and agar was stained with 0.03% Congo Red followed by destaining with 1M NaCl. The formation of the hydrolysis zone around colonies indicated the presence of cellulolytic activity [11]. Proteolytic activity was determined by hydrolysis of gelatin. Briefly, yeasts were cultivated in YNB broth containing 5 g/l of glucose and 100 g/l gelatin for 3 weeks at 25-26^{0}C. Strains able to liquefy gelatin were considered positive for protease [9].

Results. pH of the obtained samples varied from 3.41 to 4.25, in good agreement with the published data [1] (Table 1).

Such low pH would inhibit the growth of most bacteria at the same time creating favourable conditions for yeast replication [1]. Yeast counts greatly varied among samples of brine and pickled cucumbers obtained from the local markets. The highest yeast number was observed in samples F and H – $9.3x10^5$ CFU/ml and $9.15x10^5$CFU/g or ml respectively and in sample B – cucumber brine obtained from the market in Kiev - $3.86\pm0.23x10^5$ CFU/ml. Two samples of brine and cucumbers (G and J) did not produce any yeast counts while the remaning samples produced yeast counts ranging from $3.63\pm0.2x10^2$ to $2.23\pm0.15x10^4$ CFU/g or ml.

Table 1

Yeast counts in brine and pickled cucumbers samples

Sample	Source	pH of the sample	Yeast count (CFU/g or ml of sample) (±standard deviation)	
			YPD	YPD+6% NaCl
A	cucumber brine, market in Kiev	4,25	$6.23\pm0.25\times10^3$	$2.76\pm0.11\times10^2$
B	cucumber brine, market in Kiev	3,45	$3.86\pm0.23\times10^5$	$2.81\pm0.27\times10^4$
C	pickled cucumbers, market in Kiev	3,81	$2.23\pm0.15\times10^4$	$5.3\pm0.57\times10^1$
D	pickled cucumbers, market in Kiev	3,41	$9.23\pm0.25\times10^2$	$3.53\pm0.2\times10^2$
E	pickled cucumbers, market in Kiev	4,25	$3.63\pm0.2\times10^2$	-
F	cucumber brine, market in Bila Tserkva	3,65	$9.3\pm0.17\times10^5$	$4.07\pm0.15\times10^5$
G	cucumber brine, market in Bila Tserkva	3,91	-	-
H	pickled cucumbers, market in Bila Tserkva	3,54	$9.15\pm0.62\times10^5$	-
J	pickled cucumbers, market in Bila Tserkva	3,9	-	-

Note: "-" – absence of yeast growth

The number of yeast counts grown on plates with YPD supplemented with 6% NaCl was mostly one or more orders of magnitude lower than those observed on YPD plates (Table 1, Samples A-C). No yeast growth was observed on YPD-NaCl plates inoculated with samples E and H which means that either halotolerant yeast counts in these samples were too low to detect or they were altogether absent.

23 yeast isolates with distinct morphotype were selected, purified and characterized by their morphology, ability to assimilate and ferment carbon and nitrogen sources and other phenotypic traits. According to these characteristics most isolated yeast strains belonged to species *Debaryomyces hansenii* (26.1% isolated strains), *Wickerhamomyces (Pichia) anomalus* (30.4% isolated strains) and to genus *Pichia* (Table 2). Several different yeast strains were found in the most selected samples - from 2 to 6 isolates in each sample, excluding the sample H, there the single yeast strain identified as *Pichia* sp was isolated.

Most of isolated yeasts could survive the presence of 10% NaCl both in agar and broth medium, though MIC of salt in agar medium were somewhat higher compared to that in broth increasing even 2-fold for the strain *Pichia* sp. H1 (Table 3). While 69.5% of the isolates tolerated the presence of 15% NaCl in agar medium only 4 yeast strains (17.4%) were able to grow in the broth containing 150 or more g/l NaCl.

Table 2

Phenotypic identification of yeasts isolated from pickled cucumbers

Yeast strain	Yeast species/genus	Yeast strain	Yeast species/genus
A1	*Debaryomyces hansenii*	D1	*W. (Pichia) anomalus*
A2	*Pichia membranifaciens*	D2	*W. (Pichia) anomalus*
A3	*Pichia sp.*	D4	*P. membranifaciens*
A4	*Candida sake*	E1	*D. hansenii*
B1	*Wickerhahomyces (Pichia) anomalus*	E2	*Pichia sp.*
B2	*W. (Pichia) anomalus*	E3	*Kregervanrija fluxuum (Candida vini)*
B4	*W. (Pichia) anomalus*	F1	*Pichia sp.*
B5	*D. hansenii*	F3	*D. hansenii*
B7	*W. (Pichia) anomalus*	F4	*D. hansenii*
B8	*W. (Pichia) anomalus*	F5	*Torulaspora sp.*
C1	*D. hansenii*	H1	*Pichia sp.*
C2	*Torulaspora delbrueckii*		

Table 3

NaCl tolerance of the isolated yeasts in agar and broth media

Yeast strain	MIC in agar medium, g/l	MIC in broth medium, g/l	Yeast strain	MIC in agar medium, g/l	MIC in broth medium, g/l	Yeast strain	MIC in agar medium, g/l	MIC in broth medium, g/l
A1	100	>150	B7	120	>150	E2	120	>150
A2	100	150	B8	150	>150	E3	70	100
A3	100	150	C1	150	>150	F1	120	150
A4	150	>150	C2	100	>150	F3	180	>150
B1	150	>150	D1	150	>150	F4	180	>150
B2	150	>150	D2	120	>150	F5	120	150
B4	150	>150	D4	120	120	H1	50	100
B5	180	>150	E1	180	>150			

Sodium chloride effect on living cells is mostly determined by toxicity of specific ions and osmotic shock [12]. As high glucose concentrations result in similar hypertonic effect osmotolerance of the isolated yeasts has been studied, e.g. the ability to grow at 50-80% glucose concentration. None of the studied yeasts could survive in 60-80% glucose broth. Most of isolated yeasts were able to grow at 50% glucose concentration either on agar or broth medium. After 1 month cultivation under static conditions in 50% glucose broth cell numbers varied from zero (*T. delbrueckii* C2, *K. fluxuum* E3) to 3.48×10^8 CFU/ml (Table 4). The highest yeast concentration in 50% glucose YPD broth was observed for some isolates at 14-17-th day of cultivation with the gradual decrease in yeast numbers after that period (Fig. 1).

The isolated yeasts were tested for their tolerance at pH 3.5 and 5.5 to weak acid preservatives commonly used in food industry for the long-term preservation of food products, e.g. propionic, sorbic, acetic and benzoic acids

(Fig. 2). The antimicrobial effect of these substances is most pronounced at low pH, as the content of undissociated acid considerably decreases with pH increase, e.g. for sorbic acid from 99% at pH 3.5 to 37% at pH 5.5 [13]. Sorbic acid was the most inhibitory for the isolated yeasts among all the tested substances – MIC of sorbic acid was 0.1-0.25 g/l at pH 3.5 and 0.5-1 g/l at pH 5.5 (Fig. 2, A).

Table 4

Yeast numbers in 50% glucose broth after 4 weeks cultivation

Yeast strain	Yeast numbers, CFU/ml	Yeast strain	Yeast numbers, CFU/ml	Yeast strain	Yeast numbers, CFU/ml
A1	4,38x10^7	B7	7,27 x10^7	E2	5,62 x10^7
A2	1,32 x10^8	B8	2,87 x10^8	E3	0
A3	5,03x10^7	C1	2,12 x10^8	F1	5,53 x10^7
A4	1,68 x10^8	C2	0	F3	8,1 x10^7
B1	3,48 x10^8	D1	2,42 x10^8	F4	1,32 x10^8
B2	1,57 x10^8	D2	3,3 x10^8	F5	1,42 x10^7
B4	2,89 x10^8	D4	3,47 x10^7	H1	4,37 x10^7
B5	1,22 x10^8	E1	6,1 x10^7		

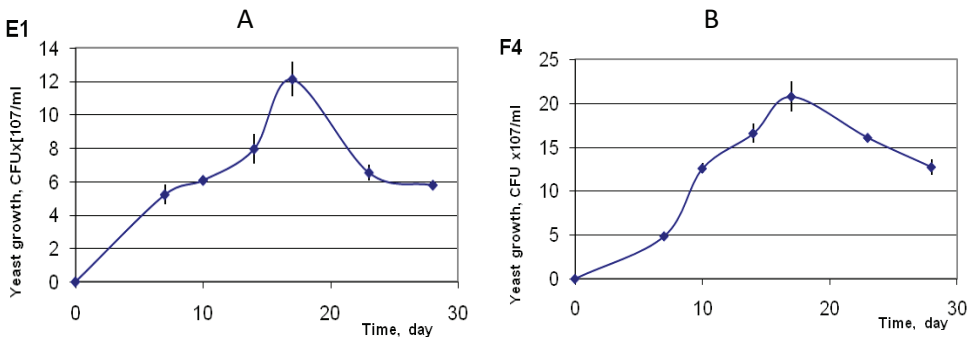

Figure 1. Growth of halotolerant yeasts *D. hansenii* E1 (A) and *D. hansenii* F4 (B) in YPD broth containing 50% glucose under static conditions

Sodium benzoate is considered safe for use in food industry in concentrations up to 1 g/l [13]. The growth of the majority of the isolated yeasts was inhibited by 0.5-5 g/l of sodium benzoate at pH 3.5 while at pH 5.5 MIC of sodium benzoate for the studied yeasts increased 10-folds and higher (Fig. 2, C). The studied yeasts were highly tolerant to sodium propionate - most could grow at 40 g/l propionate at pH 3.5 and 5.5 (Fig. 2, D).

We have determined the ability of the isolated yeasts to ferment carbohydrates (namely glucose) and hydrolyze such substrates as lipids, ethers, proteins and cellulose (Fig. 3). None of the isolated yeasts exhibited cellulolytic activity, 95.6% were lipolytic, while only one strain *Pichia* sp. A3 was esterase-positive, proteolytic acitivity was found in 13% yeast strains. Glucose fermentation was detected in 56.5% yeast isolates.

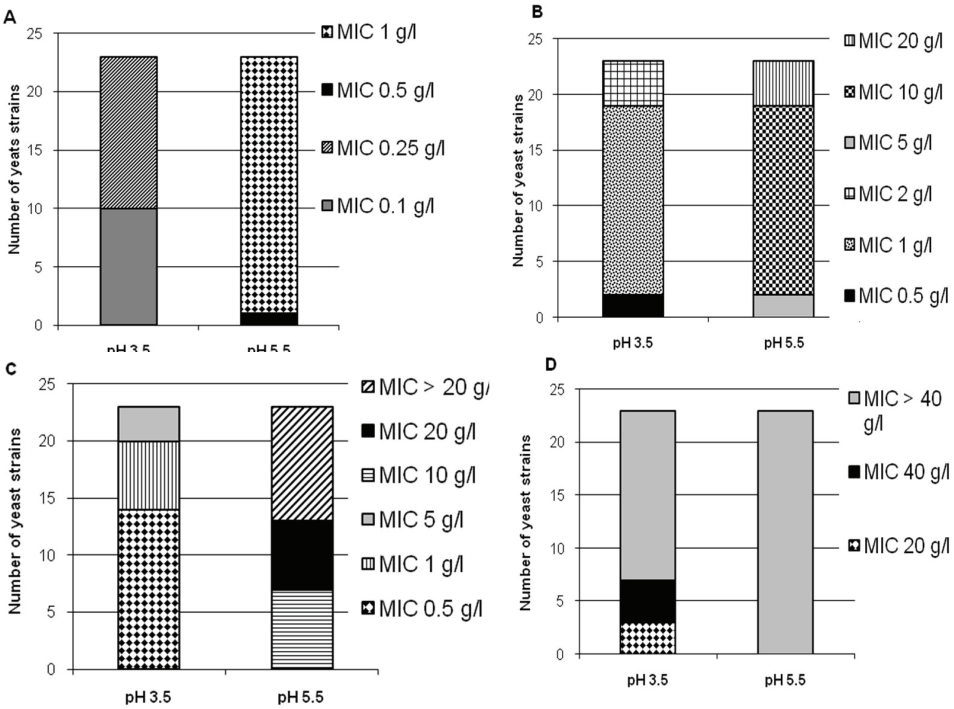

Figure 2. Tolerance of the isolated yeasts to weak organic acids: sorbic acid (A), acetic acid (B), sodium benzoate (C), sodium propionate (D).

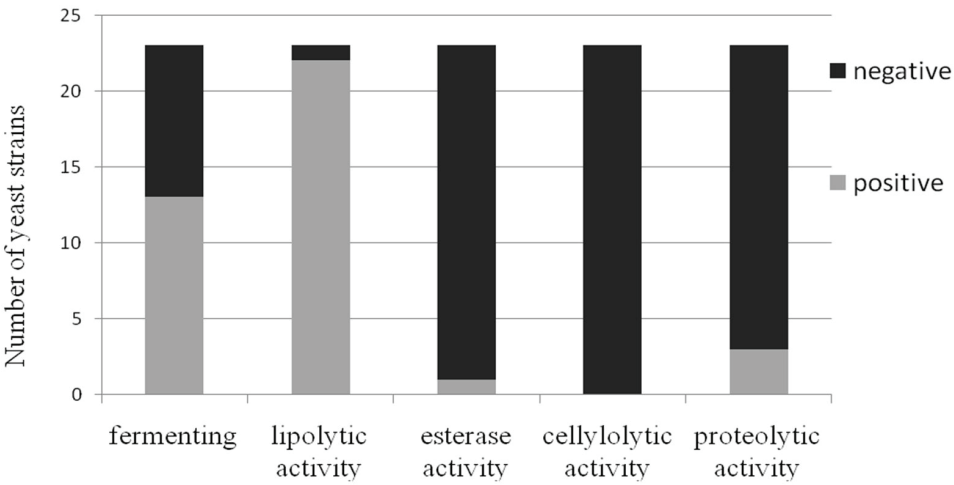

Figure 3. Hydrolytic and fermenting properties of isolated yeasts

Discussion. The various studies on yeast microbiota of pickled cucumbers produced varied yeast numbers – from complete absence of yeast growth [14] to 10^5-10^6cells/ ml or gram of the product [15]. As Stratford has noted the yeast numbers in food products up to 10^4 cells/ml do not apparently influence the quality and taste of the product and food spoilage would be caused by significantly higher yeast populations (10^5-10^6 cells per ml or gram and higher) [7]. Most of samples obtained from Kiev and Bila Tserkva produced yeast counts in the range of

10^4 CFU/g or ml or lower therefore yeast populations in these samples should not negatively affect the quality of the product however two samples of brine and one sample of pickled cucumbers contained higher numbers of yeasts thereby potentially causing the spoilage of the product.

Yeasts we isolated from pickled cucumbers predominantly belonged to *D. hansenii*, *W.anomalus* and to genus *Pichia.* The representatives of genera *Debaryomyces* and *Pichia* are frequently found in various food products [16]. *D. hansenii* is a well-known spoilage yeast inhabiting foods with low water activity and high salt content. *Pichia* sp. strains are persistently isolated from processed foods and can cause spoilage of processed fruits and pickles. The yeast microbiota of high-salt cucumber brines comprised the representatives of genera *Candida, Pichia, Torulaspora, Zygosaccharomyces* and others, while film-forming yeasts belonged to *D. hansenii, Pichia ohmeri, Isaatchenkia orientalis* and *Zygosaccharomyces rouxii* [17]. So yeast microbiota from the obtained samples of pickled cucumbers comprised yeasts frequently isolated from food products including foods with high salt content.

Sodium chloride is one of the earliest known food preservatives. Usually salt content in brine for vegetable fermentation is 4-6% although may rise up to 10% [3] thus allowing growth of mainly halotolerant or halophilic microorganisms. The ability to survive salt stress is one of the known characteristics of spoilage yeasts [7]. The majority of the isolated yeasts could tolerate high salt concentration (10% NaCl or more) in the medium, although we observed the variation in MICs of sodium chloride in both and agar media. Such discrepancy was observed for microorganisms when determining resistance to antimicrobial substances, where MICs on solid medium could be considerably higher than in broth [18].

Weak organic acids (sorbic, acetic, propionic etc.) are well-known food preservatives used to inhibit the growth of undesirable microbiota. The effect of propionic, sorbic, acetic and benzoic acids on yeasts isolated from pickled cucumbers has been studied. The tolerance of the isolated strains to sorbic acid was similar to that of *Pichia guilliermondii* and *Candida halophila* strains isolated from spoiled products with high sugar content by Martorell et al. [10] while MIC of acetic acid for yeasts isolated by us was several times lower. At pH 4 MIC of sorbic acid for *Saccharomyces cerevisiae* was 0,34-0.39 g/l while for the spoilage yeast *Zygosaccharamyces bailii* - 2-3-folds higher [19].

Shimazaki et al. observed a 10-fold increase in MIC of sodium benzoate for *Saccharomyces cerevisiae* from 0.8 g/l at pH 4 to 8 g/l at pH 5 and 15-fold increase in MIC at pH 6 – to 12 g/l, the similar pattern was observed for *Candida albicans* [20]. Garnier et al studied tolerance to food preservatives of various yeasts and moulds isolated from dairy products at pH 5 and MICs of sodium benzoate for such yeasts as *Candida parapsilosis, Meyerozyma (Pichia) guillermondii, Rhodotorula mucilaginosa* was 1-2 g/l [21] while for the most isolated by us yeasts MIC of sodium benzoate at pH 5.5 comprised 20 g/l or higher. At the same time spoilage yeasts belonging to *Zygosaccharomyces* known for their remarkable resistance to food preservatives exhibited a much higher tolerance to weak organic acids e.g. at pH 4 MIC of sorbic acid was 0.3-1 g/l, acetic acid – 6.4-33.6 g/l, however tolerance to benzoic acid was compared to that of the studied isolates (MIC 0.4 g/l or higher) [10]. MIC of sodium benzoate for *Pichia anomala* and *Candida albicans* at pH 5.2 was

0.1-1 g/l [22]. Therefore we may conclude that yeasts isolated from pickled cucumbers exhibited high tolerance to benzoic acid at pH 3.5 and 5.5.

Propionate is known for its antimold activity at the same time being much less effective against yeasts and bacteria [13]. Garnier et al. determined MIC of calcium propionate higher than 3 g/l for spoilage yeasts [21] while MIC of 0.5-5 g/l of propionic acid was observed for *P. anomala* and *C. albicans* at pH 3.6 [22], some registered the lack of inhibitory effect of propionate against yeasts altogether [23]. Interestingly Moon observed growth inhibition of several yeast strains by comparatively low concentrations of propionate, e.g. less than 10 g/l at pH 4.5 [24]. Thus, yeasts isolated from pickled cucumbers exhibited a comparatively low tolerance to sorbic and acetic acids while being resistant to benzoate and propionate action.

The major spoilage of fermented vegetables caused by yeasts is because of gas formation (bloating) or the changes in flavor characteristics due to hydrolytic activities of microorganisms [7]. Usually pickled cucumbers can contain up to 3.7% carbohydrates, 0.39% lipids, 0.5% proteins [25]. The majority of the isolated yeasts were lipolytic and could ferment glucose. So, it could be concluded that yeasts isolated from nine samples of pickled cucumbers and brine could potentially cause the spoilage by gas formation or lipid hydrolysis but mostly lack hydrolytic activities that could affect the final quality of the product.

Conclusion. Microbiota of fermented vegetables could play a positive role in the product development producing desirable flavours and substances and inhibiting the growth of the unwanted microorganisms. However in some cases, especially in acidic and high salt or sugar foods where bacterial reproduction is inhibited yeast activity can result in undesirable changes in the food quality. The obtained results conclude that yeasts isolated from pickled cucumbers are tolerant to salt and osmotic stress while are comparatively sensitive to such food preservatives as sorbic and acetic acids, especially at low pH. The fermenting activity of the isolated yeasts could result in gas formation thus leading to the spoilage of the product.

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СТІЙКІСТЬ ДО СТРЕСОВИХ ФАКТОРІВ ДРІЖДЖІВ, ІЗОЛЬОВАНИХ З СОЛОНИХ ОГІРКІВ

Резюме

Метою цієї роботи було дослідити склад дріжджової мікробіоти солоних огірків, стійкість дріжджів до різних стресових факторів (сольового та осмотичного стресу, харчових консервантів) та їх гідролітичні властивості, що можуть призвести до псування продукту. **Методи.** Культури дріжджів з солоних огірків були ізольовані методом серійних розведень та ідентифіковані відповідно до фенотипових ознак. Було визначено стійкість ізольованих дріжджів до стресових факторів та їх гідролітичні властивості. **Результати.** 23 штами дріжджів було ізольовано з 9 зразків солоних

огірків та розсолу. Кількість дріжджів в зразках варіювала від нуля до $9.3x10^5$ КУО/мл. Більшість ізольованих штамів належали до видів *Debaryomyces hansenii* (26,1%), *Wickerhamomyces anomalus* (30,4%) та до роду *Pichia* (26,1%). Виділені дріжджі демонстрували різний рівень стійкості до харчових консервантів – слабких органічних кислот: були доволі чутливі до дії сорбінової та оцтової кислот, але проявляли високу стійкість до пропіонату та бензоату. Сорбінова кислота мала найбільш пригнічуючу дію серед досліджених консервантів. Більшість ізольованих дріжджів були гало- та осмотолерантними, ряд з них проявляли високу стійкість до хлориду натрію (МІК вище 15% NaCl). МІК хлориду натрію для ряду ізолятів була в 1,5-2 рази вище на агаризованому середовищі, ніж в рідкому. 22 з 23 ізолятів гідролізували трибутирин, жоден не виявляв целюлолітичних властивостей, 13% штамів були протеолітичними, 56,5% були здатні зброджувати глюкозу. **Висновки.** В роботі досліджений склад дріжджової мікробіоти солоних огірків**,** представлена стійкість дріжджів до сольового та осмотичного стресів та чутливість до таких харчових консервантів, як сорбінова та оцтова кислоти. Ізольовані дріжджі потенційно можуть викликати псування продукту через газоутворення або гідроліз ліпідів, але, переважно, не мають гідролітичних властивостей, що можуть вплинути на якість продукту

Ключові слова: солоні огірки, дріжджі, стійкість до стресових факторів, гідролітичні властивості.

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УСТОЙЧИВОСТЬ К СТРЕССОВЫМ ФАКТОРАМ ДРОЖЖЕЙ, ВЫДЕЛЕННЫХ ИЗ СОЛЕНЫХ ОГУРЦОВ

Резюме

Целью данной работы было исследовать состав дрожжевой микробиоты соленых огурцов, стойкость дрожжей к различным стрессовым факторам (солевому и осмотическому стрессу, пищевым консервантам) и гидролитические свойства дрожжей, которые могут привести к порче продукта. **Методы.** Штаммы дрожжей были выделены из соленых огурцов методом серийных разведений и идентифицированы согласно фенотипическим признакам. Были определены устойчивость выделенных дрожжей к стрессовым факторам и их гидролитические свойства. **Результаты.** 23 штамма дрожжей было выделено из 9 образцов соленых огурцов и рассола. Количество дрожжей в образцах варьировало от нуля до $9.3x10^5$ КОЕ/мл. Большинство выделенных штаммов было отнесено к видам *Debaryomyces hansenii* (26,1%), *Wickerhamomyces anomalus* (30,4%) и роду *Pichia* (26,1%). Выделенные дрожжи проявляли разную степень устойчивости к пищевым консервантам – слабым органическим кислотам: были чувствительны к сорбиновой и уксусной кислоте, но демонстрировали высокую устойчивость к пропионату и бензоату. Сорбиновая кислота проявляла наибоее ингибирующее действие среди исследованных консервантов. Большинство изолятов были гало- и осмотолерантными, ряд из них демонстрировали высокую устойчивость к хлориду натрия (МИК выше 15% NaCl). МИК NaCl для некоторых выделенных дрожжей были в 2-3 раза выше на агаризованной среде, чем в бульоне. 22 из 23 штаммов гидролизовали трибутирин,

13% штаммов были протеолитическими, 56,5% могли сбраживать глюкозу, целлюлазной активности не обнаружено ни у одного из изолятов. **Выводы.** В работе изучен состав дрожжевой микробиоты соленых огурцов, представлены данные по устойчивости дрожжей к осмотическому и солевому стрессу и чувствительности к таким пищевым консервантам, как сорбиновая и уксусная кислоты. Выделенные дрожжи могут, вероятно, приводить к порче продукта путем газообразования или гидролиза липидов, но, в основном, не обладают гидролитическими свойствами, которые могут повлиять на качество продукта

Ключевые слова: соленые огурцы, дрожжи, устойчивость к стрессовым факторам, гидролитические свойства.

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