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Comparison Phytotsenotichesky Characteristic of Populations and *Bupleurum longifolium* L. subsp. *aureum* (Fisch. Ex Hoffm.) Soo. at the ridges thoroughwax Ivanovsky and Ubinscy

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Abstract

This article presents the results of studies of populations Bupleurum longifolium L. subsp. aureum (Fisch. ex Hoffm.) Soo ridges on the Ivanovsky and Ubinscy. Presents data on stocks of biological and ecological characteristics of *Bupleurum longifolium* L. subsp. *aureum* (Fisch. Ex Hoffm.) Soo. Based on the analysis of the results we found that the most promising is cenopopulation *bupleurum-dactylis* phytocoenosis, yield of 950 kg/ha. The remaining coenopopulations of interest to industrial pieces of medicinal raw materials.

Keywords: Ivanovsky Ridge, Ridge Ubinscy, *Bupleurum longifolium* L. subsp. *aureum* (Fisch. ex Hoffm.) Soo, Phytotsenotic characteristic, yields, phytocenoses, population

Введение

Казахстанский Алтай – по видовому составу и запасам лекарственных растений является самым богатым по всему Казахстану и может служить сырьевой базой для нужд фармацевтической промышленности всей страны. Правильное построение заготовок лекарственных растений может послужить фундаментом для разработки научно-обоснованного алгоритма рационального использования растительных богатств, как на региональном, так и на общенациональном уровнях.

Bupleurum longifolium L. subsp. *aureum* (Fisch. ex Hoffm.) Soo – Многолетник, содержит флавоноиды, углеводы, алифатические спирты, витамины; терпеноиды, лиганы, хромоны, фталиды, глицины, фенольные и жирные кислоты. Используется как капилляроукрепляющее, слабительное, ранозаживляющее, детоксикационное, противоамебное [1, 2], секреторное [3]. В народной медицине отвар травы пьют как слабительное, при лихорадке, при нервных болезнях, листья прикладывают к резаным ранам [4].

Распространён Европ. часть СССР, Зап. и Вост. Сибирь, Зап. Китай, Монголия. В Казахстане встречается в 11а. Карк., 22. Алтай, 23. Тарб., 24. Джунг. Алат., 25. Заил. Кунг. Алат., 25 а. Кетм. Терск. Алат., 27. Кирг. Алат. [5].

Материалы и методы

Исследования проводились маршрутно – рекогносцировочным методом [6]. При составлении фитоценотической характеристики ценопопуляции использовался классический метод с визуальной оценкой количества особей по шкале Друде [7]. Статистическую обработку материала проводили согласно рекомендаций Г.Н. Зайцева [8].

Обсуждения результаты

Володушка золотистая распространена по всей территории Юго-Западного Алтая, за исключением степных растительных сообществ. На Юго-Западном Алтае, поднимается в горы до 1900 м над у. м. по склонам разной крутизны, преимущественно юго-восточных, северо-восточных и северо-западных экспозиций [9].

В Казахстанской части Юго – Западного Алтая были обследованы популяции *Bupleurum longifolium*L. subsp. *aureum* (Fisch. exHoffm.) Soo на хр. Ивановский и Убинский.

Убинская популяция находится на юго – восточных отрогах хр. Убинский, между сёлами Быструха и Зимовье, в долине реки Топкуша на юго – западном микросклоне. Склон пологий уступчатый. В данной популяции выделено 2 фитоценоза. Общая площадь популяции составляет 70 га.

Ценопопуляция ежово – володушкового (*Bupleurum longifolium* L. subsp. *aureum* (Fisch. ex Hoffm.) Soo, *Dactylis glomerata* L.) фитоценоза входит в состав злаково – разнотравных лугов. Рельеф выровненный. Почвенный слой хорошо развит 60-70 см толщины. Напочвенный покров хорошо выражен 4-6 см, вес опада 120 гр/м². Растительный покров хорошо развит. Древесный и кустарниковый ярус не сформирован. Общее проективное покрытие 100%. Травостой четко трехъярусный.

Первый ярус выстой 140-200 см, сформирован с доминированием *Dactylis glomerata* L – cop_2, на его долю в покрытии приходится 25%. Из сопутствующих видов в первом ярусе встречаются: *Crepis sibirica* L. – sol, *Serratula coronata* L. – sp, *Alfrediacernua* (L.) Casso – s, *Rumex confertus* Willd. – s, *Heracleum sibiricum* L. – sol, *Sanguisorba officinalis* L. – sol. Сомкнутость первого яруса составляет 02.

Второй ярус, высотой 80 – 150 см, образован доминирванием *Bupleurum longifolium* L. subsp. *aureum* (Fisch. ex Hoffm.) Soo – cop_3, на его долю в покрытии приходится 30% от общего. Из второстепеных видов во втором ярусе встречаются *Bunias orientalis* L. – sp, *Clematis integrifolia* L. – sol, *Lavatera thuringiaca* L. – sol, *Elytrigia repens* (L.) Nevski – sp, *Poa pratensis* L. – sol, *Bromopsis inermis* (Leys.) Holub – sp, *Phleum pratense* L. – sol, *Tanacetum vulgare* L. – sol. Сомкнутость второго яруса составляет 05.

Третий ярус высотой 20-65 см, сформирован *Galium verum* L. – sp, *Euphorbia pilosa* L. – sol, *Origanum vulgare* L – sol, *Potentilla chrysantha* Trev. – sp, *Hypericum perforatum* L. – sol, *Trifolium pratense* L. – sol сомкнутость третьего яруса составляет 03.

Растения *Bupleurum longifolium* L. subsp. *aureum* (Fisch. ex Hoffm.) Soo по площади популяции размещены обильно – рассеяно. Количество генеративных особей на 1 м² – 2,13±0,27 шт., V – 30%. Количество побегов на одну генеративную особь – 4,40±0,32 шт., V – 42%. Высота генеративных побегов 133,13±6,11 см, V – 17%. Вес зеленой массы генеративных побегов составил 0,25 кг/м². Коэффициент усушки составил 62%. Урожайность воздушно сухого сырья составил 950 кг/га. Эксплуатационный запас воздушно сухого сырья наземной массы генеративных побегов составил 66,5 т. Ежегодно возможный объем заготовки сырья составил 16,62 т.

Ценопопуляция разнотравно-володушково-кустарникового (*Rosa acicularis* Lindl., *Lonicera tatarica* L., *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó, разнотравье) фитоценоза встречаются по юго-восточным предгорьям хр. Убинский, занимая значительные межгорные понижения, долины рек и склоны низкогорий. В кустарниковом ярусе часто встречаются *Rosa acicularis* Lindl., *Lonicera tatarica* L., *Viburnum opulus* L., *Padus avium* Mill., *Sambucus racemosa* L., *Salix caprea* L. Сомкнутость 04-1. Володушка растет по

опушкам кустарника, чаще и обильнее на обширных полянах среди лесного высокотравья. Общее проективное покрытие 100%. Общая площадь популяции 50 га.

Травяной покров хорошо развит, богат в видовом отношении. По данным 5 описаний насчитывает боле 60 видов, зачастую доминирует *Calamagrostis epigeios* (L.) Roth., в некоторых местах на его долю в покрытии приходится до 55%, субдоминантами чаще всего выступают *Serratula coronata* L. – cop_2-sp, *Crepis sibirica* L. – sp, *Cirsium incanum* (S.G. Gmel.) Fisch. – sp. Они формируют первый ярус в травостое, 150-190 см выс. Здесь также можно встретить *Alfredia cernua* (L.) Cass. – s, *Brachypodium sylvaticum* (Huds.) Beauv. – sp- cop_2, *Dactylis glomerata* L. – sp, их покрытие не более 5-12%. Ярусность нечётко выражена.

Первый ярус образует лесное высокотравье 190-240 с выс. с незначительным перемешиванием ксеромезофитов: *Serratula coronata* L. – sp, *Cirsium incanum* (S.G. Gmel.) Fisch. - cop_2, *Crepis sibirica* L. – sp, *Artemisia vulgaris* L.- s, *Alfredia cernua* (L.) Cass. – s, *Elymus caninus* (L.) L. – sol и др. Зачастую кустарники и растения и растения первого яруса увиты *Calystegia sepium* (L.) R. Br., образуя трудно проходимые заросли.

Второй ярус очень богат во флористическом разнообразии, 100-11 см выс., где возможно в роли доминантов выступают *Brachypodium sylvaticum* (Huds.) Beauv. – cop_1-sp, *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó, - sp-cop, *Calamagrostis epigeios* (L.) Roth – sp-cop. В травостое яруса злаки базово представлены: *Elytrigia repens* (L.) Nevski – sp-sol, *Elymus mutabilis* (Drob.) Tzel. – sol, *Elymus caninus* (L.) L. – sp-sol, *Poa sibirica* Roshev. – sol, *P. angustifolia* L. – s, *Festuca pratensis* Huds. – s, *Phleum pratense* L. – s, *Agrostis gigantea* Roth – s. Они представляют интерес как пастбищные и сенокосные угодья.

Кусты *Bupleurum longifolium* subsp. *aureum* мощно развиты, 110-120 см выс., побеги разветвлённые, хорошо облиственные. Листья длинные и широкие. Количество генеративных особей на 1 м² – 2,4±0,21 шт., V – 35%. Количество побегов на одну генеративную особь – 1,5±0,39 шт., V – 20%. Высота генеративных побегов 110±2,23 см., V – 30%. Урожайность зеленой массы составил 0,097 кг/м². Коэффициент усушки составил 62%. Вес воздушно сухого сырья составил 370 кг/га. Эксплуатационный запас воздушно сухого сырья наземной массы генеративных побегов составил 18,5 т. Ежегодно возможный объем заготовки сырья составил 4,62 т.

Ивановская популяция *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) размещена на хр. Ивановский. Здесь выделено 3 фитоценоза: в долине р. Быструха, и в ур. Серый Луг.

Ценопопуляция разнотравно-злаково-володушкового (*Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm. Soó, *Brachypodium pinnatum* (L.) Beauv., *Dactylis glomerata* L., *Milium effusum* L., разнотравье) фитоценоза находится в долине р. Быструха, в районе кордона Босяково, где *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó, входят в разреженный елово-кедровый лес с сомкнутостью около 02. в высотном пределе 1228 м над ур. м. (50°20'33" с.ш., 83°44'04" в.д.). Общая площадь занимаемая *Bupleurum longifolium* subsp. *aureum* – 40 га.

Травяной покров хорошо развит, с четко выраженной четырехъярусной структурой. Общее проективное покрытие до 100%. Площадь популяции 35 га.

Фитоценоз полидоминантен, из злаков доминируют *Calamagrostis obtusata* Trin. - soc, *Brachypodium pinnatum* (L.) Beauv. – cop, в роли судоминантов выступают *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó – cop_2, *Ranunculus grandiflorus* L. - cop_2, *Trollius altaicus* C.A. Mey. – cop, из второстепенных отмечено около 30 видов (*Solidago virgaurea* L., *Polemonium caeruleum* L., *Helictotrichon pubescens (*Huds.) Pilg., *Pedicularis proboscidea* Stev. и др.). В экологическом отношении травянистые растения сообщества в основном сложены горно-лесными и луговыми мезофитами.

Первый ярус, 120-150 см выс., сомкнутость до 04, представленным высокотравьем: *Cirsium helenioides* (L.) Hill – sol, *Milium effusum* L. - sol, *Dactylis glomerata* L. - sp, *Delphinium elatum* L. – sp и др.

Второй ярус, 90-110 см выс., составлен мезофильными луговыми видами: *Polemonium caeruleum* L. - sol, *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó – cop_2, *Geranium pseudosibiricum* J. Mayer – sp и др.

Третий ярус 45-70 см выс. представлен *Ptarmica ledebourii* (Heimer) Klok. et Krytzka - sp, *Solidago virgaurea* L. - sol, *Rumex acetosella* L. – sol, *Poa sibirica* Roshev. – sol, *Viola disjuncta*

W. Beck. – sp и др., сложен луговыми мезофильными видами. Четвертый ярус 25-35 см выс. беден в видовом отношении, обычны и постоянны *Euphrasia altaica* Serg. – sp, *Dianthus superbus* L. – sol, *Carex macroura* Meinch. – cop_2, *Trifolium pratense* L. – sol, *Amoria hybrida* (L.) C. Presl – sp и др.

В покрытии на долю *Bupleurum longifolium* subsp. *aureum* приходится 3-5%, Растения хорошо развиты. Количество генеративных особей на 1 м² – 3,8±0,23 шт., V – 36%. Количество побегов на одну генеративную особь – 1,7±0,40 шт., V – 46%. Высота генеративных побегов 55,36±1,11 см., V – 25%. Урожайность зеленой массы составил 0,086 кг/м². Коэффициент усушки составил 62%. Вес воздушно-сухого сырья генеративных особей составил 320 кг/га. Эксплуатационный запас воздушно-сухого сырья наземной массы генеративных побегов составил 11,20 т. Ежегодно возможный объем заготовки сырья составил 2,8 т.

Ценопопуляция ежово-володушково-гераниевого (*Geranium pratense* L., *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó, *Dactylis glomerata* L.) фитоценоза размещено на северо-восточном склоне хр. Ивановский, ур. Серый Луг, на высоте 1020 м над ур. м. (50°22'21" с.ш., 83°50'56" в.д.). Общее проективное покрытие 100%. Общая площадь около 70 га.

Кустарниковый ярус отсутствует. Травяной покров развит с трехъярусной структурой, образован мезофильными луговыми видами. В травяном покрове доминируют *Dactylis glomerata* L. – soc, *Geranium pratense* L. – cop, *Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó – cop_1. В покрытии на их долю приходится до 45%, в том числе *Bupleurum longifolium* subsp. *aureum* до 9%. Здесь в качестве субдоминанта выступает *Anthriscus sylvestris* (L.) Hoffm.- cop_1-sp.

Первый ярус 170-190 см выс. образован лесным высокотравьем, помимо доминантов, присутствуют *Crepis sibirica* L. – sol, *Tephroseris integrifolia* (L.) Holub – sol, *Cirsium incanum* (S.G. Gmel.) Fisch. – sol, *Artemisia vulgaris* L.- s, *Thalictrum simplex* L. - sol, *Aconitum septentrionale* Koelle – sol и др. Покрытие до 7%.

Второй ярус, 80-100 см выс., составлен луговыми видами, в видовом отношении очень разнообразен. Наиболее часто встречаются *Origanum vulgare* L. – s, *Filipendula ulmaria* (L.) Maxim. – sol, *Rumex acetosella* L. – s, *Galium boreale* L. – sol, *Hypericum perforatum* L. – sol и др. Сомкнутость яруса до 05, покрытие до 45%. Здесь на долю *Bupleurum longifolium* subsp. *aureum* в покрытии приходится до 9%.

Третий ярус 35-40 см выс., сложен такими видами как *Lathyrus pratensis* L. – sol, *Pulmonaria mollis* Wulf. ex Hornem. – s, *Fragaria viridis* (Duch.) Mill. - cop_2, *Viola hirta* L. – sol и др.

В покрытии на долю *Bupleurum longifolium* subsp. *aureum* приходится 3-5%, Растения хорошо развиты. Количество генеративных особей на 1 м² – 2,6±0,24 шт., V – 36%. Количество побегов на одну генеративную особь – 2,4±0,30 шт., V – 41%. Высота генеративных побегов 74,36±1,3 см., V – 25%. Урожайность зеленой массы составил 0,10 кг/м². Коэффициент усушки составил 62%. Вес воздушно сухого сырья генеративных особей составил 380 т/га. Эксплуатационный запас воздушно сухого сырья наземной массы генеративных побегов составил 26,6 т. Ежегодно возможный объем заготовки сырья составил 6,65 т.

Ценопопуляция кипрейно-вейниково-володушковое (*Bupleurum longifolium* subsp. *aureum* (Fisch. ex Hoffm.) Soó, *Calamagrostis purpurascens* R. Br., *Chamaenerion angustifolium* (L.) Scop.) фитоценоза размещено на юго-восточном и северо-восточном склонах г. Листвяга в высотном пределе 1176 м над ур. м. Общая площадь сообщества с участием *Bupleurum longifolium* subsp. *aureum* до 110 га. Общее проективное покрытие 100%. В покрытии на долю *Bupleurum longifolium* subsp. *aureum* приходится около 8%. Кустарниковый ярус развит слабо, включает один вид - *Spiraea media* Franz Schmidt с сомкнутостью 02-03.

Травянистый покров довольно разнообразен в видовом отношении, насчитывает более чем 50 видов с четкой четырехъярусной структурой. Сложен видами лесного высокотравья и луговыми мезофильными видами. В нем доминируют: *Chamaenerion angustifolium* (L.) Scop. – soc, *Calamagrostis purpurascens* R. Br. – cop, как субдоминанты обычно выступают *Brachypodium pinnatum* (L.) Beauv. – cop_2, *Crepis sibirica* L. - cop_2, *Bupleurum longifolium*

subsp. *aureum* (Fisch. ex Hoffm.) Soó - cop_2, из сопутствующих следует отметить *Thalictrum simplex* L. - sol, *Geranium pseudosibiricum* J. Mayer – sp, *Lathyrus gmelinii* Frirsch – sol, *Poa sibirica* Roshev. – sol, *Pleurospermum uralense* Hoffm. – sol, *Anthriscus sylvestris* (L.) Hoffm. – sol, *Paeonia anomala* L. – sol, *Artemisia vulgaris* L. – sol, *Alfredia cernua* (L.) Cass. – s и др.

Первый ярус составлен исключительно лесным высокотравьем 200-250 см выс.: *Dactylis glomerata* L. – cop, *Alfredia cernua* (L.) Cass. – s, *Hesperis sibirica* L. – sol, *Aconitum septentrionale* Koelle – sol и др.

Второй ярус 110-120 см выс.: *Chamaenerion angustifolium* (L.) Scop. – soc, *Cacalia hastata* L. – s, *Elymus caninus* (L.) L. – sol, *Calamagrostis langsdorffii* (Link) Trin. – sp и др.

Третий ярус 90-100 см выс., образуют *Filipendula ulmaria* (L.) Maxim. –sol, *Crepis sibirica* L. – sol, *Lilium martagon* L. – s, *Calamagrostis obtusata* Trin. – sol и др.

Четвертый ярус 80-90 см выс. составлен *Origanum vulgare* L. – sol, *Hypericum perforatum* L. – sol, *Lathyrus gmelinii* Frirsch – sol, *Poa angustifolia* L. – sol и др.

Особи *Bupleurum longifolium* subsp. *aureum* в отличном жизненном состоянии, генеративные побеги крепкие, умеренно разветвлённые в верхней части, у основания с розетками из зеленых листьев летней генерации. Количество генеративных особей на 1 м² – 4,9±1,27 шт., V – 31%. Количество побегов на одну генеративную особь – 3,4±0,45 шт., V – 35%. Высота генеративных побегов 137,13±6,11 см, V – 17%. Урожайность зеленой массы составила 0,19 кг/м². Коэффициент усушки составил 62%. Вес воздушно сухого сырья составил 720 т/га. Эксплуатационный запас воздушно сухого сырья составил 79,42 т. Ежегодно возможный объем заготовки сырья составил 19,85 т.

Таблица 1.

Запасы сырья володушки золотистой выявленных на хр. Ивановский и Убинский

Вид растений, заготовляемая часть	Популяции	Местонахождение и координаты	Площадь, га		Урожайность воздушно-сухого сырья, кг/га	Эксплуатационный запас воздушно-сухого сырья, т	Объём возможных ежегодных заготовок, т
			общая	Занимаемая видом			
Bupleurum longifolium L. subsp. *aureum* (Fisch. exHoffm.) Soo, трава	Убинская популяция	Ценопопуляция ежово – володушкового фитоценоза	85	70	950	66,5	16,62
		Ценопопуляция разнотравно-володушково-кустарникового фитоценоза	66	50	370	18,5	4,62
	Ивановская популяция	Ценопопуляция Разнотравно-злаково-володушкового фитоценоза	48	35	320	11,20	2,8
		Ценопопуляц	80	70	380	26,6	6,65

		ия ежово-володушково-гераниевого фитоценоза					
		Ценопопуляция кипрейно-вейниково-володушкового фитоценоза	150	110	720	79,2	19,85

Выводы

Ивановская популяция занимает обширные территории и представляет интерес для промышленных заготовок лекарственного сырья. Все ценопопуляции полночленные, нормального типа, с преобладанием генеративных особей.

Ценопопуляция разнотравно-злаково-володушкового фитоценоза урожайностью воздушно-сухого сырья 320 кг/га и ценопопуляция ежово-володушково-гераниевого фитоценоза урожайностью 380 кг/га характеризуются средней урожайностью и представляют интерес для промышленных заготовок для обеспечения фармацевтической промышленности лекарственным сырьем.

Ценопопуляция кипрейно-вейниково-володушкового фитоценоза характеризуется высокой урожайностью воздушно сухого сырья до 720 т/га. Эксплуатационный запас на площади 110 га составляет 79,42 т. Данная ценопопуляция перспективна для отбора форм, промышленной заготовки лекарственного сырья и сбора семян для интродукционных испытаний в культуре.

Убинская популяция представляет интерес для промышленной заготовки лекарственного сырья. Оба ценопопуляции расположены на обширных площадях с хорошим семенным и вегетативным возобновлением условия обитания вида оптимальные для удержания и захвата территории.

Ценопопуляция разнотравно-володушково-кустарникового фитоценоза характеризуется средней урожайностью воздушно сухого сырья 370 кг/га эксплуатационный запас на площади 50 га воздушно сухого сырья составил 18,5 т.

Ценопопуляция ежово-володушкового фитоценоза характеризуется самой высокой урожайностью из всех описанных ценопопуляций и составляет 950 кг/га. Эксплуатационный запас на общей площади 70 га, составил 66,5 т воздушно сухого сырья. Растения володушки хорошо развиты и достигают до 2-х метров высоты. Здесь целесообразно проводить селекционно-генетический отбор, сбор семян, производить заготовку лекарственного сырья в промышленных масштабах и интродуцировать в культуру.

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Примечания:

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Сравнительная фитоценотическая характеристика и запасы популяций володушки золотистой на хребатах Ивановский и Убинский

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Аннотация. В данной статье представлены результаты исследований популяций володушки золотистой на хребтах Ивановский и Убинский. Представлены сведения по запасам и эколого-биологической характеристике *Bupleurum longifolium* L. subsp. *aureum* (Fisch. ex Hoffm.) Soo. На основе анализа полученных результатов выяснили что наиболее перспективным является ценопопуляция ежово-володушкового фитоценоза урожайностью 950 кг/га. Остальные ценопопуляции представляют интерес для промышленных заготовок лекарственного сырья.

Ключевые слова: хребет Ивановский, хребет Убинский, володушка золотистая, запасы, фитоценотическая характеристика, урожайность, фитоценоз, популяция.

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Metabolism and Physiology of Halophiles

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Abstract

Halophiles (*lat.* “salt-loving”) is the taxonomic group of extreme aerobic obligate Gram-negative microorganisms that live in conditions of high salinity – in the seas, salt lakes, saline soils etc. These microorganisms are known to reddish patina on products, preserved with using large quantities of salt (NaCl). Halophiles were isolated for the first time at the beginning of the XX century from the marine flora estuary mud, but their systematic study was started only at the end of the second decade of the XX century. The internal environment of the human body is not suitable for existence of halobacteria, since none of them are known to have pathogenic forms. Halobacteria have great practical potential for using in molecular bioelectronics and bio-nanotechnology due to their unique ability to convert the energy of sunlight into electrochemical energy of protons H^+ due to the presence in their cells a special photo transforming retinal containing integral protein – bacteriorhodopsin, the mechanism of action of which has been currently studied in detail. The paper describes the characteristics of the metabolism and physiology of halophilic bacteria, as well as a method of biosynthesis and preparation of bacteriorhodopsin from purple membranes of cells of the extreme photoorganotrophic halobacterium *Halobacterium halobium*.

Keywords: halobacteria, bacteriorhodopsin, purple membranes, biosynthesis.

Introduction

The halophiles, related according to the taxonomic classification to the ancient archaea *Archebacteria* genera are single-celled microorganisms with no marked nucleus and expressed membrane organelles, occupy a special place among other microorganisms [1]. These are the only microorganisms that can exist in environments with a high salt content – on salt crystals in the coastal strip, on the salt marsh, in the salt brine etc. (Figure 1). In the Dead Sea (Israel), for example, the salt concentration reaches 26–27 %, in some years, rising to 30 %, whereas at 35 %

NaCl precipitates from the salt solution into the sediment [2]. The biochemical apparatus of the cell, enzymes and ribosomes of halophilic bacteria due to the peculiar cell osmoregulation system and the structure of the cell wall, consisting of proteins and amino sugars, is not only insensitive to such high salt concentrations, but on the contrary needs NaCl and functions effectively only in saturated solutions with 15–20 % of NaCl [3]. For maintainance of cell stability of halophiles primarily NaCl is required. Wherein, Na^+ cations interact with the negatively charged cell wall of halobacteria imparting the necessary stiffness. Inside the cell, the concentration of NaCl is low. Potassium cations (K^+), in conjunction with chlorine anions (Cl^-) are needed to maintain the ionic equilibrium inside and outside of the cells, to stabilize enzymes and other cellular membrane structures of halobacteria. While removing the halobacteria from salinity environment, their cell wall is dissolved and the cytoplasmic membrane disintegrates into smaller fragments.

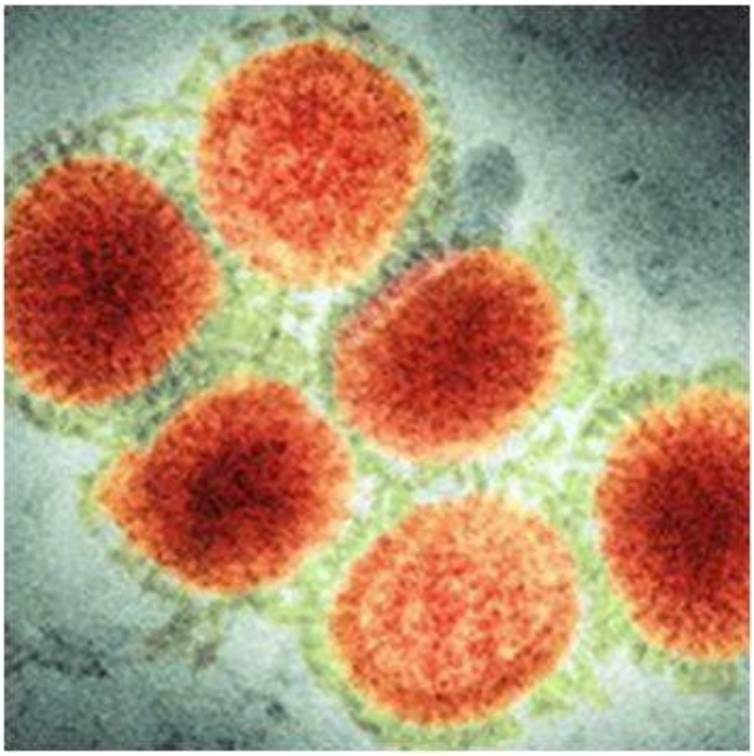

Figure 1: Typical red colored colonies of extreme aerobic photoorganotrophic halobacteria in the salt marsh of the Dead Sea (Israel)

The family of halobacteria (lat. *Halobacteriaceae)* includes about 20 genera, including *Halobacterium*, *Halococcus*, *Haloarcula*, *Natrococcus*, *Natrobacterium* and others. Extremely halophilic forms of halobacteria are referred to the genera of *Halobacterium* and *Halococcus* (15–32 % NaCl), whereas less halophilic forms – to the genera of *Haloarcula*, *Natronobacterium* and *Natronococcus* (5–20 % NaCl). Members of the halobacteria family are presented by coccoid or rod-shaped forms, as well as by mobile or stationary forms of microorganisms, most of which are painted Gram-positively. Some halobacteria have gas vacuoles for keeping the buoyancy control. The halobacteria usually do hot form spores. According to the nutrition type halobacteria are predominantly aerobic microorganisms, e.g. they require oxygen for the growth, but they also can tolerate the very low oxygen content in growth media (hemoorganotrophs) [4]. Furthermore, halobacteria can use a wide range of organic compounds for the growth as amino acids, carbohydrates and organic acids. They possess a few changes in biochemical pathways of assimilation of sugars via complete citric acid cycle. With a lack of oxygen halobacteria are able to evolve the photoorganotrophic pathway by synthesizing the photo-transforming membrane protein bacteriorhodopsin (BR), which allows use solar energy for the growth.

The halobacteria are oftnen refered as organisms “living on the edge of physiological capabilities”. They have virtually no competitors that could exist under the same conditions, and therefore halophiles freely evolved throughout the evolution of life on Earth. The interesting fact is

that some modern bacteria under the growth in extreme conditions acquire the features of halophiles and other ancient archaebacteria by losing the more rigid upper layer in the cell membrane. It is assumed that archaebacteria had lost this layer under the influence of high salt concentrations. The changes in the structure of their cell membranes are caused by the need to ensure the necessary protection for the cells from aggressive external environment. The process of formation of adaptive protective systems in halobacteria demanded the synthesis of specific substances and cellular systems, which do never almost occur in other microorganisms.

Halobacteria have lived on Earth since the Archean age – 3,0–3,5 billion years, almost without changing. Fossilized remains of these organisms are found in ancient rocks aged 2,7 billion years and in Precambrian formations. The oldest of these bacterial remains were found in Isuan green-stone belt in the west of Greenland, where there have been the oldest on Earth, sedimentary rocks formed 3,8 billion years ago. It is possible that the archaebacteria were the first forms of life on Earth in the first period of its evolution [5]. One of the main arguments in favor of this hypothesis is the fact that the representatives of numerous species of archaebacteria use as the sole carbon source for the biosynthesis of components of the cell biomass a mixture of amino acids, i.e., they are heterotrophs.

It is argued that the archaea, bacteria and eukaryotes are submitted by three separate taxonomic lines which were early separated from the ancestral group of microorganisms [6]. Perhaps it was occurred even before the evolution of the cell, wherein the lack of the cell membrane made it impossible to unlimited transfer and exchange of genes that is why the ancestors of the three domains are differed in lockable sets of genes. It is very likely that the common ancestor of archaea and bacteria was a thermophile, it gives a reason to us to assume that low temperatures were “extreme environment” for archaea, and organisms adapted to them, appeared a little later. Indicating the relationship between these three domains is a crucial for understanding the origin of life. The majority of metabolic pathways, which involves most of the genes are similar in bacteria and archaea, whereas the genes responsible for the expression of other genes are very similar in archaea and eukaryotes. According to the cellular structure the archaea are closest to Gram-positive bacteria: their cells are covered with the plasma membrane, the additional outer membrane, characteristic for Gram-negative bacteria is absent; the cell walls are of varying composition and as a rule are usually thick.

Table 1 below shows some of the main features of the archaea, characteristic or inherent to other domains, to demonstrate their relationship.

Table 1: The general features characteristics of bacteria, archaea and eukaryotes

Typical for archaea and bacteria	**Typical for archaea and eukaryotes**	**Typical only for archaea**
The absence of the nucleus and membrane organelles	The absence of peptidoglycan (murein)	The structure of the cell wall (cell walls of some archaea contain psevdomurein)
Ring chromosome	DNA is associated with histone protein	The cell membrane lipids contain ether linkage bond
Genes are combined into operons	Translation of protein begins with the methionine residue	The structure of flagellin
No introns and RNA processing	Similar RNA polymerase and other components of the transcription	The structure of the ribosome (some signs closer to the bacteria, while some others - with eukaryotes)
Polycistronic mRNA	Similar mechanisms of replication and repair of DNA	The structure and metabolism of tRNA
Cell size (more than 100 times less than in eukaryotes)	A similar ATPase (type V)	No fatty acid synthase

The genetic apparatus of the archaea is represented by a single circular chromosome with the size of 5751492 bp found in *Methanosarcina acetivorans*, having the largest known gene among the archaea [7]. On the contrary in *Nanoarchaeum equitans* 1/10 the size of the genome composes 490 885 bp, having the smallest known genome among the archaea; it contains only 537 genes, encoding different proteins [8]. The archaea also contains smaller molecules of DNA, known as plasmids. Probably the plasmids can be transferred via contact between the cells, in a process similar to bacterial conjugation.

However, evolutionary relationship between archaea and eukaryotes remains to be unclear. Besides the similarities in the structure and functions of cells, there are similarities at the genetic level. It was found that a group of archaea – *Crenarchaeota* are closer to eukaryotes than to other types of archaea – *Euryarchaeota* [9] The most common is a hypothesis that the ancestor of eukaryotes early separated from the archaea, and eukaryotes appeared as a result of the merger of archaea and eubacteria; the later became the cytoplasm and the nucleus of the new merged cell [10]. This hypothesis explains the various genetic similarities, but has some difficulty in explaining the cell structure.

The recent information on the genetic diversity of the archaea is fragmentary, and the total number of species could not be evaluated fully. Comparative analysis of the 16S structures of rPHK of archaea allowed assuming the existence of 18–20 phylogenetic groups of the archaea [11]. Numerous of these groups are known only from a single sequence of rRNA, which suggests that the limits of the diversity of these organisms remain to be unclear. Many halobacteria have never been cultured under laboratory conditions, which makes their identification difficult.

The aim of the research was the investiging of the metabolism and physiology of extreme halophilic bacteria *Halobacterium halobium* and searching for new biotechnological applications of the synthesized by this bacterium photo-transforming integral membrane protein bacteriorhodopsin (BR).

Material and methods

Bacterial objects

As a BR producer was used a carotenoid strain of extreme photo-organotrophic halobacterium *Halobacterium halobium* ET 1001, obtained from Moscow State University (Russia). The strain was modified by selection of individual colonies on solid (2% (w/v) agarose) media with peptone and 4,3 M NaCl.

Growth conditions

BR (yield 8–10 mg from 1 g biomass) was obtained in synthetic (SM) medium (g/l): *D,L*-alanine – 0,43; *L*-arginine – 0,4; *D,L*-aspartic acid – 0,45; *L*-cysteine – 0,05; *L*-glutamic acid – 1,3; *L*-lycine – 0,06; *D,L*-histidine – 0,3; *D,L*-isoleucine – 0,44; *L*-leucine – 0,8; *L*-lysine – 0,85; *D,L*-methionine – 0,37; *D,L*-phenylalanine – 0,26; *L*-proline – 0,05; *D,L*-serine – 0,61; *D,L*-threonine – 0,5; *L*-tyrosine – 0,2; *D,L*-tryptophan – 0,5; *D,L*-valine – 1,0, AMP – 0,1; UMP – 0,1; NaCl – 250; $MgSO_4 \cdot 7H_2O$ – 20; KCl – 2; NH_4Cl – 0,5; KNO_3 – 0,1; KH_2PO_4 – 0,05; K_2HPO_4 – 0,05; Na^+-citrate – 0,5; $MnSO_4 \cdot 2H_2O$ – $3 \cdot 10^{-4}$; $CaCl_2 \cdot 6H_2O$ – 0,065; $ZnSO_4 \cdot 7H_2O$ – $4 \cdot 10^{-5}$; $FeSO_4 \cdot 7H_2O$ – $5 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$ – $5 \cdot 10^{-5}$; glycerol – 1,0; biotin – $1 \cdot 10^{-4}$; folic acid – $1{,}5 \cdot 10^{-4}$; vitamin B_{12} – $2 \cdot 10^{-5}$. The growth medium was autoclaved for 30 min at 0,5 atm, the pH value was adjusted to 6,5–6,7 with 0,5 *M* KOH. Bacterial growth was performed in 500 ml Erlenmeyer flasks (volume of the reaction mixture 100 ml) for 4–5 days at +35 ^oC on Biorad shaker ("Birad Labs", Hungary) under intense aeration and monochromatic illumination (3 lamps × 1,5 *lx*). All further manipulations for BR isolation were carried out with the use of a photomask lamp equipped with an orange light filter.

Isolation of purple membranes (PM)

Biomass (1 g) was washed with distilled water and pelleted by centrifugation on T-24 centrifuge ("Carl Zeiss", Germany) (1500 g, 20 min). The precipitate was suspended in 100 ml of

dist. H_2O and kept for 3 h at 4 °C. The reaction mixture was centrifuged (1500 g, 15 min), the pellet was resuspended in 20 ml dist. H_2O and disintegrated by infrasound sonication (22 kHz, 3 times × 5 min) in an ice bath (0 °C). The cell homogenate after washing with dist. H_2O was resuspended in 10 ml of buffer containing 125 mM NaCl, 20 mM $MgCl_2$, and 4 mM Tris-HCl (pH = 8.0), then 5 mg of RNA-ase (2–3 units of activity) was added . The mixture was incubated for 2 h at 37 °C. Then 10 ml of the same buffer was added and kept for 10–12 h at 4 °C. The aqueous fraction was separated by centrifugation (1500 g, 20 min), the PM precipitate was treated with 50 % (v/v) ethanol (5 times × 7 ml) at 4 °C followed by separation of the solvent. This procedure was repeated 6 times to give a colorless washings. The protein content in the samples was determined spectrophotometrically on DU-6 spectrophotometer ("Beckman Coulter", USA) by the ratio D_{280}/D_{568} ($\varepsilon_{280} = 1{,}1 \cdot 10^5$; $\varepsilon_{568} = 6{,}3 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$) [12]. PM regeneration is performed as described in the article [13]. Yield of PM fraction – 120 mg (80–85 %).

Isolation of BR

Fraction PM (in H_2O) (1 mg/ml) was dissolved in 1 ml of 0,5 % (w/v) sodium dodecyl sulfate (SDS-Na), and incubated for 5–7 h at 37 °C followed by centrifugation (1200 g, 15 min). The precipitate was separated, than methanol was added to the supernatant in divided portions (3 times × 100 ml) at 0 °C. The reaction mixture was kept for 14–15 h in ice bath at 4 °C and then centrifuged (1200 g, 15 min). Fractionation procedure was performed three times, reducing the concentration of 0,5 % SDS-Na to 0,2 and 0,1 %. Crystal protein (output 8–10 mg) was washed with cold ^2H_2O (2 times × 1 ml) and centrifuged (1200 g, 15 min).

Purification of BR

Protein sample (5 mg) was dissolved in 100 ml of buffer solution and placed on a column (150 × 10 mm), stationary phase – Sephadex G-200 ("Pharmasia", USA) (specific volume packed beads – 30–40 units per 1 g dry of Sephadex) equilibrated with buffer containing 0,1 % (w/v) SDS-Na and 2,5 mM ETDA. Elution proceeded by 0,09 M Tris-buffer containing 0,5 M NaCl, pH = 8,35 at a flow rate of 10 ml/$cm^2 \cdot$ h. Combined protein fraction was subjected to freeze-drying, in sealed glass ampoules (10 × 50 mm) and stored in frost camera at -10 °C.

Quantitative analysis of the protein

The procedure was performed in 12,5 % (w/v) polyacrylamide gel (PAAG) containing 0,1 % (w/v) SDS-Na. The samples were prepared for electrophoresis by standard procedures (LKB protocol, Sweden). Electrophoretic gel stained with Coomassie blue R-250 was scanned on a CDS-200 laser densitometer (Beckman, USA) for quantitative analysis of the protein.

Absorption spectrometry

Absorption spectra of pigments were recorded on the programmed DU-6 spectrophotometer ("Beckman Coulter", USA) at $\lambda = 280$ nm and $\lambda = 750$ nm.

Scanning electron microscopy

The structural studies were carried out with using scanning electrom microscopy (SEM) on JSM 35 CF (JEOL Ltd., Corea) device, equiped with X-ray microanalyzer "Tracor Northern TN", SE detector, thermomolecular pump, and tungsten electron gun (Harpin type W filament, DC heating); working pressure: 10^{-4} Pa (10^{-6} Torr); magnification: 100000, resolution: 3,0 nm, accelerating voltage: 1–30 kV; sample size: 60–130 mm.

Results and Discussion

The structure of BR

The mechanism of converting the solar energy into the chemical energy of ATP used by halobacteria is different from the classic photosynthetic mechanism realized by plants and green algae containing chlorophyll. For this purpose halobacteria use a special chromophore protein with a molecular weight of 26 kDa, designated bacteriorhodopsin (BR) by analogy with the photo-sensitive protein of mammalian visual apparatus – rhodopsin providing visual perception in animals and humans [14]. BR was firstly isolated in 1971 from the cell membrane of extreme photoorganotrophic halobacteria *Halobacterium halobium*, inhabiting saline geothermal lakes and seas, including the Dead Sea (Israel) [15]. This photo-transforming protein is represented by a chromoprotein associated by aldimine bond with the amino acid residue lysine-216. As a chromophore group the BR contains an equimolar mixture of 13-*cis*- and 13-*trans-retinal* – an analogue of vitamin A defining purple-red color of colonies of halobacteria. Along with the BR the cell membrane of halophiles contains other carotenoid pigments, the main of which bakterioruberin, causes the colorage of halobacteria from pink to red and red-orange [16]. The presence of these pigments has to the halophiles an important meaning as a means of protection against excessive solar radiation, as their habitats are characterized by high luminosity, and these pigments are able to delay radiation.

The cell membrane of halophiles also contains two sensory rhodopsins, which provide positive and negative phototaxis in cells [17]. These proteins absorb different wavelengths of light, causing a cascade of signals that eventually control the flagella of halobacteria. For example, the absorption of a photon of red light leads to the generation of a signal on receiving of which halobacteria begin to move toward the light source. By the absorption of a photon of blue light, it is occurred the opposite reaction. The maximum optical effect is achieved in both cases at wavelengths of $\lambda = 565$ and $\lambda = 370$ nm, respectively [18]. Thus, the photosensor reaction provides the optimal for the cell spatial orientation. Cells leave areas, which penetrates detrimental shortwave solar radiation and by means of flagella or gas vacuoles are concentrated in a favorable light condition area. This mechanism provides optimal conditions for the growth and vital function of halobacteria. Furthermore, the cell membrane of halovacteria contains another membrane protein, halorhodopsin serving as a light-dependent pump of chlorine ions (Cl^-), the main function of which is the transport of Cl^- into the cell [19]. Life in environments with high concentrations of NaCl has resulted in the development in halobacteria an effective system of active transport of Na^+ and K^+, whereby Na^+ is pumped out the cell and K^+, on the contrary, is pumped into the cell. As a result, the Na^+ content in the cytoplasm is maintained at a low level.

Despite the similarity of the mechanism of action of BR with the visual animal protein – rhodopsin, the amino acid sequence of BR differs from the animal rhodopsin that suggests their independent evolutionary origins. This is confirmed by the fact that the BR molecule forms 13-*cis, trans*- configuration rather than 11-*cis, trans*-configuration as in the animal rhodopsin [20]. However, the conformation of BR indicates that the protein belongs along with the rhodopsin to the family of transport G-proteins that involved in a large number of biochemical signal processes in the cell.

According to the structure and location in the cell membrane BR refers to integral transmembrane proteins, penetrating the entire thickness of the cell membrane of halophiles, which is divided into three main fractions: yellow, red and purple. The purple fraction, containing 75 % of BR, carotenoid and phospholipid (mainly phospho glycerol with a small amount of non-polar lipids and isoprenoids) and water forms natural two-dimensional crystals that can be investigated with using electron microscopy techniques and diffraction analysis – X-ray scattering and the scattering of electrons and neutrons on the surface of PM crystals [21]. These methods proved the existence in the BR molecule 7 α-helical protein segments in the middle of which is symmetrically located the chromophore moiety as a retinal residue (Figure 2).

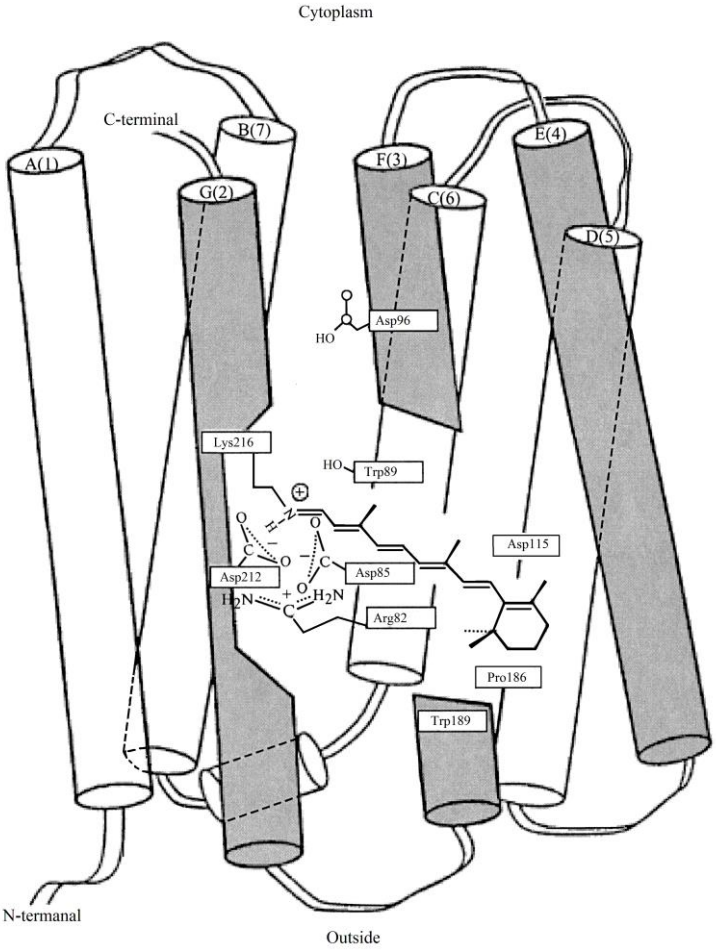

Figure 2: Location of the protein moiety of the BR and the retinal residue in the cell membrane of halobacteria *Halobacterium halobium* according to computer simulation [20]: protein fragments of the BR molecules in the form of 7 penetrating the cell membrane α-helical segments are indicated in Latin characters; dark color designated the segments responsible for binding the retinal residue to the protein part of the BR molecule.

The polypeptide chain of BR consists of 248 amino acid residues, 67 % of which are hydrophobic [22], while 33 % – hydrophilic residues formed by aspartic and glutamic acids, arginine and lysine (Figure 3a). These residues play an important structural and functional role in the spatial orientation of α-helical segments of the BR molecule, which is organized in the purple membrane in an orderly manner in the form of trimmers with an average diameter of about 0,5 mm and a thickness of 5–6 nm. Each trimmer is surrounded by six others so that a proper hexagonal crystal lattice is formed (Figure 3b). The individual BR molecule consists of 7 α-helix segments arranged in the direction perpendicular to the plane of the cytoplasmic membrane (Figure 3c). Hydrophobic domains represent transmembrane segments, whereas hydrophilic domains protrude from the membrane and connect the individual intra membranous α-helical segments of protein segments in the BR molecule [23].

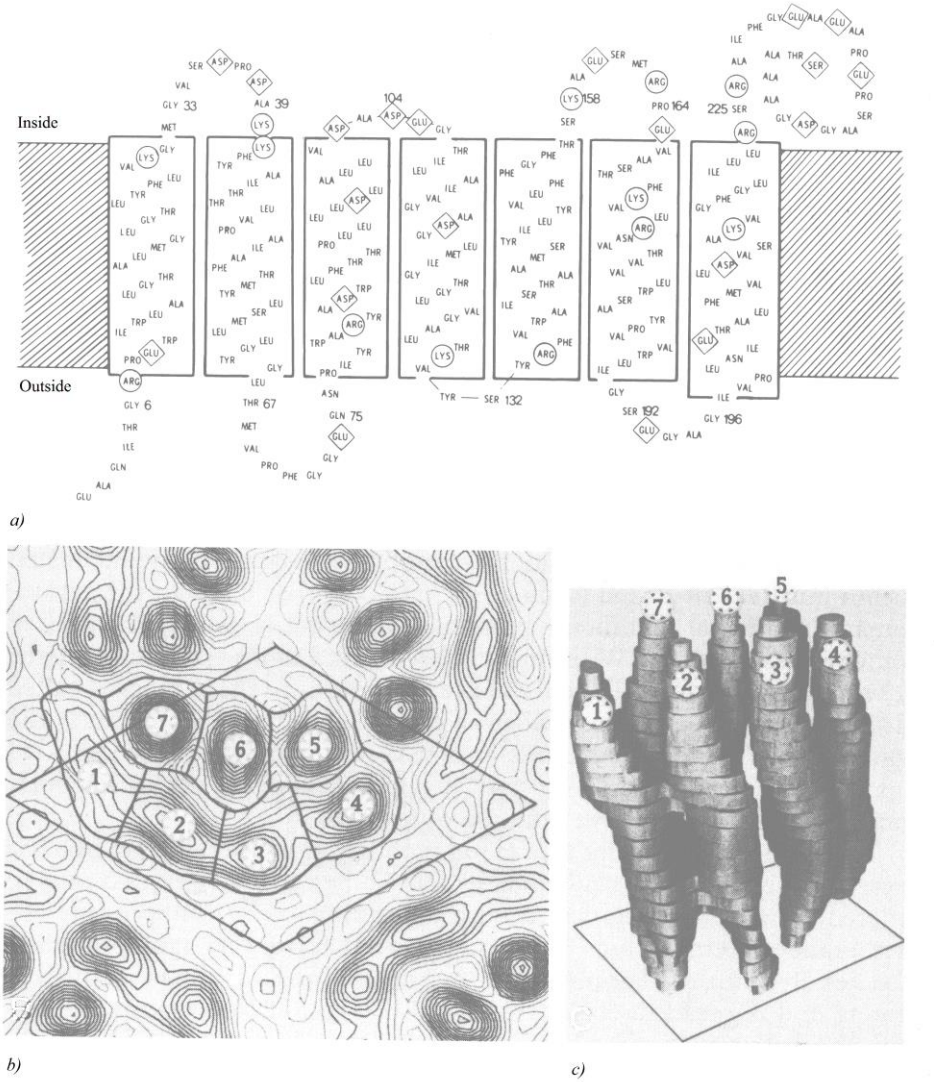

Figure 3: The structure of the BR molecule according to diffraction analysis [21]: a) – the primary structure of the BR molecule: amino acids indicated in Latin characters, circles and rhombs show the functionally important amino acids responsible for spatial orientation of α-helical segments of the protein residue of BR and formation of channels for the transfer of protons H^+ across the cell membrane; b) – electron density map of PM (a single molecule of the protein is encircled in the center). Numbers 1–7 are designated α-helical segments of BR: 1 – A-segment; 2 – B-segment; 3 – C-segment; 4 – D-segment; 5 – E-segment; 6 – F-segment; 7 – G-segment; c) – the spatial structure of the BR molecule: 1 – A-segment; 2 – B-segment; 3 – C-segment; 4 – D-segment; 5 – E-segment; 6 – F-segment; 7 – G-segment.

The mechanism of functioning of BR

BR acts as a light-dependent proton pump, pumping protons across the cell membrane and generates an electrochemical gradient of H^+ on the surface of the cell membrane, which energy is used by the cell for the synthesis of ATP in the anaerobic photosynthetic phosphorylation [24]. The mechanism of ATP synthesis is called “non-chlorophyll photosynthesis”, in contrast to the plant photosynthesis with the participation of chlorophyll. In this mechanism, at absorption of a light photon the BR molecule becomes decolorized by entering into the cycle of photochemical reactions, resulting in the release of a proton to the outside of the membrane, and the absorption of proton from intracellular space. The formation of concentration gradient of H^+ leads to the fact that the illuminated halobacteria cells begin to synthesize ATP, i.e. convert light energy into the energy of

chemical bonds. Due to this, the pH value inside the cytoplasm keeps the constant value – about 3 units and only slightly dependent on the pH of the exterior medium, which can reach 10–12 units.

The mechanism of subsequent sequential proton transfer of H^+ with the participation of the BR molecule across the cell membrane includes a chain of hydrogen bonds formed by the side residues of hydrophilic amino acids extending through the entire thickness of the protein. This H^+ proton transfer through the protein chain is carried out providing the protein consists of two parts and contains a photochrome functional group capable under the influence of light to change its microenvironment and thereby sequentially "lock" and "unlock" sites of binding of H^+ and its further transfer across the cell membrane. The role of such a "shuttle" mechanism between two conductors of H^+, one of which communicates with the exterior, and the other – with the cytoplasmic surface of the cell membrane plays a retinal residue linked by the aldimine bond (as in the visual pigments of animals) with a lysine-216 residue of the protein. Retinal has a 13-*trans* conformation and is located in the membrane tunnel between protein α-segments of the BR molecule, blocking the flow of protons. By the absorption of a light photon it occurs reversible isomerization of 13-*tras*-BR (λ_{max} = 548 nm) (the quantum yield 0,03 at t = +20 ^oC) into the 13-*cis*-BR (λ_{max} = 568 nm) [25], initiating a cascade of photochemical reactions lasting from 3 ms to 1 ps with the formation of transitional intermediates J, K, L, M, N, and O, followed by separation of H^+ from the retinal residue of BR and the connection of H^+ from the side of cytoplasm (Figure 4). In this process, the retinal residue is specifically bent in the membrane tunnel forming the transmembrane transport H^+ channel from the cytoplasm to the outside environment, and carries a proton H^+ from the inner cytoplasmic membrane to the outer membrane of the cell. In this case, a proton H^+ from the retinal residue is transferred to the Asp-85-residue, after that the resulting vacancy is filled with a proton H^+ transferred from the residue Asp-96. As a result, between the internal and external surface of the membrane forms a concentration gradient of H^+, which leads that illuminated cells begin to synthesize ATP, i.e. convert light energy into energy of chemical bonds. This process is reversible and in the dark flows in the opposite direction. In this way the BR molecule behaves as a photochromic carrier with a short relaxation time – the transition from the excited state to the ground state. Optical characteristics of BR vary depending on the method of preparation of PM embedded onto the polymer matrix.

An interesting feature of the metabolism of halobacteria is at that the presence of oxygen and the organic compound (amino acids, peptones), which can be used as growth substrates and sources of energy, halophiles can grow in the dark, by switching on a heterotrophic photosynthetic metabolism [26]. However, with a lack or even in the complete absence of oxygen and under the bright light in the cell membrane of halobacteria is synthesized BR, allowing them to use solar energy for growth and ATP synthesis. Thus, halobacteria are capable to carry out the synthesis of ATP molecules as due to chemical energy released in the process of oxidation in the respiratory chain, as well as using the light energy absorbed by BR.

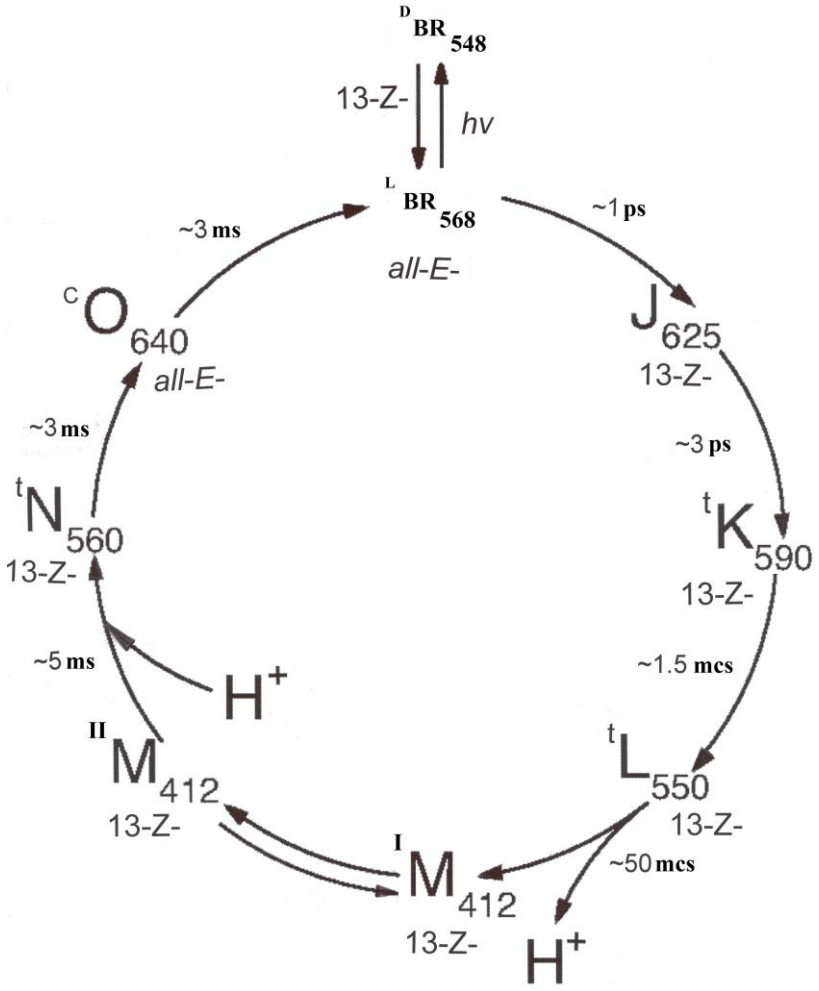

Figure 4: Photocycle scheme of BR (aqueous solution, pH = 7,2; t = 20 °C). Latin numbers J, K, L, M, N, O denote spectral intermediates of BR. ^{I}M and ^{II}M represent spectral intermediants of *meta*-bacteriorhodopsin with the protonated (^{I}M) and deprotonated (^{II}M) aldimine bond. L and D denote dark and light forms of pigments. The subscripts correspond to the position of the absorption maximum in the photocycle intermediates (nm).

The spheres of practical application of BR in bionanotechnology

BR is the focus of bio-and nanotechnology because of its high sensitivity and resolution, and is used in molecular bioelectronics as natural photochromic material for light-controlled electrical regulated computer modules and optical systems [27]. In addition, BR is very attractive as a model for studies related to the research into the functional activity and structural properties of photo-transforming membrane proteins embedded into the native and photo-converting membranes [28].

Nanofilms produced using the BR-containing PM were first obtained and studied in this country in the framework of the project “Photochrome” [29], when it was demonstrated effectiveness and prospects for the use of BR as photochromic material for holographic recording (Figure 5).

The main task for the manufacture of BR-containing nanofilms is the orientation of PM between the hydrophobic and hydrophilic media. Typically, to improve the characteristics of the BR-containing films use multiple layers of PM that are applied to the surface of the polymeric carrier and dried up, preserving their natural structure. The best results are achieved in the manufacture of nanofilms embedded onto the gelatin matrix [30]. This allows to achive high

concentration of BR (up to 50 %) in nanofilms and avoid aggregation of cell membrane fragments and destruction of BR in the manufacturing process [31].

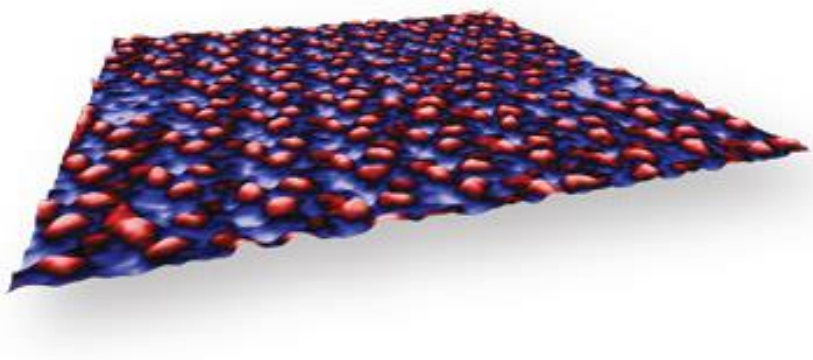

Figure 5: Artificial membrane based on the BR-containing PM in scanning electron microscope (SEM): scanning area – 100×100 mm; resolution – 50 nm; magnification – 100000 times. PM shown in purple, BR – in red color.

Embedded into a gelatin matrix BR-containing PM fragments are durable (~10^4 h) and resistant to solar light, the effects of oxygen, temperatures greater than +80 ºC (in water) and up to +140 ºC (in air), pH = 1–12, and action of most proteases [32]. The dried PM is stacked on the top of each other, focusing in the plane of the matrix, so that a layer with 1 μm thickness contains about 200 monolayers [33]. When being illuminated such nanofilms exert the electric potential at 100–200 mV, which coincides with the membrane potential of living cells [34]. These factors are of great practical importance for integration of PM into polymeric nanomatrix with keeping photochemical properties.

Biosynthesis of BR

The technology of preparation of BR consists in growing of halobacteria on liquid artificial synthetic media with 15–20 % NaCl, containing synthetic amino acids or on natural media with peptones or protein-vitamin concentrate (PVC) of yeast [35].

Artificial synthetic media are represented by mixtures of synthetic essential amino acids (glycine, alanine, arginine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, valine), nucleotides (adenosine-5-monophosphate, uridine-5-monophosphate), inorganic salts and vitamins.

Peptones – are products of partial hydrolysis of animal proteins (milk, meat), consisting of mixtures of different polypeptides; also contain di- and tripeptides, and free amino acids. Peptones are formed by the action of proteolytic enzymes of the gastric and pancreatic juices (pepsin, trypsin) on natural proteins, as well as by mild hydrolysis of animal proteins by acids and alkalis. The source of the peptone protein depends on the species from which it is derived: meat, fish, egg peptones etc.

PVC is a dry biomass of feed yeast of saccharomyces genus of *Saccharomyces cerevisiae*, grown on hydrocarbons - oil paraffins (paprin) or natural gas (gaprin). It contains approx. 50% of protein, a full set of vitamins, a large number of trace elements (iron, manganese, iodine, magnesium, sodium, zinc) and amino acids.

Depending on the needs of a particular type of halobacteria in sources of growth substrates use artificial synthetic nutrient media or media prepared on the basis of natural peptons of PVC of yeast.

For the biosynthesis of BR often use the extreme aerobic photo-organotrophic halobacterium *Halobacterium halobium* [36]. This bacterium is grown on the synthetic liquid complex medium containing 18 amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine,

tyrosine, tryptophan, valine), nucleotides (adenosine-5-monophosphate, uridine-5-monophosphate), inorganic salts of sodium, magnesium, manganese, copper, calcium, zinc, iron, potassium, ammonium, phosphorus) and biotin, folic acid and vitamin B_{12}.

The process of growing the halobacteria is conducted on an orbital shaker in flat-bottomed flasks in condition of intensive aeration under the light of monochrome fluorescent lamps. Bacterial growth was measured by optical density of the cell suspension at λ=620 nm on a spectrophotometer. As is shown in Figure 6 under optimal growing conditions (incubation period 4–5 days, temperature t = +35 °C, illumination with monochromatic light at λ = 560 nm) in cells is synthesized the purple carotenoid pigment, characterized as BR by the spectral ratio of protein and chromophore fragments D_{280}/D_{568} = 1,5:1,0 in the molecule [37]. The subsequent isolation of BR from the PM fraction is carried out by a combination of physical, chemical and enzymatic methods [38].

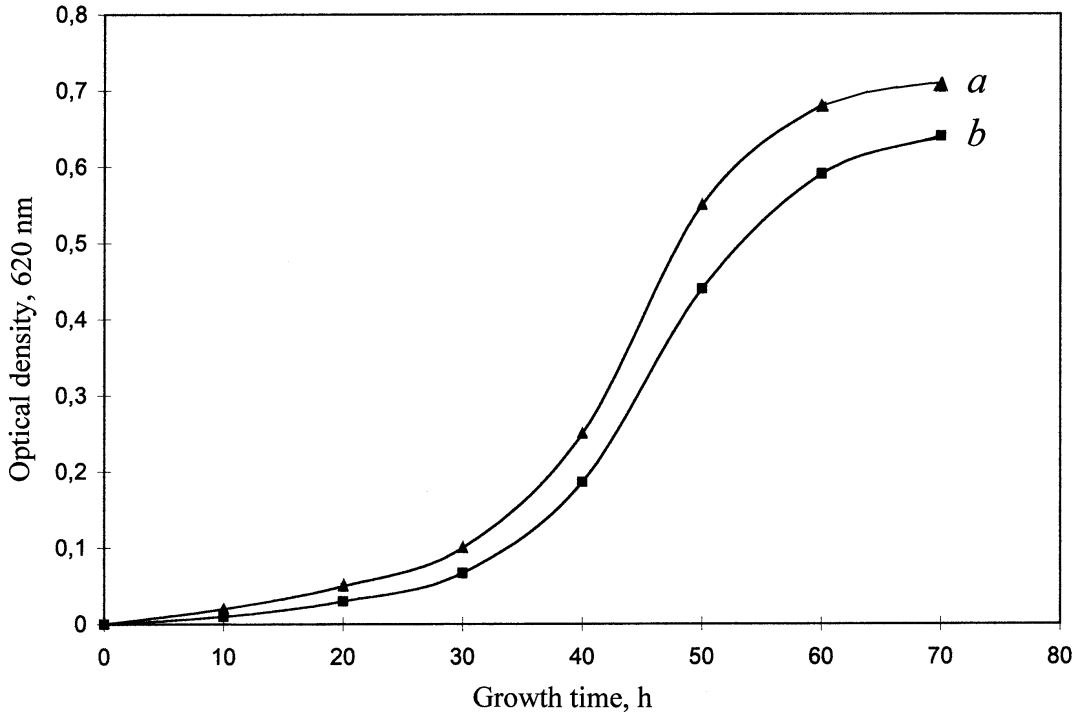

Figure 6: Growth dynamics of *H. halobium* under different conditions: *a)* – the peptone medium; *b)* – complex synthetic medium. Growing conditions: incubation period of 4–5 days at t = +35 °C; illuminating by monochromatic light with a wavelength of λ = 560 nm.

Isolation of BR

Isolation and purification of BR from PM fraction is carried out with using a light-shielding lamp equipped with an orange color filter as BR is very sensitive to light and light isomerization.

The main stages for obtaining BR are:

- Growing the halobacterium *H. halobium* on artificial or natural nutrient media;
- Cell disruption and lysis of cell walls;
- Allocation fraction of PM;
- Cleaning of PM from low- and high molecular weight impurities, cellular RNA and carotenoids;
- Dissolving the PM fraction in a 0,5 % solution of the ionic detergent sodium dodecyl sulfate (SDS-Na) to form a microemulsion;
- Precipitation of BR from the microemulsion by methanol;
- Gel Permeation Chromatography (GPC) on Sephadex G-200;
- Electrophoresis in 12,5 % polyacrylamide (PAGE) gel.

The protein is localized in the PM; the release of low molecular weight impurities and intracellular contents was reached by osmotic shock of cells with distilled water in the cold after the removal of 4,3 *M* NaCl and the subsequent destruction of the cell membrane by ultrasound at 22 kHz. For the destruction of cellular RNA the cellular homogenate was treated with Rnase I. PM fraction along with the desired protein in a complex with lipids and polysaccharides also contained impurity of related carotenoids and proteins. Therefore, it was necessary to use special methods of fractionation of the protein without damaging its native structure and dissociation. That required applying the special methods of purification of carotenoids and lipids, and the subsequent GPC on Sephadex G-200. Removing of carotenoids, consisting in repeated treatment of PM with 50 % (v/v) EtOH at t = +4 °C, was a routine but necessary step, in spite of the significant loss of chromoprotein. It was used five treatments by 50 % (v/v) EtOH to obtain the absorption spectrum of purified from carotenoids PM suspension (4) and (5) (degree of chromatographic purity of 80–85 %), as shown in Figure 7 at various processing stages (*b*) and (*c*) relative to the native BR as a control (*a*). The formation of retinal-protein complex in the BR molecule leads to a bathochromic shift in the absorption spectrum of PM (Figure 7*c*) – the main bandwith (1) with the absorption maximum at λ = 568 nm is caused by the light isomerization of the chromophore by the C13=C14 bond is determined by the presence of 13-*trans*-retinal residue in BR_{568}; additional low-intensity bandwith (2) at λ = 412 nm characterizes a minor impurity of a spectral form of *meta*-bacteriorhodopsin M_{412} (formed in the light) with deprotonated aldimine bond between 13-*trans*-retinal residue and protein; the total bandwith (3) at λ = 280 nm is determined by the absorption of aromatic amino acids in the polypeptide chain of the protein (for the native BR – D_{280}/D_{568} = 1,5:1,0).

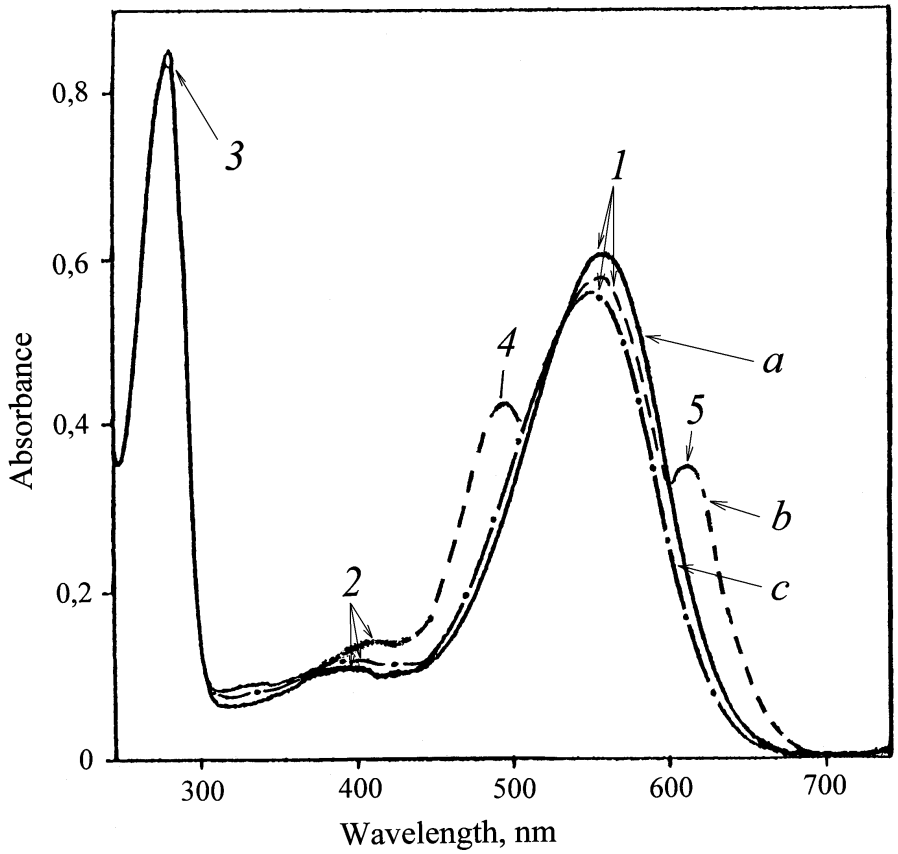

Figure 7: The absorption spectra of the PM (50 % (v/v) EtOH) at various stages of processing: (*a*) – the natural BR (control); (*b*) – PM after intermediate treatment; (*c*) – PM purified from carotenoids. The bandwith (1) is the spectral form of BR_{568}; (2) – impurity of spectral form of *meta*-bacteriorhodopsin M_{412}; (3) – the total absorption bandwith of aromatic amino acids; (4) and (5) – extraneous carotenoids. As a control used the native BR

The fractionation and chromatographic purification of BR

The fractionation and chromatographic purification of the protein was the next necessary step of the research. As BR, being an integral membrane protein intricately penetrates bi-lipid layer in form of seven α-helices, the use of ammonium sulfate and other conventional agents to salting out did not give a positive result for isolation of the protein. The resolving was in the translation of the protein to a soluble form by the colloidal dissolution (solubilization) in an ionic detergent. Using as the ionic detergent SDS-Na was dictated by the need to carry out the solubilization of the protein in a native, biologically active form in complex with 13-*trans*-retinal, because BR solubilized in 0,5 % (v/v) SDS-Na retains a native α-helical configuration [39]. Therefore, there is no need to use organic solvents as acetone, methanol and chloroform for purification of lipids and protein, and precipitation and delipidization is combined into a single step, which significantly simplifies the further fractionation. A significant advantage of this method is that the isolated protein in complex with lipids and detergent molecules was distributed in the supernatant, and other high molecular weight impurities – in unreacted precipitate, easily separated by centrifugation [40]. Fractionation of the protein solubilized in a 0,5 % (w/v) SDS-Na and its subsequent isolation in crystalline form was achieved at t = +4 ^{o}C in three steps precipitating procedure with treatment by methanol, reducing the concentration of detergent from 0,5; 0,25 and 0,1 % (w/v) respectively.

The final stage of BR purification involved the separation of the protein from low-molecular-weight impurities by using GPC-method. For this purpose the BR containing fraction was passed twice through a chromatography column with dextran Sephadex G-200 balanced with 0,09 *M* Tris-buffer (pH = 8,35) containing 0,1 % (w/v) SDS-Na and 2,5 *mM* EDTA. The elution was carried out at t = 20±25 ^{o}C with 1 mM Tris-HCl buffer (pH = 7,6) at rate of 10 ml/cm^{2}·h. The data on purification of BR of phospholipids and carotenoids are shown in Table 2. As it is demonstrated in Table 2, 84 % of phospholipids was removed by five washes (65, 70 and 76 % was removed by 1st, 2nd and 3nd wash respectively). The total endogenous phospolipid removal on the BR peak was 92 % relative to the native PM.

Table 2: Summary results for the isolation and purification of BR by various methods

Sample	PM content, mol PM/mol BR	Phospholipid and carotenoid removal, %	BR yield*, %
PM fraction	20,5	–	–
PM washed with EtOH			
1 wash	16,9	65	93
2 wash	15,1	70	90
3 wash	14,5	76	88
4 wash	13,6	81	84
5 wash	13,2	84	80
BR crystallised from MeOH	12,9	86	75
BR from GPC on Sephadex G-200	10,2	92	86

Notes:

* Percentage yield is indicated in mass.% relative to BR solubilized in 0,5 % SDS-Na before concentration.

Conclusions

Haobacteria is a taxonomic group of extreme aerobic microorganisms having great practical bionanotechnological potential. BR synthesized by these microorganisms is the focus of bio-and nanotechnology because of its high sensitivity and resolution, and may be used in molecular bioelectronics as natural photochromic material for light-controlled electrical regulated computer

modules and optical systems. The technology of BR biosynthesis allows obtain milligram quantities of pure crystal protein. The main advantage is that BR retains its natural configuration and the ability to undergo photochemical transformations. By this method is possible to obtain similar to BR transmembrane proteins of halobacteria – sensorodopsin and halorodopsin. The unique properties of these natural bacteriorhodopsins provide a wide range of optical applications in which they can be applied, because the integration of these proteins in the most advanced technical optical systems is very simple.

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Метаболизм и физиология галофилов

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Аннотация. Галофилы (*лат.* “любящие соль”) – таксономическая группа экстремальных аэробных грам-положительных облигатных микроорганизмов, обитающие в условиях высокой солёности – в морях, солёных озерах и засоленных почвах. Человечеству они известны довольно давно по красноватому налету на продуктах, консервируемых с использованием больших количеств поваренной соли (NaCl). Впервые галофилы были выделены в начале XX столетия из микрофлоры морской лиманной грязи, однако их систематическое изучение началось только в конце второго десятилетия XX века. Внутренняя среда человека не пригодна для жизнедеятельности галобактерий, поэтому среди них нет ни одной патогенной формы. Галобактерии обладают большим потенциалом использования в молекулярной биоэлектронике и био-нанотехнологии вследствие уникальной способности преобразовывать энергию солнечного света в электрохимическую энергию протонов H^+ за счет наличия фотопреобразующего ретинальсодержащего мембранного белка – бактериородопсина, механизм функционирования которого в настоящее время детально изучен. В данной статье рассмотрены особенности метаболизма и физиологии галофильных бактерий, а также приведен метод биосинтеза и выделения бактериородопсина из пурпурных мембран клеток экстремальной фотоорганотрофной галобактерии *Halobacterium halobium*.

Ключевые слова: галобактерии, бактериородопсин, пурпурные мембраны, биосинтез.

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Dynamics of Bioelements Contents of Organs and Tissues of Animals Organism under Influence of Xenobiotics

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Abstract

The content of bioelements in the heart, liver, kidneys, adrenals, spleen, serum of blood in the prolongued experiment was studied on white rats aftter 45 days of poisoning info mouth with water based liquids of neonols 1/100 and 1/10 DL_{50}. The results talk about the breach of adaptation of organisms to harmful influence of xenobiotics.

Keywords: bioelements, metaloenzyms, xenobiotics, homeostasis, stress, adaptation, stabilization, rats of the Vistar population.

Введение

Многие заболевания человека сопровождаются или вызваны нарушением биоэлементного баланса организма [1]. Причиной дисбаланса могут быть различные экзогенные факторы, в том числе и токсические химические соединения, в частности синтетические поверхностно-активные вещества (ПАВ), которые являются одними из самых распространённых загрязнителей поверхностных и подземных источников водоснабжения. Низкая эффективность очистки воды от ПАВ на современных очистных сооружениях, техногенные катастрофы являются причиной появления ксенобиотиков в питьевой воде, что естественно влияет на здоровье населения [2]. Вследствие этого сравнительная оценка специфических изменений фонда биоэлементов организма представляет большой интерес для изучения перестройки регуляции организма непосредственно на клеточном, гуморальным, органном, организменном уровнях под действием вредных факторов окружающей среды [3, 4]. На современном этапе развития представлений об адаптации обнаружено, что обратная реакция на последовательные множественные влияния стресс-факторов зависит от накопления данным организмом в процессе адаптации метаболической памяти [5]. Таким образом, формируется стрессовое состояние в организме, которое модулирует физиологические функции в связи с вновь создавшимися условиями существования [6].

Материалы и методы

Изучали содержание биоэлементов в сердце, печени, почках, надпочечниках, селезёнке, сыворотке крови половозрелых крыс (самцов) популяции Вистар, подвергавшихся

воздействию новых групп ПАВ в подостром эксперименте. Вещества на основе оксиэтилированных алкил- и изононилфенолов марок АФ 9-12 и АФС 9-6 КМ вводили ежедневно утром натощак с помощью металлического зонда перорально в дозах 1/10 и 1/100 DL_{50} в течение 45 суток. Элементный состав определяли атомно-абсоробционным методом, основанным на определении поглощения света атомами определённого макро- или микроэлемента, находящимися в газообразном состоянии. Для испарения элементов использовали пламя газовой горелки. Наличие и количество элемента определяли по степени поглощения света с определённой длиной волны, которую поглощает анализируемый элемент [7]. Для проведения анализа микроэлементов органы и ткани подвергали предварительному озолению и экстрагированию по Е.А. Лойко и Г.О. Бабенко [8, 9]. Полученный экстракт подавали в спектрофотометр и с его помощью определяли содержание биоэлементов. Полученные результаты сравнивали с результатами эталонных образцов. Исследовали такие элементы: Na, K, Ca, Mg, Zn, Cu, Fe, то есть те, которые выполняют кофакторную функцию и обеспечивают каталитическую активность многих ферментов, таких как Ca^{2+}, Mg^{2+}-зависимые АТФазы, K^+, Na^+-зависимые АТФазы, моноаминооксидаза, цитохромоксидаза, церулоплазмин, лактатдегидрогеназа, малатдегидрогеназа, сукцинатдегидрогеназа, глюкозо-6-фосфатдегидрогеназа, каталаза, пероксидаза. Достоверность результатов оценивали с помощью t = критерия Стьюдента. Величину $p < 0,05$ считали статистически значимой.

Обсуждение результатов

Результаты опытов показали, что неонолы групп АФ 9-12 и АФС 9-6 КМ, действуя на организм, приводят, в основном, к перераспределению биоэлементов в органах и тканях экспериментальных животных.

Динамика содержания биоэлементного состава в организме белых крыс под влиянием ксенобиотиков в дозе 1/100 DL_{50} (мг/100 г ткани)

Орган	Группы животных	Микроэлементы, (M ± m)						
		K	Na	Ca	Mg	Cu	Zn	Fe
Сыворотка крови	Опыт	8,33 ±0,56*	145,01 ±8,37*	3,67 ±0,35*	1,77 ±0,18	63,41 ±4,23*	17,22 ±0,87	53,75 ±2,37*
	Контроль	6,82 ±0,25	100,42 ±2,6	2,92 ±0,1	1,47 ±0,03	46,43 ±0,79	15,91 ±0,29	40,30 ±2,53
Печень	Опыт	6,98 ±0,23*	8,75 ±0,35	3,55 ±0,23*	4,95 ±0,45*	9,35 ±0,45	0,82 ±0,13	1,15 ±0,09
	Контроль	8,27 ±0,25	8,80 ±0,46	4,37 ±0,19	6,67 ±0,27	9,85 ±0,37	0,81 ±0,04	1,26 ±0,05
Надпочечники	Опыт	2,43 ±0,35	267,52 ±7,85	29,32 ±2,33	34,73 ±1,35	51,22 ±2,43	30,90 ±2,57*	8,75 ±0,43
	Контроль	2,35 ±0,06	271,01 ±7,70	29,61 ±1,8	35,72 ±2,82	48,23 ±1,82	37,74 ±1,76	9,02 ±0,46
Почки	Опыт	2,53 ±0,05	213,74 ±8,67*	2,60 ±0,09	4,93 ±0,08	17,33 ±1,77	0,56 ±0,06	8,00 ±0,43
	Контроль	2,63 ±0,06	255,01 ±4,6	2,60 ±0,08	4,85 ±0,06	17,72 ±1,67	0,54 ±0,03	7,78 ±0,36
Сердце	Опыт	4,27 ±0,25	3,66 ±0,27	1,63 ±0,07	5,01 ±0,1*	0,53 ±0,05	2,55 ±0,07	25,33 ±1,73
	Контроль	4,39 ±0,5	3,84 ±0,19	1,67 ±0,06	4,46 ±0,13	0,75 ±0,04	2,60 ±0,02	23,12 ±2,09
Селезёнка	Опыт	2,14 ±0,22	0,83 ±0,07	2,17 ±0,13*	1,36 ±0,07	0,37 ±0,03	2,00 ±0,05	9,96 ±0,55
	Контроль	2,14 ±0,08	0,82 ±0,04	2,81 ±0,03	1,38 ±0,03	0,36 ±0,03	2,65 ±0,02	10,01 ±0,49

∗ Отличие от контроля достоверно ($p < 0,05$)

Более значимые изменения динамики микроэлементов обнаружены под влиянием АФС 9-6 КМ, который повышал в сыворотке крови содержание K^+ на 22 %, Na^+ на 44 %, Ca^{2+} на 26 %, Mg^{2+} на 20 %, Cu^{2+} на 36,5 %, Zn^{2+} на 8 %, Fe^{3+} на 33 % *(таблица)*. Изменения фонда биогенных элементов могут объясняться способностью детергентов к комплексообразованию с макро- и микроэлементами и последующим перераспределением этих веществ в клетках, тканях и организме в целом. Нарушения натрий-калиевого гомеостаза могут приводить к изменениям электрохимического градиента и связанных с ним клеточных процессов; гиперкалиемия может быть причиной угнетения сердечно-сосудистой системы, что было характерно для сыворотки крови экспериментальных животных. При изменении содержания ионов натрия в организме происходят нарушения функций нервной системы, гладких и скелетных мышц.

В печени обнаружено снижение содержания K^+ на 15,6 %, Mg^{2+} на 25,8 %. С нарушением магниевого гомеостаза может существенно изменяться ряд ферментативных процессов – окислительное фосфорилирование, аденилатциклазный каскад, протеосинтез, что нашло подтверждение в исследованиях [10, 11]; а так же может нарушаться работа Ca^{2+}, Mg^{2+} и Na^+, K^+ АТФ-зависимых транспортных белков плазматической, митохондриальной мембраны и эндоплазматической сети, которые осуществляют трансмембранный перенос двух- и одновалентных ионов Ca^{2+}, Mg^{2+}, Na^+, K^+ и протонов, вследствие чего электрохимические градиенты поддерживаются на уровне функционирования клеток в норме [12, 13]. Увеличение содержания Ca^{2+} тормозит образование гормонов паратирина и кальцитриола, причём одновременно возрастает количество неактивных продуктов метаболизма этого соединения. С помощью Ca^{2+} и путём фосфорилирования осуществляется регуляция ряда ключевых ферментов метаболизма: гликогенсинтазы, глицерол-3-фосфатдегидрогеназы, пируватдегидрогеназы, пируваткиназы, пируваткарбоксилазы и др. [1]. Снижение уровня Ca^{2+} наблюдалось в печени на 18,8 % и селезёнке на 22,3 % на фоне снижения активности ферментов в печени Ca^{2+}-зависимой АТФазы на 14 % и Mg^{2+} - зависимой АТФазы на 22,7 %, в почках соответственно на 21 % и 35,5 %. Снижение активности данных ферментов свидетельствует о нарушении структурно-функционального состояния мембран и срыве защитных механизмов под действием токсических веществ, которые являются стрессорным фактором для организма. Снижение содержания Cu^{2+} на 29,3 % в органах и тканях явилось одной из причин инактивации моноаминоксидазы, что, в свою очередь, может привести к уменьшению дезинтоксикации протеиногенных аминов, нарушению процессов дыхания и окислительного фосфорилирования, а также нарушению обмена биогенных моноаминов, которые являются медиаторами действия регуляторных систем. При действии неонолов снижалась активность и других медьсодержащих ферментов – церулоплазмина и цитохромоксидазы, которые играют большую роль в окислительно-восстановительных процессах в организме.

Некоторые дегидрогеназы, функционирующие с никотинамидными коферментами, содержат ион цинка, в частности алкогольдегидрогеназа печени и глицеральдегид-3-фосфатдегидрогеназа скелетных мышц [1]. Повышение содержания данного иона в сыворотке крови свидетельствует о высвобождении его из описанных ферментов и их дисфункции. Снижение содержания Zn^{2+} в надпочечниках на 18,1 % и в селезёнке на 24,5 % может свидетельствовать о ингибировании активности цинксодержащих ферментов в данных органах.

В надпочечниках отмечено снижение содержания Fe^{3+} и снижение активности железосодержащих ферментов: пероксидазы на 12,3 %, каталазы на 41 %, глутатионпероксидазы на 31,1 %. Действием этих ферментов регулируется разложение перекиси водорода восстановленным глутатионом в эритроцитах. И таким образом обеспечивается защита липидов мембран и гемоглобина от окисления перекисями [14, 15]. При изменении уровня Fe^{3+} изменяется активность фермента сукцинатдегидрогеназы, которая переносит восстановленные эквиваленты от субстрата непосредственно на дыхательную цепь, катализирует первое дегидрирование сукцината в цикле трикарбоновых кислот, тем самым учавствуя в обеспечении окислительно-восстановительных процессов [1].

Выводы

Оксиэтилированные алкил- и изононилфенолы в дозах 1/10 и 1/100 $ДЛ_{50}$ снижают содержание биоэлементов, что влечет за собой изменение активности металлоферментов во внутренних органах. Это подтверждает существование комплекса взаимосвязанных механизмов снижения и перераспределения биоэлементов в органах и тканях под воздействием ксенобиотиков. В большей степени нарушение фонда биоэлементов обнаруживалось в печени и сыворотке крови, что тесно коррелируют с активностью металлоферментов в органах и тканях.

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Динамика биоэлементного состава тканей и органов животного организма при действии ксенобиотиков

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Аннотация. Исследован состав биоэлементов в сердце, печени, почках, надпочечниках, селезёнке, сыворотке крови в подостром опыте на белых крысах популяции Вистар после 45 суточной пероральной затравки водными растворами 1/10 и 1/100 $ДЛ_{50}$ неонолов. Выявлено, что под пролонгированным действием ксенобиотиков снижается содержание микроэлементов в органах и тканях организма, что свидетельствует о нарушении адаптации организма к вредному воздействию ксенобиотиков. Но в продолжении эксперимента организм животных выходит на уровень стабилизации параметров биоэлементного состава.

Ключевые слова: биоэлементы, металлоферменты, ксенобиотики, гомеостаз, стресс, адаптация, стабилизация, крысы популяции Вистар.

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On Localization of Ancient Bearers of Y-DNA R1a Haplotype in Eastern Europe Neolithic Cultures

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Abstract

The work considers the problems of genetics, archeology, and anthropology connected with problem of localization of R1a* Y-DNA haplotype bearers in Meso- and Neolithic **Pre-Corded Ware** archaeologic sites. Based on the analysis of findings of 2014-2015 years (described in other works) this paper proposes a hypothesis that the areas of Comb Ware cultures of Eastern Europe could be the possible area of archaic Y-DNA R1a1 subclades spread. The ancient R1a1 Y-haplotype (M198-, M459+) bearers could be possibly accompanied with those with J2b Y-DNA haplotype. As for pre-Mesolitic time, the situation remains unclear as more Eastern and more Southern localizations of R1a* and R1a1* bearers are possible according to modern data.

Keywords: Y-DNA haplotype**,** R1a1, J2b, Mesolithic, Yuzniy Oleni Ostrov, Khvalynsk, Serteya, paleogenetics, paleolinguistics, subclades.

Introduction

The interest in the origin and early localization of carriers of Y-DNA haplogroup R1a1 is serious, since this Y-DNA subclade is inherent to the significant percentage of the population of Central and Eastern Europe, India, Middle East. It is widely recognized and already proven in terms of archeology and paleogenetics that a significant concentration of R1a1 Y-DNA haplotype was inherent to the population of European Corded Ware culture (authors note that there also existed less famous East Asian Corded Ware culture group **(8)**). However, the location of R1a1 bearers of Pre-Corded Ware horizons causes debates due to a lack of data. However, over the last year such data emerged, allowing formulating a data-based hypothesis.

Materials and Methods

The main materials for the research are data from paleogenetic samples described in other works:

Sample	Y-DNA	MtDNA	Source
R1a1			
Yuzniy Oleni Ostrov burial № 125, 5500-5000 BCE.	R1a1*-M459+, M198-	C1g (formerly C1f)	**7, p 25**
Serteya archeological site, middle of V-IV mill. BCE	R1a1	H2	**10, p 294**
Khvalynsk-II burial 5200-4000 BCE.	R1a1, preliminary classification was determined as R1a1*-M459+, M198- **(14)**	U5a1i	**9**
J			
Yuzniy Oleni Ostrov burial № 40, 5500-5000 BCE.	J	U4a	**9**
Satsurblia burial (Georgia), Upper Paleolithic	J	K3	**12**
Kotias burial (Georgia), Mezolithic	J2a	H13c	**12**

The main research method of this paper is the interpretation of recently obtained genetic data which are compared with archaeological cultures distribution.

Discussion and Results

R1a1*, M459+, M198- on Yuzhniy Oleni Ostrov burial (North-Western Russia)

One of the most well studied Eastern European archeological sites is the Yuzhniy Oleni Ostrov burial on the shores of Lake Onega (Karelia, Russia) and it is dated back to the developed and late Mesolithic period of VII-V millennium BC. Three individuals from the Yuzhniy Oleni Ostrov, who lived 7500 years ago (UZOO-7, 8 and UZOO-UZOO-74), possessed a non-existing now in Europe mitochondrial haplogroup C1f **(4)**. Also among the burials of Yuzhniy Oleni Ostrov were found mitochondrial haplogroups U4, U2e, U5a **(5)**, J and H **(6, p.36)**. The Mesolithic inhabitant of Yuzhniy Oleni Ostrov (burial № I0061) possessed Y-chromosome haplogroup R1a1 (SRY10831.2, M198- subclade) **(7)** and mitochondrial haplogroup C1g (formerly C1f) **(7)**. The other Mesolithic inhabitant of Yuzhniy Oleni Ostrov (I0221 / UZ0040) possessed Y-chromosome haplogroup J, and mitochondrial haplogroup U4 **(9)**.

The author of one the most detailed publication on Yuzhniy Oleni Ostrov antropology V.P.Yakimov adhered to the Eurocentric point of view on the formation of the Mesolithic Onega inhabitants. He suggested that their origins are linked to the Paleolithic population of Eastern Europe, who moved along the glacier to the northern and northeastern directions **(2)**. But some cross-breeding with Eastern population was also confirmed: «*Later, it was concluded that it belonged to the described in the southern edge of the region so-called «flint» Mesolithic culture associated by origin with cultures of the Volga-Oka area, and (since the appearance in the VIII millennium BCE) coexisted with an earlier (since the X millennium BCE) local «quartz-slate» culture created by people from the North Urals and Trans-Urals and related to Finnish Askola – Suomusyarvi*» **(3)**. Currently, researchers emphasize that «*Yuzhniy Oleni Ostrov burial site is as an archaeological source extremely multifaced*» **(ibid)** and represents a particular genetic type, different from the classical Mongoloid and Caucasoid **(ibid).** A very heterogeneous composition of the population is now well proven by the presence of Y-chromosome haplogroup J, which indicates the influence of the southern areas and communication of Yuzhniy Oleni Ostrov people with populations of the Black and Caspian Sea.

On the scheme of Yuzhniy Oleni Ostrov (**13, p. 5**) burials with determined Y-DNA haplotypes are located as follows, according to (**7, p. 25), (13, p. 35)**.

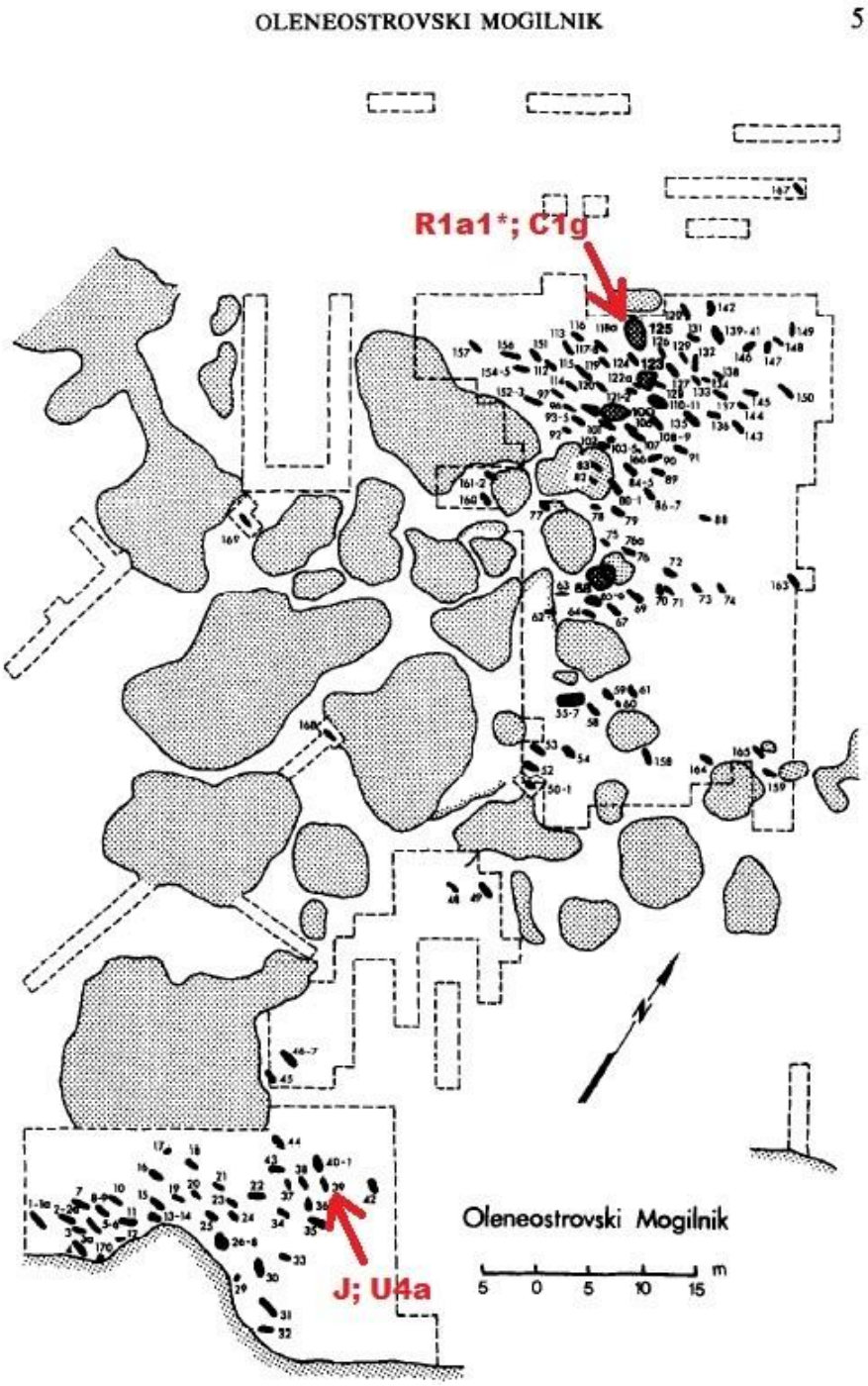


Figure 1. Y-DNA R1a1 and J typed samples in Yuzhniy Oleni Ostrov burial

The question if the culture of Yuzhniy Oleni Ostrov is non-ceramical Mesolithic or ceramical Neolithic is still open. It should be mentioned that P.N. Tretiakov in his book «Finno-Ugrians, Balts and Slavs at the Dnieper and Volga» mentioned: «*The population, which left behind the Oleni Ostrov burial ground seems to be familiar with the ware. It is proven by the bone plates with pinked edge, which must have served as ornament molds for clayware. If this statement is true, the Oleni Ostrov burial ground should be connected with the ancient culture of the Pottery Neolithic of the East Baltics – the Sperrings culture*» and further: *«Comb ware of Sperrings type (Pic. 3), according to different researchers, has the closest analogs to the Neolithic cultures of the*

Kama Region, the Cisuralian area and Trans-Urals, and we agree with this statement. Anyhow, there is no doubt that numerous Ural and Kama analogs to Sperrings ware, which have been detected in the recent years are incomparably more convincing than the Middle Dnieper one» (**15, p. 24**). This supports the version of the possible connections of Yuzhniy Oleini Ostrov people with the southern or eastern Neolithic cultures.

In addition to the local component the cultural influences on Yuzhniy Oleni Ostrov, the influences of most far-off regions have been mentioned in different works. For example, one article highlights the unexpected similarities of Yuzhniy Oleni Ostrov inhabitants and the representatives of culture Çatalhöyük **(16, p. 92)**: «*However, the distribution observed on the charts provokes a number of questions because of the convergence of typological characteristics of the groups diametrically opposed geographically and for which the likelihood of direct biological kinship and mutual contacts excluded. The most vivid illustration of this is the convergence of characteristics a series of Mesolithic Oleni Ostrov burial ground with sample from Çatalhöyük by the values of the second factor …*». But the finding of the Y-DNA haplotype J, which is associated with significantly more southern regions, only confirms ties of Yuzhniy Oleni Ostrov with the Southern cultures.

This way, the southern Neolithic influences on Yuzhniy Oleni Ostrov seem to be strongly probable and they could originated from the Neolithic tribes of Comb Ware cultures.

R1a1 in Serteya Culture (Western Russian Plain). The Usvyaty Culture or the Usvyaty level of Serteya culture is another area of finding of an R1a1 bearer of pre-Corded Ware period (**10, p. 294**). According to A.N. Mazurkevich: «*The first ceramic ware within Pskov area emerged no later than in the middle of the VI millennium BC. At this time the sites of Serteya culture were located in the Lovat-Dvina interfluve on the shores of lakes, running into the stream, connecting the basins. The population built small rectangular houses with the pillar construction of walls and sunk floor. The first clayware appeared. These are small raw clay vessels with cylinder body and conic bottom. The pots were made of the small clay 'stripes' with the beveled edge and were covered with the thin clay wash. After that the surface of the vessels was ornamented with the ornament mold, leaving the triangle, double or comb prints. The ornament was often molded in plotted manner. The similar ware emerged in forest, forest-steppe and steppe zones of the Russian Plain, as well as in the Lovat-Dvina interfluve. Other components of the material culture (stone, bone and horn artifacts) did not have the fundamental changes in time of the spread of this ware. It enables to propose the emergence of this type of ware in one center and the quick spread of the idea of the ware industry from this area. Such center was supposedly located in the Lower Volga Region and the North Caspian Sea Region in the VI millennium BC*» (**18**).

A.M. Miklyaev also gives a detailed picture of emerging the ware industry in Serteya Region «*The most ancient early Neolithic culture featuring ware development phases of the area is the Serteya Culture. This phase includes fragments of heavy-walled vessels produced through the 'overlap' method. After drying, the ribbons were jointed together and their joints were smoothed out by a* ***comb press*** *for a proper binding. The surface of ready vessels was covered with the thin clay wash and the ornament in a form of geometric composition, performed in a stroke-setback or (more rarely) in a stroke manner. The vessels were not burnt, but dried up. Judging by the ornament techniques (Smirnov, 1989), the idea for pottery manufacture came from Azov-Caspian Cultural province, but it cannot be proved by reliable sources of information so far. It should be noted that the Serteya Culture could have entered the Early Neolithic Community extending from the South of the Russian Plain to as far as Valday».* Therefore, the first stage has links to the Caspian region.

Then *«The next stage features the cauldron-type vessels. This time, the ornaments included more compositions performed by a* ***comb press*** *and the first appeared dents and cuts. As a rule, an ornament was placed in the upper part of a vessel. This stage ware has a narrower range of analogs – The Upper Dnieper tableware (Artemenko, 1954; Kalechits, 1987) and Lithuanian territory (Rimantane, 1966 and 1973). This may indicate to a separation of local groups within the above said community. In this case, it is a group located between two rivers: the Dvina and the Lovat, the Upper Dnieper and Lithuania. The links between the Upper Volga Region and the*

Left-bank Ukraine are getting weaker» (**19**). The Upper-Dnieper Culture located to the South of Serteya can be classified as a forest culture with **comb ware** traditions (**20, p. 73**) and could be considered as a more Western tradition than what had come from the North Caspian area and Lower Volga.

The genotyped carrier of R1a1 haplotypes belongs to Usvyaty culture group. According to A.M. Miklyaev «*The Usvyaty ware culture, phase g is a vivid ware tradition of uncovered f and f1 ceramic phases. Back in 1979 V.A. Semenov believed that there were links between the Usvyaty culture and the world of Line-Banded Ceramics. Now, his ideas can be confirmed by existing materials. In general, the Usvyaty culture, judging by the ornaments, should be considered as a kind of oriental-style culture of funnel beakers and globular amphoras…. a certain connection with Central Europe was being preserved. The peculiarity of flint Usvyaty industry is a reflection of this link*» (**19, p. 20**). Though mentioning of central European connections, A.M. Miklyaev notices that the ceramics of the Dubokrai-V level is still technologically close to that of Serteya. *«From technological perspective, the green ware of Dubokrai V is close to phase b and c ware of Serteya culture, but unlike the others, its ornament was placed up to the spreading of the surface with a certain substance, making it dark or even sometimes bright black*» (**ibid**).

In the text A.M. Miklyaev indicates that Serteya culture can be divided into three phases: a, b and c, followed by phase d and e of Rudnya culture. Ceramic phase and is characterized by stroked technique, in phase b **comb stamp appears**, in the next phase c **comb stamp** becomes prevailing, holes and notches appear (A.M. Miklyaev with reference to Artemenko and Rimantene relates this last phase with Upper Dnieper Early Neolithic culture and Lithuanian Neolithic) (20, pp. 16-22). Usvyaty culture dating from the late IV till the middle of the III millennium BC (21 p. 369) allows comparing its comb-stamped ceramics with a close-type comb-stamped pottery of the North European part of Russia, for example in Kargopol culture (22 p. 222). In the A. M. Miklyaev's work is emphasized the connection of the population of the region with the Narva culture, especially during the Rudnya culture stage (19, p. 24).

Periodization phases of Serteya microregion cultures is complicated by the fact that this land was attractive for multiple migrations, and comprises all types of ceramics in Eastern Europe. In this respect, we can (of course, in the first schematic approximation) see in the history of ceramics in the region of Eastern Europe Neolithic period a certain competition between the two types of pottery: **comb** inherent to the North and the North –West and **stroked**, what came from the South and the South-East, and is most likely connected with the Lower Volga Neolithic cultures. And Usvyaty culture belongs to the time **continuing the large period of comb ware domination**, having undergone the influence of european Linear Band Ceramics.

So forth, the zone of Neolithic cultures of Serteya region was within the bigger zone of comb ceramics culture of Eastern Europe. During early stages, the eastern connections were prevailing, but then the west and south-west connection became defining. Usvyaty stage emerged after Comb Ware domination and moreover has definite Central European connections.

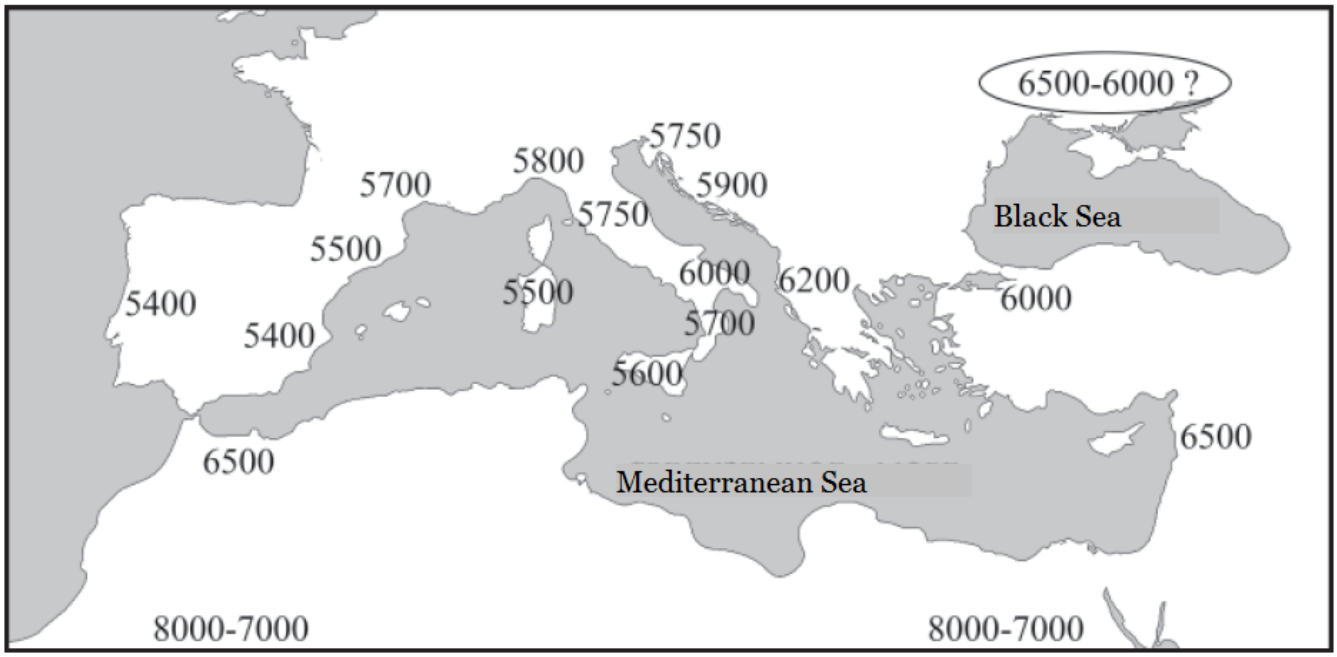
R1a1*- M459+, M198- in Khvalynsk-II burial (Steppe Volga Region). The mentioned before connection between Serteya and the Low Volga Region lead us to the analysis of Khvalynsk-II burial culture, which also belongs to the pre-Corded Ware comb-stroke ornament group: «*Khvalynsk culture can be characterized by flat-bottomed and sharp bottomed ware … Ornament composed of the rows of horizontal strokes separated by horizontal wave lines, usually cowers all the ware specimen or its upper half*» (**24, p. 39**). I.N. Vassilieva gives the characteristics to the ornaments of ware in the Khvalynsk I and Khvalynsk II burials: «*According to the opinion of I.N. Vassilieva, based on the microscopic research of the Khvalynsk ceramics technology, the ornament was made by the wicker factures… Sometimes the ornament was made by ammonite prints, strokes, short lines or* ***comb stamp***» (**25, p. 66**). The similar technique has the analogs in the b and c phases of Serteya Culture, identified by A.M. Miklyaev (**18**).

The analysis of the ceramics of Khvalynsk culture shows that it definitely does not belong to the Corded Ware areal, and can be referred as belonging to the cultures of comb-and-stroked pottery.

The areal of comb-ware cultures. Y-haplotype J2b as a possible companion of R1a1 on Neolithic sites.

The analyzed material showed that the discovery of Y-haplotype R1a1 bearers in pre-Corded Ware sites happens in the areas influenced by cultures of comb-stroked ceramic, and everywhere the presence of a comb ornament is noticeable. In the context of the analysis of cultural influences in the Eastern Europe, it is necessary to distinguish between stroked and comb pattern. Comb pattern is traditionally considered to be brought about from the north to the south of Eastern Europe, but D. L. Gaskevich in his long article «North Pontic Impresso: the origin of the Neolithic Pottery with Comb Decoration in the South Eastern Europe» (**26**) wrote the opposite. He made quite a bold assumption that runs counter to the tendency to minimize the migration, and proposed the origin of this type of pottery in the northern Black Sea coast.

«*The absolute data collected over the last 15 years in Kiev Radiocarbon Laboratory, have revealed that such ware appeared in the North-Pontic region earlier than in Upper Dnieper, Volga Region, Kama basin, Trans-Urals. However, in the steppe Pontic region it appeared earlier than in forest-steppe. All these data have proved unreliability of above mentioned hypothesis. As an alternative, the author suggests considering the Pontic region Neolithic area with* ***comb ceramic*** *ornamentation as a part of Neolithic cultures with Impresso ware from the Mediterranean region*» (**26, p 246-247**). His hypothesis was affirmed by the map (**26, p. 239**)**:**



Approximate time of appearance of ceramics Impresso in Greater Mediterranean (Messrs. Cal BC) (according Balossi, Frangipane 2002; Daugas et al. 2008; Forenbaher, Miracle 2004; Mohammed-Ali, Khabir2003; Robb 2007; Zilhao 2001).

Figure 2. The estimated time of appearance of Impresso Ceramics in Big Mideterranian Region **(26, p. 239)**.

This way, according to D. Gaskevich we see in the Eastern Europe only a small episode of a big process, which took place from Sahara to Trans-Urals and from Marocco to the Levant. Probably, the early appearance of ceramics in Samara region and in the Mideterranian area are two faces of one wave of the spread of the Neolithic technologies. The ancient subclades of Y-DNA R1b haplotype can be detected both in the Volga-Ural region and in the Northern Africa, and the Neolithic findings of R1b are attributed to the Elshanskaya (Lebyazhinka IV) and Els Trocs cave (Spain). At the moment of writing the paper the opinion about the spread of R1b Y-haplotype carriers from the Eastern Eurasia is dominating, and in moving westward the R1b (and may be

R1a) bearers of ceramic Neolithic technologies could obviously merge with the carriers of other haplogroups, J among others.

As D.L. Gaskevich refers to the initial spread of the Neolithic within the Eastern Europe, we should consider the issue of the genetic reflection of this process and specify the genetic map of the North Black Sea Region and the adjust territories in the period, preceding the Mediterranean ware adoption (though, there is another possible address of this initial spread, namely Elshanskaya culture and its derivates up to the Crimea). In VIII-VII millennia BC the North Black Sea Region was inhabited by the bearers of the Kukrek and Zimnikovskaya cultures (**27, p. 44-45**), the final Paleolithic includes Osokorovskaya culture, considered with the Caucasian Imereti one as a group of «Epi-Gravettian traditions» (**ibid, p. 43**) (Figure 3).

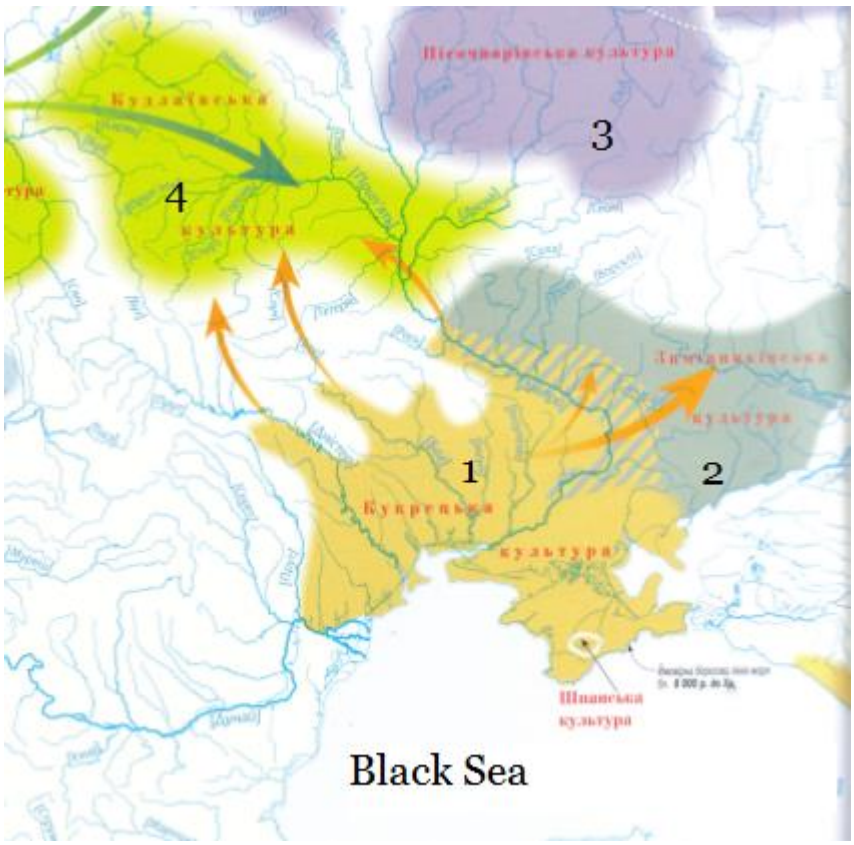


Figure 3. Black Sea area in the end of IX – first half of VII milleina BCE. (28, p. 14).
Epi-Gravettian Kukrek (1) and Imereti cultures are painted in orange.
2 - Zimnikovskaya culture, 3 - Pesochnorovskaya culture, 4 - Kudlaevskaya culture.

Pre-Neolithic Bug-Dniester culture developed on the basis of the Kukrek one. As the above mentioned Satsurblia and Kotias burial grounds, genetics of which contains the male haplogroup J refer to the habitat of Imereti culture, we can make the conclusion that one of the subclades of the haplogroup J could have been spread across the North Black Sea Region in the pre-Neolithic period (common epi-Gravettian tradition). The mentioned migration by D.L. Gaskevich is supported by genetic data, if we consider that its representatives were the bearers of subclade J2b Y-haplogroup J. Nowadays subclade J2b is widely spread within the zone of ancient migrations of cardial tribes (Figure 4).

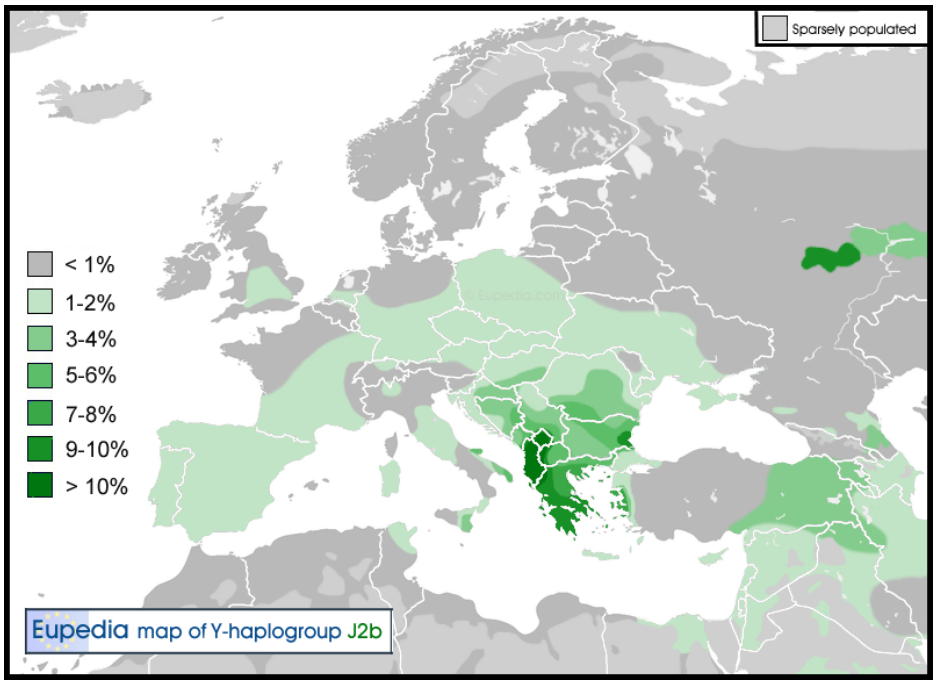


Figure 4. Distribution of haplogroup J2b (M102) in Europe, the Middle East & North Africa. http://www.eupedia.com/europe/Haplogroup_J2_Y-DNA.shtml

Those cardial tribes that could have given rise to the **comb ceramics** culture, may have been centered around Black Sea and Adriatic shores, and they could contain Y-DNA subclade which could be different with those of Georgia but related to them. The regions of the highest concentration of the haplogroup J2b bearers, namely Albania, the South-East of Bulgaria, Greece and some coastal regions of Italy (from 10 to 26 % of population) up to the Black Sea are represented on the above mentioned map.

This view is strongly supported by the spread of J2b in populations, which can be considered theoretically connected with the cultures of **comb ware** and were least of all Indo-Europeanized among all the East of Europe (nowadays they speak Uralic languages with some pre-Uralic substrate **(46)**). So, the presense of J2b in these populations may not reflect the Indo-European migrations, but earlier waves of Neolithic spread.

Firstly, the spot of the noticeable spread of **J2b (10-15% of population) is Mordovia** (central Russia) (particularly, Moksha environment (**29**)). V.V. Stavitsky in his thesis work on the theme «Neolithic, Eneolithic and the Early Bronze Age of Sura-Oka Interfluve and the Upper Prikhoper'e: Dynamics of North and South Cultures' Interrelations in the Forest-steppe Zone», says: «*The spread of comb-stroke ware across Moksha river area is apparently concerned with the advance of the definite groups of Upper-Volga population. The carriers of the stroke ware, focused on the forest-steppe landscapes, must have moved to the south-west and south-east, to Ryazan Oka and Ulyanovsk Volga Region. But some part of the previous population must have not left Moksha river area and had contacts with the Middle-Volga tribes. It is reflected in the data of the 1st Kovylyaskaya site, where the weakly ornamented ware of the Elshanskaya type lies together with the comb-stroke ceramics. The stroke group of the Inerskaya site has combined under the influence of the forest-steppe traditions. These processes are reflected in the comb-stroke ware of the settlement Gorodok 1, which is similar to the ware of Kovylyaskoe settlement. The simple motives of the ornament, consisting of horizontal rows of the slanted prints of the short low arched stamp, often interspersing with the plain sections are most common for the comb ware here. The stroke ware commonly uses the hatched zones of differently directed rows of strokes. Since recently there was an opinion that the Middle-Volga population left Moksha river area under the influence of the pit-comb ware.* ***But the examination of Ozimenki 2 site showed that the local population continued the development of the Upper-Volga traditions at the late stage of this culture existence without leaving the region.***

The ornamentation of the ware of Ozimenki 2 site widely uses the broad-toothed prints of the long stamp with the rare rows of deep patches on top, having the complete analogs in the late Upper-Volga ware»(**30**). V.A. Yurchenkov in his book, which is the review of academic research, says about the prevailing **comb nature** of *Moksha river area* ware: «There are 20 memorials with the so-called comb-stroke ware in the Moksha basin. The prints of comb stamp prevailed in the ware decoration; the share of stroke ornament is low» (**31, p 113**). Thus, the penetration of J2b into Mordovia territory can be explained by the migration from the south, and the Mordovian J2b peak can be explained by the fact that the population of comb-stroke ware had not left the territory.

Secondly, **Saami J2b phenomenon**. The Saami have the reputation of the relict, some kind «reserve» of the ancient genes of Europe (and the bearers of the considerable pre-Uralic language substrate), that is why it is not surprising that one of the first Neolithic migrations to the European continent could be preserved in the genes of this isolated northern people, unaffected by «Indo-Europeanization» (**32**). The population of the Saami within Kola Peninsula contains about **14% of haplogroup J2b (17)**. As the comb ware cultures in pure form were displaced to the north of the Eastern Europe in early Bronze Age, it is quite possible that their creators could have played an important role in the Saami formation.

The further search of R1a1 roots. Thus, we can suppose that haplotype R1a1 could be found in the wide range of comb-stroke cultures, especially comb ware cultures often accompanied by J2b (at the north-west area – in Karelia and Mordovia exactly comb cultures exist in the considered period) in the Neolithic horizons. Besides, it is also possible that the epicenter of the spread and divergence of modern most widespread subclades R1a1 (M198+, M417+) was located to the west of Serteya, which is indicated by the relations of Serteya culture with the funnel beaker (developed on the basis of Ertebelle) and Narva cultures (Fig. 5.).

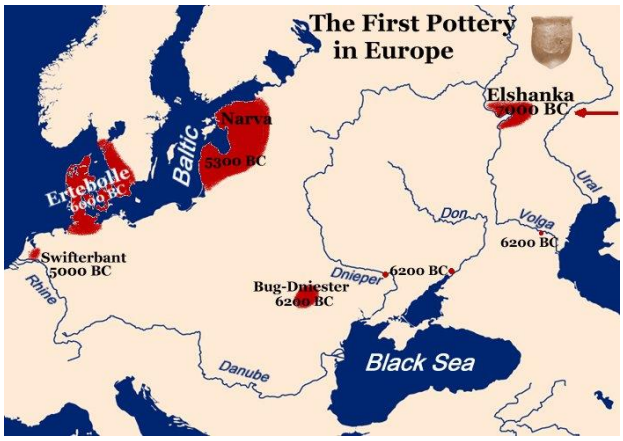


Figure 5. Main Ceramic Neolithic Cultures of Europe

But the issue of R1a1 bearers' Upper Paleolithic origin is debatable. Firstly, the variant of the Black Sea Region origin is still possible, as this haplotype can be present in Bug-Dniester culture and further southward. This fact is supported by the detection of basal haplotype in the population of the Middle East Region: «*... more basal (R1a-M420*) Y-chromosomes have been detected in Iran and eastern Turkey. Overall, our detection of haplogroup R1a1 in a northwest Russian hunter-gatherer establishes the early presence of this lineage in eastern Europe, and is consistent with a later migration from eastern Europe into central Europe which contributed such haplogroups to the Corded Ware population*» (**7**).

Some data enable to consider that it also can have an origin not necessarily concerned with the Middle East via north Black Sea Region. The choice of candidate cultures is rather small, as many cultural layers (Ienevo, for instance) of the Eastern Europe have post-Swiderian or post-Ahrensburg type have Western European origin, while the present consensus considers the deep emergence of R1 in the Paleolithic in the eastern part of Eurasia. If we accept the theory, concerning the Neolithic nature of the Yuzhniy Oleni Ostrov, the R1a1 haplotype could have come with the first group of ware bearers to Karelia. If we accept the pre-ware theory of R1a1 emergence

in Karelia, it is possible in terms of Butovo or Kunda culture, but, taking into consideration the (partly) western post- Swiderian nature of these cultures' origin, we should search for the eastern substrate in them. And there exists one. According to M.G. Zhilin: «*The first significant penetrations of the population of Swiderian cultural tradition into the Upper Volga trace back to the middle of pre-Boreal period (the site Tikhonovo). The site Mar'ino-4, the goods of which are similar to Tikhonovo-1, should be dated back the same time, or a bit earlier. But the major cultures of the Upper Volga Region, namely Ienevo and Butovo have already formed by that time. Probably, the certain groups of the population of Swiderian tradition, having penetrated into the Upper Volga Region, were quickly assimilated by the population of Butovo culture*» (**33, p. 7-16**).

And the primary pre-Swiderian population of the Butovo culture could have the occidental origin. When the researchers found the archeological site Chernoozer'e II, located 140 km to the north of Omsk, they were surprised by the similarity with the Krasnyi Yar site at the Angara, which referred to the cultural groups of Malta-Buret community (containing proven Y-haplotype R*). The distance between these sites is 1800 kilometers, but it was not a problem for the migration of mobile Paleolithic hunters (**34, p. 303, p. 310**). A little bit later in early 1980s, the Chernoozer'e was estimated as the unique site within the West Siberia, but «having proximity with the Kazakhstan late Paleolithic» (ibid**, p. 310**). If we speak of the neighboring cultures, the famous Shikaevka II, which artifacts are close to the Caspian Sea Region, but different from Yangelskaya culture, is located to the west of Chernoozer'e (ibid). This subtlety enables to distinguish Shikaevka from the Yangelskaya culture of the south Caspian and later – Zarzian origin and compare it with pre-Zarzian late Paleolithic of Kazakhstan, which originated the part of the population of Chernoozer'e. In the period of late and final Paleolithic, the creators of Shikaevka and Chernoozer'e inhabited the south coast of Mansi periglacial sea-lake (**35**). The tie of Chernoozer'e with Malta-Buret cultures enables to consider the late Paleolithic migrations of the latter to the west and estimate the impact of these migrations on the ethnic and general archeologic history of Ural and the adjacent territories: «*The middle Ural culture was formed on the basis of the emergence of new groups of the population in 16–17 millenia BC, genetically connected with the north-Asian Paleolithic*» (**36, p. 54**). Migration of the creators of Chernoozer'e is not the only one, concerned with Malta-Buret community. The Upper Paleolithic Chulym, located at the border of the West and Middle Siberia, was also created by the expatriates from the Angara banks (**37**). Consequently, we deal with the rather wide migration from the Baikal Region to the west of the late Upper Paleolithic period, which had a great affect on the North Urals. In general, a great group of the sites of the West Siberia, Urals and the north of the European part of Russia: Byzovaya, Shikaevka, Il'murzinskaya, Talitskogo, Deukovskaya, Ust-Kamskaya, Karacharovo, Altynovo and Zolotoruch'e, as well as Kapova and Medvezhya Peshchery are combined into the so-called 'north-western area of the late Paleolithic', equivalent by the proximity of Podonskaya cultures or South-Western areas. Microplated industries of sites, named after Talitsky, Shirovanovo and Medvezhya Peshchera have direct links with the similar techniques of the Middle Siberia (Malta-Buret ones), dated back to the XXV-XV millennia BC. (**38, p. 43**). Concerning Altynovo and Zolotoruch'e sites, which are located at the edge western flank of the above mentioned late Paleolithic area and are situated on the east of the present Yaroslavl Region, they were compared with the middle Volga Region sites Syukeevsky Vvoz and Postnikov Ovrag, as well as Gornaya Talitsa site (**39**). Syukeevsky Vvoz and Altynovo are the basis for the development of the influences, coming from the west, which are called «the east Federmesser» (**40, p. 205**). A.Kh. Khalikov considered that the early Mesolithic sites of the Middle Volga belong to the Siberian circle, which is indicated by the data of the sites Syukeevsky Vvoz and Postnikov Ovrag (**37**).

We can make the justified conclusion that the western influences of the early Mesolithic (like eastern Federmesser and Swider), having formed the Butovo culture, were combined with the occidental ones in the Upper and Middle Volga Region, concerned with the migrations of the Siberian groups, having created Altynovo and Zolotoruch'e in the final Palerolithic Age. Thus, the Upper Volga Region became the zone of intense Paleo-ethnic and Paleo-genetic contacts approximately in XI-VIII millennia BC (Fig. 6).

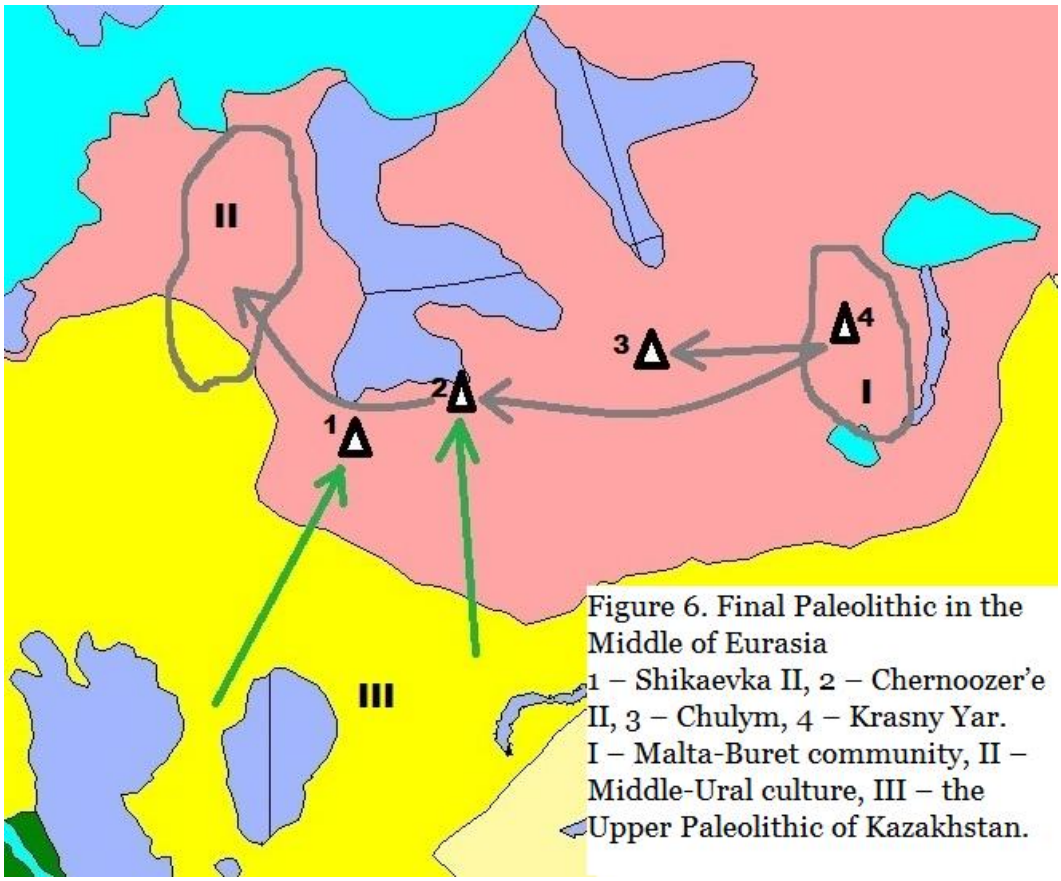


Figure 6. Final Paleolithic in the Middle of Eurasia
1 – Shikaevka II, 2 – Chernoozer'e II, 3 – Chulym, 4 – Krasny Yar. I – Malta-Buret community, II – Middle-Ural culture, III – the Upper Paleolithic of Kazakhstan.

The landscapes of the XVI thousand years BC are highlighted in colors: glaciers in blue, tundra in pink, «mammoth steppes» in yellow; source (41)**.**

Proceeding from the fact that the north-eastern area of the late Paleolithic extended to the Upper Volga and the North Dvina, we can consider that it may also be ancestral for the creators of the Oleniy Ostrov burial ground, the bearers of R1a1-M198-, C1f. The east-Eurasian origin of both male and female haplotype indicates this possibility. If we consider that the bearers of this type came from the east, but via the Black Sea Region and penetrated into the Oleni Ostrov from the south, we can compare their mt DNA with the mitochondrial C of the Neolithic time found southward. There are some mtDNA haplogroup C findings in the cultures of Dnieper-Donetsk circle, but they refer to a different subclade C4 (**42**), having parted with C1 in Paleolithic Age. In other words, the population of Oleni Ostrov and the bearers of Dnieper-Donetsk cultures have related, but different mtDNA haplotypes C subclades.

But there's an argument against the eastern trace. The above mentioned migration came across Urals, having left Ilmurzinskaya culture. There are statements that Romanovo-Ilmurzinskaya culture could be relevant to the Elshanskaya one: «*The issue of the origin of the Elshanskaya culture should be solved, firstly, by the comparison of its flint industry with the relevant industries of the earlier, Mesolithic era. …… Nevertheless, the data of such sites as Krasnyi Yar I, Chekalino II, Staro-Torskaya and some other memorials shed light on this problem. The flint collection of these memorials has definite similarity with the Elshanskaya one… Basing on the similarity of these industries with the Elshanskaya flint complexes, we come to the conclusion that the roots of the local Neolithic culture should be sought in its Mesolithic traditions of Volga-Kama and perhaps some adjacent areas, located to the north or to the north-west from the Elshanskaya memorials' area, rather than to the south*» (**43**).

The genetics of the Elshanskaya and related cultures is well-studied by now. The burial of Lebyazhinka IV possessed mtDNA U5a1d (**7, p. 25**). Also «*Bramanti et al. (2009) tested*

Mesolithic remains from several locations across Europe, and found one haplogroup U5a(9,800 ybp) at the Chekalino site in the Volga-Ural region of Russia, one U5a1 (10,000 to 8,000 ybp)» (**44**). The Khvalynsk culture contains mitochondrial haplotype U5a (together with U4 and H) (**9**). If the migrants moved via the mentioned territories, the found subclades of mt-DNA C in Mesolithic-Neolithic remains could be the evidence (as they preserved in Yuzhniy Oleni Ostrov and Dnieper-Donetsk area), but there are no such findings. According to (**45**), C1f is rather frequent in Yuzhniy Oleni Ostrov and Bolshoi Oleni Ostrov (also the north of Russia). Thus, C mt-DNA haplotype has been preserved there till late Mesolithic and Neolithic and was displayed in the first genotyped findings in the North of Russia but is still absent in contemporal findings in the Volga-Ural area. Probably, in case if R1a1 has come to the North-East Europe from the East, rather than from the South, we deal with another population, different from the one, having formed the Romanovo-Ilmurzinskaya culture, concerned with the late Upper Paleolithic of Kazakhstan and Urals. But the strong presence of haplotype C in the Black Sea Region in the Neolithic time demand consideration of the variant of R1a1 migration from the East via the south regions (Near East or Black Sea).

Thus, we can conclude that the presence of haplotype R1a1 is strongly probable in the cultures of Comb Stamp Ware (with J2b bearers as well) in the Neolithic context, but the origin and the trace of R1a1 migration is still unclarified and more data are needed.

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Introduction *Freylinia Lanceolata* (L.F.) G.Don. on the Black Sea Coast of the Caucasus

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Abstract

Freylinia lanceolata (L. f.) G. Don is the only woody plant of South Africa, which successfully introduced on the Black sea coast of the Caucasus. *Freylinia lanceolata* regularly, abundantly and long lasting blooms in the winter, sets seed, can withstand the average of the absolute minimums (-5,6°C), does not suffer from summer droughts in the humid subtropics of Russia. Research of methods introduction based on the results of acclimatization *Freylinia lanceolata* conducted. This analysis has allowed to identify promising principles of selection of objects of introduction from the Cape region.

Keywords: introduction, Western Cape region, *Freylinia lanceolata (*L.f.) G.Don, Sochi Dendrarium, the Black sea coast of Caucasus.

Введение

Сочинский «Дендрарий» является интродукционным пунктом в зоне влажных субтропиков России. С 1892 года здесь было испытано свыше 10 000 видов и форм древесных и кустарниковых растений. Основное внимание уделяется видам, происходящим из субтропических зон. В Южной Африке сухой субтропический климат, который имеет схожие с Черноморским побережьем Кавказа показатели [8]. Капская область характеризуется обилием экзотических эндемичных растений [9], представляющих интерес для интродукции. Экспериментально доказано, что культивирование южноафриканских древесных растений в условиях открытого грунта Сочи бесперспективно.

Единственным древесным видом Капской области, успешно интродуцированным во влажные субтропики Черноморского побережья Кавказа является *Freylinia lanceolata* (L.f.) G.Don сем. *Scropulariaceae*.

Результат интродукции этого вида подлежит изучению и анализу. Является ли это случайной удачей прямого эксперимента или его можно объяснить с помощью научных методов прогонозирования.

Материалы и методы

Объектом исследования являются растения *Freylinia lanceolata* (L.f.) G.Don в коллекции сочинского «Дендрария».

Таксономическое определение осуществляли по определителю «Деревья и кустарники СССР» [1].

Для оценки морозоустойчивости была использована модифицированная шкала зимостойкости Н.К. Вехова [6], характеризующая степень повреждения растений отрицательными температурами: I – повреждений нет (растение не обмерзает); II – обмерзает не более половины длины однолетних побегов; III – обмерзают однолетние побеги полностью; IV – обмерзают двулетние и более старые части растений; V – обмерзает вся надземная часть; VI – растение вымерзает полностью.

Оценку акклиматизации давали по 5-бальным шкалам зимостойкости, засухоустойчивости, репродуктивности, устойчивости к вредителями и болезнями, разработанные Ростовским ботаническим садом [3].

Успешность интродукции *Freylinia lanceolata* (L.f.) G.Don оценивали с позиций метода климатических аналогов Мауг'а (Майр Г., Бекетов А.); метода агроклиматических аналогов (Селянинов Г.Т.);ботанико-географического и исторического метода (Краснов А.Н.); ботанико-географического методаинтродукции растений (Вавилов Н.И.); методафлорогенетического анализа (Малеев В.П.) и его модификация для древесных растений (Кормилицин А.М.); метода потенциальных ареалов Good'а (Гуд Д.); метода палеоботанической теории Seward'а (Сьюорд А.Ч.); метода эколого-исторического анализа флор или экогенетического анализа рода (Культиасов М.В.); эколого-биоморфологического метода интродукции с позиций эволюционного учения и экологии растений (Лаптев А.А.); эколого-системногометода (Чекалин С.В.); метода филогенетических или родовых комплексов (Русанов Ф.М.); метода геоботанических эдификаторов (Русанов Ф.Н. – Быков Б.Н.); фитоценотического метода анализа растительных сообществ (фитоценозов) (Карписонова Р.А.); метода репрезентативной интродукции растений природной флоры; метода предварительного отбора интродуцентов: интродукция без изменения природы растений и интродукция с существенным изменением наследственности растений (Соколов С.Я.); метода изучения интродуцентов в природе (Кучеров Е.В.); метода выбора материала для интродукции в зависимости от индивидуальных свойств видов растений (Базилевская Н.А.); графического метода многолетних фенологических спектров (Аврорин Н.А.); метода морфофизиологического анализа годичных ритмов интродуцируемых растений (Сергеева Л.И. и Сергеева К.А.); метода прогнозирования результатов интродукционной работы (Соболевская К.А.); метода учёта опыта акклиматизации за прошлое время (Аврорин Н.А.); категории интродукционной практики (Шлыков Г.М.) [2, 4, 5, 7].

Перечисленные методы можно объединить в несколько групп по принципу подбора интродуцентов: 1) поиск сходных условий произрастания вида (как первичных, так и вторичных пунктов-доноров); 2) анализ истории формирования и развития вида; 3) выявление внутривидового генетического разнообразия; 4) анализ экспериментальных результатов.

Результаты

На Черноморское побережье *Freylinia lanceolata* (L.f.) G.Don впервые попал с синонимичным названием *Freylinia cestroides* Colla в Сухуми или Батуми на рубеже XIX–XX веков. Это крупный (в условиях Сочи до 3 м), вечнозелёный кустарник.

Молодые побеги 4-х-гранные, зелёные, слабо опушённые. Зрелые побеги без граней, относительно тонкие (1,5 см в диаметре), маловетвистые, покрытые гладкой, желтовато-коричневой корой. Листорасположение супротивное. Листья ланцетные, 7-17 см длины и 0,4-1,5 см ширины, цельнокрайние, напоминающие листья ивы. Сверху голые, блестяще – зелёные, снизу тусклые из-за слабогоопушения. Листья заострённые на верхушке, с клиновидным основанием, почти сидячие.

Цветки мелкие, снаружи кремовые, внутри оранжевато-жёлтые. Чашечка 2-3 мм длиной, глубоко 5-лопастная, венчик 1,3 см длиной и 3 мм шириной, трубчатый, с

5 треугольными лопастями (рис. 1). Тычинок 5, они скрыты внутри венчика. Завязь верхняя, 2 гнездная. Цветки, собраны в пирамидальные метёлки длиной 10-15 см.

Рис. 1. Соцветие *Freylinia lanceolata* (L.f.) G.Don

В одном соцветии обычно по 50-60 цветков. Метёлки располагаются в пазухах листьев верхней части побегов текущего года. Аромат цветков в сочинских условиях неприятный, несмотря на упоминание этого растения как «медовые колокольчики» за сладкий запах. В условиях Черноморского побережья Кавказа сейчас цветение длится с ноября по март, хотя в 1960-е годы оно цвело в августе–сентябре.

Плодоносит фрейлиния в июле-августе. Плод – двустворчатая, многосемянная коробочка до 7 мм длины и 3 мм ширины, яйцевидной формы.

Freylinia lanceolata ценится в зелёном строительстве за обильное продолжительное цветение в холодный период года. На родине цветение также проходит в зимний период, в соответствии для Южного полушария с июня (зимы) до августа (ранней весны). Это, как и наличие плодоношения, свидетельствует об акклиматизации растения.

Рис. 2. Декабрьское цветение *Freylinia lanceolata* (L.f.) G.Don

Самому крупному цветущему экземпляру фрейлинии ланцетной в сочинском «Дендрарии» 30 лет. С укрытием он выдержал несколько зим с понижением температуры воздуха до -8˚С. Без укрытия отмерзает до корневой шейки, но потом отрастает от пня. [8]. В декабре 2013 года отмечен средний из абсолютных годовых минимумов, равный для Сочи -5,5°С [6]. Произрастающие в защищённых от ветра местах парка взрослые растения *Freylinia lanceolata* не пострадали даже без укрытия. У молодых растений (до 8 лет) полностью обмёрзли однолетние побеги (степень морозостойкости III), но крона хорошо восстановилась в последующий вегетационный период.

Зимостойкость растения 3 балла, засухоустойчивость 3 балла, устойчивость к вредителям и болезням 4 балла, репродуктивная оценка 3 балла. Коэффициент адаптации 65 – ограниченно перспективная [3].

Freylinia lanceolata легко черенкуется. В зимний период несколько растений выращивается в оранжерее, для сохранения таксона в случае наступления сильных морозов.

Фрейлиния – быстрорастущий кустарник, предпочитающий освещённые участки, с плодородной, влажной, дренированной почвой. Для культивирования хорошо подходят берега ручьёв на склонах юго-западной экспозиции.

Freylinia lanceolata (L.f.) G.Don способна выдерживать продолжительные летние засухи и затяжные зимние ливни, характерные для Черноморского побережья Кавказа. В последние годы отмечен полный цикл развития растений. Они ежегодно цветут и плодоносят.

Обсуждение результатов

Растения *Freylinia lanceolata* (L.f.) G.Don адаптировались в условиях влажных субтропиков России. Фрейлиния предпочитает богатые, дренированные, хорошо увлажнённые почвы и солнце. Хорошо размножается черенкованием. Относится к быстрорастущим кустарникам.

Род *Freylinia* является эндемичным для Южной Африки и насчитывает по разным источникам от 4 до 8 видов. Прямых исторических, флористических, генетических связей с Черноморским побережьем, как основы для интродукции нами не обнаружено.

Наиболее приемлемым объяснением успешной интродукции *Freylinia lanceolata*, вероятно, следует считать ступенчатую акклиматизацию вида. Впервые интродуцированная в Италию в сад графа Фрейлино в 1817 году она получила распространение во вторичных интродукционных пунктах. По оценкам европейских специалистов *Freylinia lanceolata* не может выносить морозы ниже -2-5˚С. Но, те экземпляры, по сути, внутривидовые формы, которые прошли акклиматизацию в интродукционных пунктах Черноморского побережья выявили скрытые возможности вида. Наиболее отвечают данному положению методы климатических аналогов и интродукционной практики.

Одним из синонимов *Freylinia lanceolata* является название *Buddleja glaberrima* Loisel. Возможно, что генетическая близость родов может быть причиной устойчивости данного вида на Черноморском побережье, где культивируются различные виды буддлей из Восточной Азии и Южной Америки (метод филогенетических комплексов).

Данный вид на территории России имеется только в коллекции сочинского «Дендрария». Фрейлинию при массовом озеленении можно рассматривать только как дополнительный ассортимент.

Заключение

Дальнейший интродукционный поиск объектов Капской области следует вестиво вторичных пунктах-донорах исходных условий произрастания, анализируя филогению рода и вида, фитоценотические связи, с учётом выявляемого внутривидового разнообразия.

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Интродукция *Freylinia Lanceolata* (L.F.) G.Don. на черноморское побережье Кавказа

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Аннотация. *Freylinia lanceolata* (L.f.) G.Don единственное древесное растение Южной Африки, которое успешно интродуцировано на Черноморское побережье Кавказа. В условиях влажных субтропиков России *Freylinia lanceolata* регулярно, обильно и продолжительно цветёт в зимний период, завязывает семена, выдерживает средний из абсолютных минимумов (-5,6°С), не страдает от летних засух. Проведён анализ методов интродукции с учётом результатов акклиматизации *Freylinia lanceolata,* позволившие выявить перспективные принципы подбора объектов интродукции из Капской области.

Ключевые слова: интродукция, Капская область, *Freylinia lanceolata (L.f.) G.Don*, сочинский «Дендрарий», Черноморское побережье Кавказа.